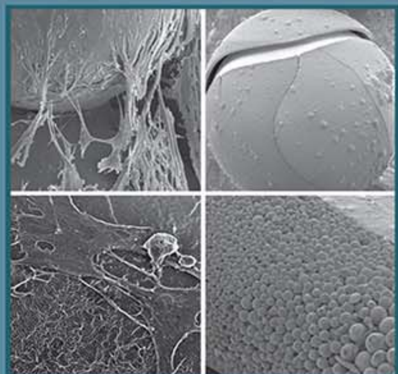
Woodhead Publishing Series in Biomaterials



Biomaterials for Oral and Dental Tissue Engineering

Edited by Lobat Tayebi and Keyvan Moharamzadeh

WP
WOODHEAD PUBLISHING

Biomaterials for Oral and Dental Tissue Engineering

Related titles

Preprosthetic and Maxillofacial Surgery: Biomaterials, Bone Grafting and Tissue Engineering (ISBN 978-1-84569-589-7)

Non-Metallic Biomaterials for Tooth Repair and Replacement
(ISBN 978-0-85709-244-1)

Stem Cell Biology and Tissue Engineering in Dental Sciences
(ISBN 978-0-12-397157-9)

Nanobiomaterials in Clinical Dentistry
(ISBN 978-1-4557-3127-5)

Emerging Nanotechnologies in Dentistry
(ISBN 978-1-4557-7862-1)

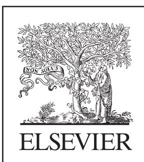
Woodhead Publishing Series in Biomaterials

Biomaterials for Oral and Dental Tissue Engineering

Edited By

Lobat Tayebi

Keyvan Moharamzadeh



WP
WOODHEAD PUBLISHING
An imprint of Elsevier

Woodhead Publishing is an imprint of Elsevier
The Officers' Mess Business Centre, Royston Road, Duxford, CB22 4QH, United Kingdom
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, OX5 1GB, United Kingdom

Copyright © 2017 Elsevier Ltd. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: www.elsevier.com/permissions.

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

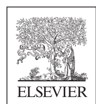
To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

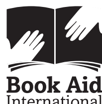
British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

ISBN: 978-0-08-100961-1 (print)
ISBN: 978-0-08-100967-3 (online)

For information on all Woodhead Publishing publications
visit our website at https://www.elsevier.com/books-and-journals





Working together
to grow libraries in
developing countries

www.elsevier.com • www.bookaid.org

Publisher: Matthew Deans
Acquisition Editor: Laura Overend
Editorial Project Manager: Natasha Welford
Production Project Manager: Poulouse Joseph
Cover Designer: Christian J. Bilbow

Typeset by MPS Limited, Chennai, India

Contents

List of contributors **xiii**
Preface **xix**

Part I Biomaterials for Oral and Dental Tissue Engineering **1**

1 Introduction to oral and dental tissue engineering **3**
Lobat Tayebi and Keyvan Moharamzadeh
References 5

2 Bioactive glasses and calcium phosphates **7**
Mohammadreza Tahriri, Rizwan Bader, Wiley Yao, Shima Dehghani, Kimia Khoshroo, Morteza Rasoulianboroujeni and Lobat Tayebi
2.1 Introduction 7
2.2 Bioactive glasses 8
2.3 Calcium phosphates 13
2.4 Conclusions 17
References 17

3 Polymers for oral and dental tissue engineering **25**
Nasim Salehi-Nik, Maryam Rezai Rad, Pantea Nazeman and Arash Khojasteh
3.1 Introduction 25
3.2 Different types of polymeric scaffolds 25
3.3 Methods for scaffold fabrication 34
3.4 Conclusion 39
References 39

4 Hydrogels in craniofacial tissue engineering **47**
Ehsan Zahedi, Sahar Ansari, Benjamin M. Wu, Sompop Bencharit and Alireza Moshaverinia
4.1 Introduction 47
4.2 Hydrogel biomaterials 48
4.3 Summary: current status and future prospects 58
References 58

5 Dental biocomposites **65**
Touraj Nejatian, Zohaib Khurshid, Muhammad S. Zafar, Shariq Najeeb, Sana Zohaib, Masoud Mazafari, Louise Hopkinson and Farshid Sefat
5.1 Introduction 65
5.2 Classification of biocomposites 65
5.3 Natural biocomposites 67
5.4 Synthetic biocomposites 68
5.5 Unique properties and adaptability of biocomposites 69
5.6 Applications of biocomposites materials in dentistry 72
5.7 Restorative applications 73
5.8 Bone cements 73
5.9 Allogeneic bone grafts 76
5.10 Xenografts 76
5.11 Alloplastic grafts 77
5.12 Shortcomings of existing biocomposites and recent developments 77
5.13 Conclusions 80
References 80
Further Reading 84

6 Surface modification of dental implants **85**
Jonathan Wirth, Mohammadreza Tahriri, Kimia Khoshroo, Morteza Rasoulianboroujeni, Andrew R. Dentino and Lobat Tayebi
6.1 Introduction 85
6.2 Surface treatment methods 86
6.3 Prospective surface modification methods 90
6.4 Conclusion 94
References 94

7 Characterization of biomaterials **97**
Meisam Omidi, Atena Fatehinya, Masomeh Farahani, Zahra Akbari, Saleheh Shahmoradi, Fatemeh Yazdian, Mohammadreza Tahriri, Keyvan Moharamzadeh, Lobat Tayebi and Daryoosh Vashaee
7.1 Introduction 97
7.2 Chemical characterization techniques 97
7.3 Physical characterizations techniques 102
7.4 Biological characterization technique 108
Acknowledgment 112
References 112

8 Biocompatibility of dental biomaterials **117**
Mohamed S. Ibrahim, Noha A. El-Wassefy and Dina S. Farahat
8.1 Introduction 117
8.2 Concepts of biocompatibility testing 117
8.3 In vitro tests 118

8.4 Animal tests 121
8.5 Usage tests 123
8.6 Combining in vitro, animal, and usage tests together 124
8.7 Biomaterials for tissue engineering 126
8.8 Conclusion 135
References 135

9 Processing and preservation of biomaterials and regulatory issues 141
Keyvan Moharamzadeh
9.1 Introduction 141
9.2 Processing of biomaterials 142
9.3 Sterilization of biomaterials 145
9.4 Preservation and storage 146
9.5 Regulatory issues 147
9.6 Conclusions 149
References 150

Part II Tissue Engineering Strategies 155

10 Specific considerations in scaffold design for oral tissue engineering 157
Amir Yadegari, Farahnaz Fahimipour, Morteza Rasoulianboroujeni, Erfan Dashtimoghadarm, Maysam Omidi, Hossein Golzar, Mohammadreza Tahriri and Lobat Tayebi
10.1 Introduction 157
10.2 Scaffold fabrication techniques 158
10.3 Multi-tissue scaffolds for oral and dental regeneration 169
10.4 Applications of scaffolds in oral tissue engineering 173
10.5 Conclusion 177
References 178

11 Stem cells from oral and maxillofacial tissues 185
Mohammadreza Tahriri, Rizwan Bader, Kimia Khoshroo, Morteza Rasoulianboroujeni and Lobat Tayebi
11.1 Introduction 185
11.2 Types of stem cells extracted from oral and maxillofacial tissues dental pulp stem cells 186
11.3 Application of clinically relevant stem cells in oral and maxillofacial surgery 187
11.4 Future trends 188
References 189

12 Bioreactor design for oral and dental tissue engineering 193
Pouya Amrollahi, Farzaneh Moghadam and Lobat Tayebi
12.1 Introduction 193
12.2 Fundamentals of bioreactors and bioreactor design 194

12.3 Different types of bioreactors 196
12.4 Application of bioreactors for tissue engineering of oral tissue 197
12.5 Conclusion 201
References 201

13 Growth factors for oral and maxillofacial regeneration applications 205
Mohammadreza Tahriri, Morteza Rasoulianboroujeni, Rizwan Bader, Daryoosh Vashaee and Lobat Tayebi
13.1 Introduction 205
13.2 Platelet-derived growth factor 206
13.3 Insulin-like growth factors 207
13.4 Transforming growth factor beta 208
13.5 Bone morphogenic proteins 209
13.6 Platelet rich plasma 211
13.7 Vascular endothelial growth factor 212
13.8 Future trends 213
References 213

Part III Oral and Dental Soft Tissue Engineering 221

14 Oral mucosa tissue engineering 223
Keyvan Moharamzadeh
14.1 Introduction 223
14.2 Normal human oral mucosa 223
14.3 Split-thickness oral mucosa engineering 224
14.4 Full-thickness oral mucosa engineering 225
14.5 Applications of tissue-engineered oral mucosa 232
14.6 Future trends 237
References 237

15 Tissue-engineered models of oral soft tissue diseases 245
Keyvan Moharamzadeh, Helen Colley, Vanessa Hearnden and Craig Murdoch
15.1 Introduction 245
15.2 In vitro models of dysplasia and oral cancer 245
15.3 Tissue-engineered models of radiotherapy-induced oral mucositis 247
15.4 In vitro three-dimensional models of bisphosphonate and medication-related osteonecrosis 247
15.5 Imaging and spectroscopic diagnostic techniques 248
15.6 Oral candidiasis disease modeling 249
15.7 Oral mucosal models of bacterial infection 250
15.8 Immune response studies 251
15.9 Drug delivery systems 251

15.10 Future trends 252
References 252

16 Periodontal soft tissue reconstruction 257
Lisetta Lam, Ryan S.B. Lee and Saso Ivanovski
16.1 Introduction 257
16.2 Autologous grafts 257
16.3 General considerations for biomaterials in periodontal soft tissue reconstruction 259
16.4 Allogenic materials 260
16.5 Xenogeneic materials 262
16.6 Autogenous materials 267
16.7 Tissue engineering 268
16.8 Alloplastic materials 271
16.9 Conclusion 272
References 272

17 Layered scaffolds for periodontal regeneration 279
Ourania-Menti Goudouri, Eleana Kontonasaki and Aldo R. Boccaccini
17.1 Introduction 279
17.2 Structure of periodontium 280
17.3 Requirements of a layered scaffold for periodontal regeneration 283
17.4 Current solutions available 284
17.5 Conclusions, limitations, and recommendations to readers 290
References 290

18 Dental pulp tissue engineering and regenerative endodontic therapy 297
Priyadarshni Bindal, Noor H. Abu Kasim, Thamil Selvee Ramasamy, Ali Dabbagh, Keyvan Moharamzadeh and Wen L. Chai
18.1 Introduction 297
18.2 Pulp therapy 298
18.3 Regenerative endodontic procedures 299
18.4 Conclusion 312
Acknowledgments 313
References 313

19 Oral nerve tissue repair and regeneration 319
Hossein E. Jazayeri, Farahnaz Fahimipour, Mohammadreza Tahriri, Luis Almeida and Lobat Tayebi
19.1 Introduction 319
19.2 Peripheral neuroanatomy applicable to dentistry 320
19.3 Nerve tissue engineering principles 321
19.4 Applicable stem cells in oral and maxillofacial nerve repair 327
19.5 Growth factors in oral and maxillofacial nerve repair 329
19.6 Clinical applications and future trends 330

19.7 Conclusion 330
References 331

20 Tissue engineering of salivary glands 337
Dewi Borkent and Keyvan Moharamzadeh
20.1 Introduction 337
20.2 Salivary gland function, anatomy, and histology 338
20.3 Salivary gland diseases and pathology 339
20.4 Prevention and preservation of salivary glands 341
20.5 Tissue-engineering approaches 342
20.6 Regeneration challenges 345
20.7 Conclusion 346
References 346

21 Facial muscle tissue engineering 353
Michael Del Monico, Mohammadreza Tahriri, Zach Nicholson, Kimia Khoshroo and Lobat Tayebi
21.1 Introduction 353
21.2 Anatomy and structure of facial muscles 353
21.3 Tissue engineering approaches 354
21.4 Cells 354
21.5 Scaffolds 355
21.6 Laboratory and clinical challenges 355
21.7 Conclusion/future trends 360
References 360

22 Vascularization 367
Mostafa Yazdimamaghani, Jose Gonzalez and Lobat Tayebi
22.1 Introduction 367
22.2 Craniofacial and dental tissue-engineering approaches 368
22.3 Clinical transplantation of engineered blood vessels 369
22.4 Current strategies to increment vascularization 369
22.5 Key challenges and future trend 375
References 376

Part IV Oral and Dental Hard Tissue Engineering 385

23 Bone tissue engineering in maxillofacial region 387
Thafar Almela, Ian Brook and Keyvan Moharamzadeh
23.1 Introduction 387
23.2 Natural alveolar bone structure 388
23.3 Strategies for bone tissue engineering 390
23.4 Tissue-engineered bone 391

23.5 Challenges in bone tissue engineering 398
23.6 Future trends 399
References 399

24 Periodontal and peri-implant hard tissue regeneration 405
Zahrasadat Paknejad, Maissa Jafari, Pantea Nazeman, Maryam Rezai Rad and Arash Khojasteh
24.1 Introduction 405
24.2 Conventional approaches 406
24.3 Tissue engineering 412
24.4 Conclusion and future direction 418
References 419

25 Regeneration concerns in craniofacial cartilage and bone defects 429
Aileen Crawford and Katie Bardsley
25.1 Introduction 429
25.2 Tissue engineering bone grafts 431
25.3 Craniofacial tissue engineering approaches 435
25.4 Conclusions/future trends 438
References 439

26 Craniofacial surgery, orthodontics, and tissue engineering 445
Mina D. Fahmy, Brinda Shah, Jasmine Faldu, Tarek El-Bialy, Hossein E. Jazayeri and Lobat Tayebi
26.1 Introduction 445
26.2 Scaffolds 446
26.3 Stem cells 446
26.4 Alveolar bone defects, current, and future repairing techniques 448
26.5 Periodontal ligament tissue engineering 451
26.6 Low-intensity pulsed ultrasound 455
26.7 Future developments 457
References 460

27 Tooth tissue engineering 467
Samaneh Hosseini, Shahrbanoo Jahangir and Mohamadreza Baghaban Eslaminejad
27.1 Introduction 467
27.2 Biological structure of teeth 468
27.3 Developmental process of tooth formation 469
27.4 Triad of tooth tissue engineering 471
27.5 Whole teeth regeneration: Recent strategies and techniques 485
27.6 Future trends and concluding remarks 489
References 490

28 Pharmacological agents for bone remodeling: An experimental approach **503**
Massoud Seifi, Parisa Amdjadi and Lobat Tayebi
28.1 Introduction 503
28.2 Calcium 504
28.3 Vitamin D 506
28.4 Prostaglandins 510
28.5 Thyroid hormone 513
28.6 Gonadal hormones 514
28.7 Fibroblast growth factor 516
28.8 Nonsteroidal antiinflammatory drugs (ibuprofen, aspirin) and acetaminophen 517
28.9 Bisphosphonates (pamidronic acid, zoledronic acid) 518
28.10 Conclusion 520
Acknowledgment 520
References 520
Further reading 523

Index **525**

List of contributors

Zahra Akbari Shahid Beheshti University of Medical Sciences, Tehran, Iran

Luis Almeida Marquette University School of Dentistry, Milwaukee, WI, United States

Thafar Almela University of Sheffield, Sheffield, United Kingdom

Parisa Amdjadi Marquette University School of Dentistry, Milwaukee, WI, United States; Shahid Beheshti University of Medical Sciences, Tehran, Iran

Pouya Amrollahi Arizona State University, Tempe, AZ, United States

Sahar Ansari University of California, Los Angeles, CA, United States

Rizwan Bader Marquette University School of Dentistry, Milwaukee, WI, United States

Mohamadreza Baghaban Eslaminejad Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

Katie Bardsley University of Keele, Staffordshire, United Kingdom

Sompop Bencharit Virginia Commonwealth University, Richmond, VA, United States

Priyadarshni Bindal University of Malaya, Kuala Lumpur, Malaysia

Aldo R. Boccaccini University of Erlangen-Nuremberg, Erlangen, Germany

Dewi Borkent University of Edinburgh, Edinburgh, United Kingdom

Ian Brook University of Sheffield, Sheffield, United Kingdom

Wen L. Chai University of Malaya, Kuala Lumpur, Malaysia

Helen Colley University of Sheffield, Sheffield, United Kingdom

Aileen Crawford University of Sheffield, Sheffield, United Kingdom

Ali Dabbagh University of Malaya, Kuala Lumpur, Malaysia

Erfan Dashtimoghadarm Marquette University School of Dentistry, Milwaukee, WI, United States

Shima Dehghani Tehran University of Medical Sciences, Tehran, Iran

Michael Del Monico Marquette University School of Dentistry, Milwaukee, WI, United States

Andrew R. Dentino Marquette University School of Dentistry, Milwaukee, WI, United States

Tarek El-Bialy University of Alberta Faculty of Medicine and Dentistry, Edmonton, AB, Canada

Noha A. El-Wassefy Faculty of Dentistry, Mansoura University, Mansoura, Egypt

Farahnaz Fahimipour Marquette University School of Dentistry, Milwaukee, WI, United States

Mina D. Fahmy Marquette University School of Dentistry, Milwaukee, WI, United States

Jasmine Faldu University of Pennsylvania School of Dental Medicine, Philadelphia, PA, United States

Masomeh Farahani Shahid Beheshti University of Medical Sciences, Tehran, Iran

Dina S. Farahat Faculty of Dentistry, Mansoura University, Mansoura, Egypt

Atena Fatehinya Shahid Beheshti University of Medical Sciences, Tehran, Iran

Hossein Golzar University of Tehran, Tehran, Iran

Jose Gonzalez Marquette University School of Dentistry, Milwaukee, WI, United States

Ourania-Menti Goudouri University of Erlangen-Nuremberg, Erlangen, Germany

Vanessa Hearnden University of Sheffield, Sheffield, United Kingdom

Louise Hopkinson University of Bradford, Bradford, United Kingdom

Samaneh Hosseini Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

Mohamed S. Ibrahim Marquette University School of Dentistry, Milwaukee, WI, United States; Faculty of Dentistry, Mansoura University, Mansoura, Egypt

Saso Ivanovski Griffith University, Gold Coast, Australia

Maissa Jafari Shahid Beheshti University of Medical Sciences, Tehran, Iran

Shahrbanoo Jahangir Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

Hossein E. Jazayeri University of Pennsylvania School of Dental Medicine, Philadelphia, PA, United States

Noor H. Abu Kasim University of Malaya, Kuala Lumpur, Malaysia

Arash Khojasteh Shahid Beheshti University of Medical Sciences, Tehran, Iran; University of Antwerp, Antwerp, Belgium

Kimia Khoshroo Marquette University School of Dentistry, Milwaukee, WI, United States

Zohaib Khurshid King Faisal University, Al-Ahsa, Saudi Arabia

Eleana Kontonasaki Aristotle University of Thessaloniki, Thessaloniki, Greece

Lisetta Lam Griffith University, Gold Coast, Australia

Ryan S.B. Lee Griffith University, Gold Coast, Australia

Masoud Mazafari Materials and Energy Research Center (MERC), Tehran, Iran

Farzaneh Moghadam Arizona State University, Tempe, AZ, United States

Keyvan Moharamzadeh University of Sheffield, Sheffield, United Kingdom

Alireza Moshaverinia University of California, Los Angeles, CA, United States

Craig Murdoch University of Sheffield, Sheffield, United Kingdom

Shariq Najeeb Al-Farabi Colleges, Riyadh, Saudi Arabia

Pantea Nazeman Shahid Beheshti University of Medical Sciences, Tehran, Iran

Touraj Nejatian Newcastle University, Newcastle upon Tyne, United Kingdom

Zach Nicholson Marquette University School of Dentistry, Milwaukee, WI, United States

Maysam Omidi Marquette University School of Dentistry, Milwaukee, WI, United States; Shahid Beheshti University, Tehran, Iran

Meisam Omidi Shahid Beheshti University, Tehran, Iran; Shahid Beheshti University of Medical Sciences, Tehran, Iran

Zahrasadat Paknejad Shahid Beheshti University of Medical Sciences, Tehran, Iran

Thamil Selvee Ramasamy University of Malaya, Kuala Lumpur, Malaysia

Morteza Rasoulianboroujeni Marquette University School of Dentistry, Milwaukee, WI, United States

Maryam Rezai Rad Shahid Beheshti University of Medical Sciences, Tehran, Iran

Nasim Salehi-Nik Shahid Beheshti University of Medical Sciences, Tehran, Iran

Farshid Sefat University of Bradford, Bradford, United Kingdom

Massoud Seifi Marquette University School of Dentistry, Milwaukee, WI, United States; Shahid Beheshti University of Medical Sciences, Tehran, Iran

Brinda Shah Marquette University School of Dentistry, Milwaukee, WI, United States

Saleheh Shahmoradi Shahid Beheshti University of Medical Sciences, Tehran, Iran

Mohammadreza Tahriri Marquette University School of Dentistry, Milwaukee, WI, United States

Lobat Tayebi Marquette University School of Dentistry, Milwaukee, WI, United States

Daryoosh Vashaee North Carolina State University, Raleigh, NC, United States

Jonathan Wirth Marquette University School of Dentistry, Milwaukee, WI, United States

Benjamin M. Wu University of California, Los Angeles, CA, United States

Amir Yadegari Marquette University School of Dentistry, Milwaukee, WI, United States

Wiley Yao Marquette University School of Dentistry, Milwaukee, WI, United States

Fatemeh Yazdian University of Tehran, Tehran, Iran

Mostafa Yazdimamaghani University of Utah, Salt Lake City, UT, United States

Muhammad S. Zafar Taibah University, Madinah Al Munawwarah, Saudi Arabia; Riphah International University, Islamabad, Pakistan

Ehsan Zahedi University of California, Los Angeles, CA, United States

Sana Zohaib King Faisal University, Al-Hofuf, Saudi Arabia

This page intentionally left blank

Preface

This book has been written to address the current need for a comprehensive reference on biomaterials for oral and dental tissue engineering. This topic has been evolved significantly in recent decades and tremendous progress has been made in tissue engineering and regeneration of hard and soft tissues in dentistry that has prompted the development of this title.

The book is intended to educate the readers about various biomaterials and tissue engineering strategies used in reconstruction of human oral and dental tissues. Different potential laboratory and clinical applications of the engineered oral and dental tissue equivalents have also been discussed in the relevant chapters.

A number of chapters have been written by the editors themselves and a great deal of effort has been put in over a long period of time to identify suitable and highly qualified authors to write some of the other chapters. Considerable amount of work has been carried out by both editors to optimize the quality of the chapters and to coordinate the assembly of this book.

It is hoped that this book will be useful for the students studying dentistry, biomedical sciences, bioengineering and tissue regeneration, dentists, scientists, and researchers with interest in the field of biomaterials, tissue engineering, and regeneration.

Any suggestions toward its further improvement will be greatly appreciated and incorporated in the next edition.

Acknowledgments

The completion of this book could not have been possible without the valuable contribution of the authors and the assistance of so many people whose names may not all be enumerated. We greatly acknowledge and sincerely appreciate their participation and help with this undertaking.

We would like to thank Elsevier team, specifically Natasha Welford, Laura Overend, and Lucy Beg, Poulouse Joseph, and Sandhya Narayanan for their administrative support, help and coordinating the production and publication of this title.

We are grateful to all relatives, friends, colleagues, and others who shared their support during preparation of this title.

Lobat Tayebi and Keyvan Moharamzadeh

This page intentionally left blank

Part I

Biomaterials for Oral and Dental Tissue Engineering

This page intentionally left blank

Introduction to oral and dental tissue engineering

1

Lobat Tayebi[1] *and Keyvan Moharamzadeh*[2]
[1]Marquette University School of Dentistry, Milwaukee, WI, United States, [2]University of Sheffield, Sheffield, United Kingdom

Tissue engineering can be defined as combining cells, materials, bioactive, and environmental factors to recreate functional biological tissues. Over the past decade, advances in tissue engineering have led to three-dimensional (3D) reconstruction of various human tissues such as bone (Almela et al., 2016a), cartilage (Bardsley et al., 2016), skin (MacNeil 2007), mucosa (Moharamzadeh et al., 2012), muscle (Ostrovidov et al., 2014), liver (Uygun and Yarmush, 2013), blood vessels (Rouwkema and Khademhosseini, 2016), and airway (Hertegard, 2016), and tremendous progress has been achieved in this field (Khademhosseini and Langer, 2016). In recent years, research has focused on the development of complex tissue-engineered organs with improved functionality, biomechanical properties, and vascularized structures to enhance the organ's biostability and survival (Kaushik et al., 2016).

Although the primary intention of tissue-engineered different organs has been to be able to produce suitable graft tissue for clinical transplantation and replacement of the damaged or lost parts of the body, there are still many challenges to overcome with regards to the clinical application of tissue-engineered reconstructs. These include cell-related issues such as cell sourcing, processing, and reconstruction of cell microenvironment and material-host related issues that have been highlighted in the literature (Almela et al., 2016b).

In addition to the in vivo applications, 3D models of various tissue types have also been developed for different nonclinical in vitro applications as alternatives to animal models. They can be used to evaluate the biological response of the living tissues to different biomaterials such as implanted materials (Chai et al., 2010) and the agents that come into contact with the skin or mucous membrane (Moharamzadeh et al., 2009). Using tissue-engineered models of diseased organs it can be possible to investigate the mechanisms of disease initiation and progression and to evaluate the effects of different treatments on the engineered model of the diseased tissues such as 3D cancer (Colley et al., 2011) or tissue infection models (Pinnock et al., 2014). These in vitro models can be very useful and provide valuable information as they can simulate the clinical situation as closely as possible and are very specific without the need for animal testing or complex and costly clinical trials (Benam et al., 2015).

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00001-3
© 2017 Elsevier Ltd. All rights reserved.

With the introduction of tissue engineering concepts to dentistry, many researchers have focused on developing tissue-engineered models of oral and dental tissues (Amrollahi et al., 2016). The oral cavity contains many different types of hard and soft tissues with specific functions. These include alveolar bone, oral mucosa, tooth, periodontal structures, salivary glands, nerves, muscles, and blood vessels. Successful tissue engineering of each individual specialized tissue requires many physiochemical and biochemical factors to be combined with appropriate biomaterials, cells, culture methods, and environment which are also dependent on the tissue's intended application.

Tissue-engineered alveolar bone can solve many of the clinical problems associated with the use of autologous grafts that are commonly used for reconstruction of maxillofacial bone defects. These include the paucity of the available bone to harvest intraorally and the donor site morbidity. Numerous biomaterials have been used as scaffolds for tissue engineering of bone (Jazayeri et al., 2017). Different materials and strategies for bone tissue engineering including the use of novel approaches such as 3D printing technology have been discussed in the following chapters.

Oral mucosa provides the barrier function for oral cavity and protects the underlying tissues. Tissue-engineered oral mucosal equivalents have been developed both for in vivo and in vitro applications (Moharamzadeh et al., 2007). Clinical applications have included intraoral and extraoral grafting of the reconstructed oral mucosa to replace the lost mucosal soft tissues with successful outcomes. In vitro applications such as oral biocompatibility testing models, oral disease modeling, and development of drug delivery systems have been extensively published in the literature (Moharamzadeh et al., 2012).

Attempts have been made to engineer tooth (Monteiro and Yelick, 2016), pulp (Albuquerque et al., 2014), and periodontal tissues (Berahim et al., 2011) and progress has been reported in the literature with details included later in the text. However, compared to the bone and oral mucosa, tissue-engineered tooth has attracted less popularity for clinical transplantation due to the availability of osseointegrated dental implants to replace the missing teeth with high success rate and the lower costs of titanium dental implants compared to production of a laboratory-made complex tissue-engineered tooth.

In this book, initially different types of biomaterials with potential applications in the reconstruction of oral and dental tissues have been discussed. This includes research update on biomaterials manufacturing, mechanical, biological properties, and characterization methods as well as their relative advantages, limitations, and potential future developments on their optimization for applications in tissue repair and regeneration. Tissue engineering factors such as cells, scaffold, and bioreactor design, and the role of growth factors and pharmacological agents are also included in the subsequent chapters. Furthermore, tissue engineering strategies for different types of oral and dental soft and hard tissues and the potential in vitro and in vivo applications of the 3D tissue models have been presented in details in the relevant chapters.

References

Albuquerque, M.T., Valera, M.C., Nakashima, M., Nor, J.E., Bottino, M.C., 2014. Tissue-engineering-based strategies for regenerative endodontics. J. Dent. Res. 93, 1222–1231.

Almela, T., Brook, I.M., Moharamzadeh, K., 2016a. Development of three-dimensional tissue engineered bone-oral mucosal composite models. J. Mater. Sci. Mater. Med. 27, 65.

Almela, T., Brook, I.M., Moharamzadeh, K., 2016b. The significance of cell-related challenges in the clinical application of tissue engineering. J. Biomed. Mater. Res. A. 104, 3157–3163.

Amrollahi, P., Shah, B., Seifi, A., Tayebi, L., 2016. Recent advancements in regenerative dentistry: a review. Mater. Sci. Eng. C: Mater. Biol. Appl. 69, 1383–1390.

Bardsley, K., Kwarciak, A., Freeman, C., Brook, I., Hatton, P., Crawford, A., 2016. Repair of bone defects in vivo using tissue engineered hypertrophic cartilage grafts produced from nasal chondrocytes. Biomaterials. 112, 313–323.

Benam, K.H., Dauth, S., Hassell, B., Herland, A., Jain, A., Jang, K.J., et al., 2015. Engineered in vitro disease models. Annu. Rev. Pathol. 10, 195–262.

Berahim, Z., Moharamzadeh, K., Rawlinson, A., Jowett, A.K., 2011. Biologic interaction of three-dimensional periodontal fibroblast spheroids with collagen-based and synthetic membranes. J. Periodontol. 82, 790–797.

Chai, W.L., Moharamzadeh, K., Brook, I.M., Emanuelsson, L., Palmquist, A., Noort, R., V. A.N., 2010. Development of a novel model for the investigation of implant-soft tissue interface. J. Periodontol. 81, 1187–1195.

Colley, H.E., Hearnden, V., Jones, A.V., Weinreb, P.H., Violette, S.M., Macneil, S., et al., 2011. Development of tissue-engineered models of oral dysplasia and early invasive oral squamous cell carcinoma. Br. J. Cancer. 105, 1582.

Hertegard, S., 2016. Tissue engineering in the larynx and airway. Curr. Opin. Otolaryngol. Head Neck Surg. 24, 469–476.

Jazayeri, H.E., Tahriri, M., Razavi, M., Khoshroo, K., Fahimipour, F., Dashtimoghadam, E., et al., 2017. A current overview of materials and strategies for potential use in maxillofacial tissue regeneration. Mater. Sci. Eng. C: Mater. Biol. Appl. 70, 913–929.

Kaushik, G., Leijten, J., Khademhosseini, A., 2016. Concise review: organ engineering: design, technology, and integration. Stem Cells. 35 (1), 51–60.

Khademhosseini, A., Langer, R., 2016. A decade of progress in tissue engineering. Nat. Protoc. 11, 1775–1781.

MacNeil, S., 2007. Progress and opportunities for tissue-engineered skin. Nature. 445, 874–880.

Moharamzadeh, K., Brook, I.M., Van Noort, R., Scutt, A.M., Thornhill, M.H., 2007. Tissue-engineered oral mucosa: a review of the scientific literature. J. Dent. Res. 86, 115–124.

Moharamzadeh, K., Colley, H., Murdoch, C., Hearnden, V., Chai, W.L., Brook, I.M., et al., 2012. Tissue-engineered oral mucosa. J. Dent. Res. 91, 642–650.

Moharamzadeh, K., Franklin, K.L., Brook, I.M., Van Noort, R., 2009. Biologic assessment of antiseptic mouthwashes using a three-dimensional human oral mucosal model. J. Periodontol. 80, 769–775.

Monteiro, N., Yelick, P.C., 2016. Advances and perspectives in tooth tissue engineering. J. Tissue Eng. Regen. Med.. Available from: http://dx.doi.org/10.1002/term.2134.

Ostrovidov, S., Hosseini, V., Ahadian, S., Fujie, T., Parthiban, S.P., Ramalingam, M., et al., 2014. Skeletal muscle tissue engineering: methods to form skeletal myotubes and their applications. Tissue Eng. B: Rev. 20, 403–436.

Pinnock, A., Murdoch, C., Moharamzadeh, K., Whawell, S., Douglas, C.W., 2014. Characterisation and optimisation of organotypic oral mucosal models to study Porphyromonas gingivalis invasion. Microbes Infect. 16, 310−319.

Rouwkema, J., Khademhosseini, A., 2016. Vascularization and angiogenesis in tissue engineering: beyond creating static networks. Trends Biotechnol. 34, 733−745.

Uygun, B.E., Yarmush, M.L., 2013. Engineered liver for transplantation. Curr. Opin. Biotechnol. 24, 893−899.

Bioactive glasses and calcium phosphates

2

Mohammadreza Tahriri[1], Rizwan Bader[1], Wiley Yao[1], Shima Dehghani[2], Kimia Khoshroo[1], Morteza Rasoulianboroujeni[1] and Lobat Tayebi[1]
[1]Marquette University School of Dentistry, Milwaukee, WI, United States, [2]Tehran University of Medical Sciences, Tehran, Iran

2.1 Introduction

Throughout recent history, bioceramics have enhanced the quality of life for millions of individuals (Hench, 1991). Bioactive glasses and calcium phosphate materials have been effectively utilized for the repair, reconstruction, and replacement of diseased or damaged parts of the body, particularly bone and teeth (Baino et al., 2014). Clinical success requires simultaneous accomplishment of a stable interface with the host tissue and a match of the mechanical characteristics of the implant with the tissue to be substituted (Banaszkiewicz and Kader, 2014). Bioceramics, made from a calcium phosphate material, including tiny pores, have been employed to coat metal joint implants or utilized as unloaded space fillers for bone ingrowth (Al-Sanabani et al., 2013). The ingrowth of tissue into the pores occurs with an enhancement in an interfacial region between the implant and the tissues, resulting in enhanced resistance to movement of the device within tissue (Al-Sanabani et al., 2013). As in natural bone, proteins adsorb to the calcium phosphate surface to provide the basic mediating layer through which the bone cells interact with the implanted biomaterial (Barrère et al., 2006). Resorbable bioceramics have been designed to degrade gradually over time to be substituted by the natural host tissue (Sheikh et al., 2015). Porous or particulate calcium phosphate ceramic materials [such as tricalcium phosphate (TCP)] have been effectively employed as resorbable materials for low mechanical strength applications, such as repairs of the craniomaxillofacial bone defects (Kinoshita and Maeda, 2013). Resorbable bioactive glasses are also substituted quickly with regenerated bone (Rahaman et al., 2011). Bioactive materials form a biologically active layer on the surface of the implant, which result in the creation of a bond between the natural tissues and the implanted bioceramic (Rahaman et al., 2011). An extensive variety of bonding rates and thickness of interfacial bonding layers are conceivable by changing the composition of the bioactive material (Greenspan, 1999). Bioactive materials (e.g., bioactive glasses) can be utilized in orthopedic implants (vertebral prostheses, intervertebral spacers, bone grafting), middle-ear bone replacements, and maxillofacial bone repair (Chiang and Jakus, 1999). Bioactive glass and glass–ceramic implants have been employed for more than one decade in middle-ear applications. In addition,

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00002-5
© 2017 Elsevier Ltd. All rights reserved.

they have been utilized as bone fillers around teeth with periodontal disease to regenerate the lost bone(Krishnan and Lakshmi, 2013).

2.2 Bioactive glasses

Bioactive glasses possess many characteristics that allow them to be used in multiple applications. They are synthetic, biocompatible, and osteoconductive materials (Saboori et al., 2009a,b; Ravarian et al., 2010; Mozafari et al. 2010a,b,c; Ashuri et al., 2012; Touri et al., 2013; Rezaei et al., 2014; Bizari et al., 2013; Hamedani et al., 2011; Solgi et al., 2015a; Seyedmomeni et al., 2016; Solgi et al., 2015b). In 1971, research showed that they could also integrate with bone and soft tissue (Aurégan and Bégué, 2015; Hench et al., 1971). In addition, bioactive glass contains antibacterial, osteoconductive, and angiogenic properties that make it suitable for treating bone defects in infections (Hu et al., 2009; Leppäranta et al., 2008; Rahaman et al., 2011; Day, 2005). Several studies have also indicated that using bioactive glass with antibiotic therapy can profoundly impact the treatment of osteomyelitis. Lastly, bioactive glass has the ability to repair longstanding bone defects while concurrently exhibiting potent antimicrobial properties (Aurégan and Bégué, 2015).

2.2.1 The composition of bioactive glasses

2.2.1.1 Silicate bioactive glass

45S5, commercially known as Bioglass, has been a commonly researched glass in the studies for biomedical applications (Hench, 2006). Research regarding its bone-bonding properties has been studied for nearly 40 years (Hench et al., 1971). Being a silicate glass, 45S5's structure is based on the three-dimensional (3D) glass-forming SiO_2 network in which silicon ion is fourfold coordinated to oxygen ions. Its low SiO_2 content (when compared to more chemically durable silicate glasses), high Na_2O and CaO (glass network modifiers) content, and high CaO/P_2O_5 ratio are key compositional features responsible for 45S5's bioactivity. Its bioactive and bone-bonding mechanisms have been widely studied (Huang et al., 2006a,b). Based on these studies, 45S5's bonding to bone has been attributed to a carbonate-substituted hydroxyapatite-like (CHA) layer forming on glass surfaces in contact with body fluids. The CHA layer resembles the mineral constituent of bone, allowing it to bond and adhere firmly with bone and tissue. Although the chemical and structural changes are unclear, Hench stated that the CHA layer is believed to be able to form as a result of a reaction sequence on the surface of the bioactive glass implant (Hench, 2006):

Stage 1: The silica group hydrolysis and silanol (Si−OH) group creation on the glass surface was led by rapid ion-exchange reactions between the glass network modifiers (Na^+ and Ca^{2+}) with H^+ (or H_3O^+) ions from the solution. The solution pH subsequently increased due to H^+ ion consumption.

Stage 2: The pH increase (or OH^- concentration) then resulted in the attack of the SiO_2 glass network and silica dissolution in the form of silicic acid, $Si(OH)_4$, into the solution with continued Si−OH group formation on the glass surface.

Although silica solubility is low, the products of 45S5 glass and glass−ceramic dissolution in aqueous solutions can increase Si concentration (Rohanová et al., 2011), indicating that silica dissolution is an important mechanism. However, other mechanisms could also contribute to increases in Si concentration.

Stage 3: The condensation and polymerization of an amorphous SiO_2-rich layer (typically 1−2 μm thick) on the surface of glass depleted in Na^+ and Ca^{2+}.

Stage 4: Further dissolution of the glass, coupled with Ca^{2+} and $(PO_4)^{3-}$ ion migration from the glass through the SiO_2-rich layer and the solution, leading to an amorphous calcium phosphate (ACP) layer formation on the SiO_2-rich layer surface.

Stage 5: The glass continues to dissolve, as the ACP layer incorporates $(OH)^-$ and $(CO_3)^{2-}$ from the solution and ultimately crystallizes as an CHA layer.

Along with the occurrence of the initial CHA layer formation, the biological mechanisms of bonding to bone involve growth factors, adsorption, attachment, proliferation, and differentiation of osteoprogenitor cells (Hench and Polak, 2002). Osteoblasts (bone-forming cells) can create an extracellular matrix (collagen). This allows them to mineralize and form a nanocrystalline mineral and collagen on the glass implant surface while the degradation and conversion of the glass continues over time (Ducheyne and Qiu, 1999).

45S5 glass' biocompatibility has been long established (Wilson et al., 1981). As described previously, upon implantation, 45S5 bioactive glass can undergo chemical degradation resulting in the release of ions such as Na^+ and Ca^{2+} and conversion to a CHA material. With the degradation by dissolution silicon, presumably in the form of silicic acid, $Si(OH)_4$ is also released. Other mechanisms can cause different functions, such as small pieces of silica-rich material being eaten by phagocytes and then excreted. Additionally, to determine the pathway, silicon is released during glass degradation in vivo. Silicon, from 45S5 granules, was implanted in the muscle and tibiae of rabbits (Lai et al., 2002) and after measuring the silicon released in urine and blood samples for up to 7 months postimplantation, chemical and histopathological analyses of bone tissue and other organs showed that silicon had resulted from the 45S5 degradation. It was then harmlessly excreted in a soluble form through the urine.

Although 45S5 glass has remained the gold standard for bioactive glass as a scaffold material, it has several limitations, such as the processing of 45S5 glass into porous 3D scaffolds. The complexity of using 45S5 glass and other bioactive glasses and biodegradable materials is that the local biological microenvironment can be influenced significantly by their degradation. The degradation, particularly in the early stages when the degradation rate is fast, increases the concentrations of ions such as Na^+ and Ca^{2+}, and pH changes (Yao et al., 2007; Fu et al., 2010). On the contrary, it is difficult to predict the biological effects of these changes in vitro experiments and the biological roles, toxicity, and removal of these soluble species have yet to be fully understood.

2.2.1.2 Borate bioactive glass

Recent research shows that certain compositions in other glass-forming systems, such as borate glass, are also bioactive (Day et al., 2003; Han and Day, 2007). Due to lower chemical durability, some borate bioactive glasses degrade at accelerated

levels and convert more completely to an HA-like material, compared to silicate 45S5 or 13-93 glass (Yao et al., 2007; Fu et al., 2010; Huang et al., 2006a). Converting borate bioactive glass to HA follows a similar process as 45S5 glass, but without an SiO_2-rich layer formation (Huang et al., 2006a).

Moreover, borate bioactive glasses can support cell proliferation and differentiation in vitro (Marion et al., 2005; Fu et al., 2009) and tissue infiltration in vivo (Fu et al., 2010). Borate bioactive glasses have been used to treat bone infection, where they act as a substrate for drug release (Liu et al., 2010; Jia et al., 2010). However, one limitation with using borate bioactive glass is its boron toxicity. When the glass is released into solution, it releases borate ions $(BO_3)^{3-}$ that are toxic to cells. In one study utilizing conventional "static" in vitro culture conditions, some borate glasses were toxic to cells, but this toxic nature diminished in "dynamic" culture conditions (Brown et al., 2008). In another study, borate bioactive glass scaffolds, designated 13-93B3, were found to be toxic to murine MLO-A5 osteogeneic cells in vitro; these were composed of B_2O_3, replacing all the SiO_2 in 13-93 glass (Fu et al., 2010). However, when the same scaffolds were implanted subcutaneously in rats, they did not show toxicity to the cells in the new tissue infiltration in vivo (Fu et al., 2010). Moreover, it was also noted that blood–boron concentrations were far below toxic levels when borate glass pellets had been implanted into rabbit tibiae (Zhang et al., 2010).

Recent research has also shown that bioactive degradation rates can be controlled when the composition is manipulated. For example, the degradation rates varied when SiO_2 was partially replaced in silicate 45S5 or 13-93 glass with B_2O_3 (yielding a borosilicate bioactive glass) or having SiO_2 replaced with B_2O_3 (producing a borate bioactive glass) (Yao et al., 2007). The ease to manufacture and control the degradation rates of these borate-based glasses makes them useful in promoting bone regeneration. In addition, it should be possible to match the degradation rates of borate-based bioactive glasses with the bone regeneration rates by controlling the glass composition. Another technique is to manipulate the compositional flexibility of the glass so that it can be a source of elements such as Zn, Cu, F, Mn, Sr, or B that encourage bone growth. These elements are released at a biologically active rate when the glass degrades in vivo.

2.2.1.3 Phosphate bioactive glass

Phosphate glasses, including a P_2O_5 glass-forming network with CaO and Na_2O as modifiers, are useful in biomedical applications (Uo et al., 1998; Franks et al., 2000; Ahmed et al., 2004). These glasses show a strong chemical affinity for bone as the constituent ions are present in the organic, mineral phase of bone. Moreover, these glasses may have additional clinical potential as resorbable materials as their solubility can be controlled by modifying their composition.

2.2.2 The preparation of bioactive glasses methods

2.2.2.1 Melt-derived methods

A common method to obtain bioactive glasses is by fusing together two or more component oxides followed by quenching. The glass batch is then prepared by mixing an

appropriate mole/weight fraction of ingredients. Next, the mixture is ground to break agglomerate particles and obtain a uniformly composed powder. In order to obtain a more homogenous particle size, the mixture is transferred to the ball mill and ground for 2 h in a wet, medium-like acetone. The ball milling is carried out using porcelain/agate balls in a jar. The resulting mixture is then air-dried so that the acetone evaporates out. The raw material mixture can also be ground in water before melting, if the raw materials are not highly hygroscopic. The powder can be transferred to a recrystallized alumina crucible or platinum crucible and melted in a high-temperature furnace, such as an atomized molybdenum disilicide ($MoSi_2$) furnace. The alumina crucible can be used for those glasses, which can be melted at lower temperatures.

Alumina can diffuse out of the crucible into the glass composition at higher temperatures. Thus, to make the glass composition at high temperatures, platinum crucible is instead used to melt the glasses. The glass mixture should be calcined at 500°C for 2 h so that the gaseous substances (moisture one gas) are released out of the composition. The furnace temperature can go up to 1500°C for certain aluminosilicate compositions, whereas borate and phosphate compositions melt at lower temperatures of 1200−1300°C. Before melting, however, the glass mixture should be held at the melting temperature for at least 1 h to facilitate uniform fusion and achieve a homogeneous mix of molten materials. The molten glass is then poured in a preheated graphite mold and the remaining melt is poured on a flat copper plate and quenched by another copper plate in air to obtain flakes. Glasses of various sizes and shapes can be made by pouring them into molds of different shapes (Kaur et al., 2015; Vallet-Regí, 2001).

2.2.2.2 Sol−gel methods

Sols are dispersions of colloidal particles (diameter of 1−100 nm) in a liquid (Kaur et al., 2015). A gel is a firm network made up of interconnected pores (in submicrometer) and polymeric chains (in micrometers) (Flory, 1953). The hydrolysis and polycondensation of the organometallic precursor forms an interconnected 3D network, ultimately forming a gel. Alternatively, gels can be formed from the network growth of discrete colloidal particle groups. Gels are categorized into three classes: alcogels, xerogels, and aerogels (West and Hench, 1990). The pore liquid in alcogels is typically alcohol based, whereas xerogels are made through the thermal removal of pore liquid, resulting in monolith shrinkage (Klemperer et al., 1988). Aerogels are low-density gels (80 kg/m^3) with large pore volumes (up to 98%), formed from removing pore liquid from the rigid network of solid gel to prevent the network from collapsing (Mackenzie and Ulrich, 1988). The initial step of the sol−gel process involves mixing all alkoxide or organometallic precursors. The second step involves the hydrolysis of liquid alkoxide precursors with deionized water (Brink et al., 1997). Once the silicon alkoxide is hydrolyzed by the water, silanol groups ($Si(OH)_4$) are formed. These silanol groups interact with each other to form Si−O−Si bonds, eventually forming the silica network (SiO_2) via the polycondensation process, during which the water is given out as a byproduct. The high field strength of Si 41 causes the highly nucleophilic hydroxyl ions (OH) to attack silicon ions.

The gelation process occurs after the sol is formed. A 3D network is formed via condensation and the cross-linking of silica particles and other colloids. Sol is a low-viscosity liquid, but during the gelation process, a sudden increase in viscosity occurs. At the gelation point (t_g), the gel behaves like an elastic solid as more particles interconnect. The gelation time depends on the solvent concentration, alkoxide group nature, and water amount used for hydrolysis (Zhang et al., 2010) and is relatively larger for heavy alkoxy groups. The gelation time also increases as the hydrolysis water content increases.

The next step is the syneresis and aging of a gel when the porosity decreases, increasing the strength due to a continual polycondensation process and reprecipitation of the gel network (Franks et al., 2000). Furthermore, phase transformation also occurs during the aging process, and hydrothermal treatment can accelerate the aging process (Peitl Filho et al., 1996).

The aging process also affects physical properties like pore volume, surface area, and gel density, ultimately resulting in glass formation. To dry the aged gel, pore liquid from the interconnected rigid 3D network should be removed. During the drying process, extra vigilance must be practiced to ensure that the gel does not crack due to extensive capillary stresses occurring within the aged gel network. The highly porous solid is then formed upon the silanol (Si−OH) removal/dehydration from the pore network. When the resulting gel is heated at higher temperatures, the pore elimination then results in increased network density.

2.2.3 Applications of bioactive glasses in oral and maxillofacial region

Bioactive glasses (BG) have been widely used for regeneration of tooth supporting hard tissues in intrabony defects resulting from periodontal diseases (Kuru et al., 2006; Demir et al., 2007). BG implantation has the potential to improve healing and clinical and radiological outcomes in intrabony defects. The use of BG in combination with guided tissue regeneration membranes is shown to have favorable long-term stability (up to 5 years in BG implanted sites) (Mengel et al., 2006). However, the use of BG alone may not lead to significant improvement of periodontal outcomes (Sculean et al., 2002). Addition of enamel matrix derivatives (EMDs) to BG results in formation of new cementum with an associated periodontal ligament, as well as enhanced mineralization around the BG particles (Kuru et al., 2006; Sculean et al., 2005).

BG serves as a placeholder when used in combination with EMD and prevents tissue collapse as EMD lacks sufficient structural integrity (Kuru et al., 2006). Histological and clinical outcomes have shown to be slightly more favorable for BG compared to demineralized freeze-dried bone allograft (Froum et al., 2002) and bioabsorbable membranes (Mengel et al., 2006). Also, BG preserves alveolar bone for later dental implants when implanted in extraction sites (Norton and Wilson, 2002; Froum et al., 2002). However, bone growth conduction tends to be slow in BGs (Norton and Wilson, 2002). Dental implants placed in previously BG filled extraction sites show

satisfactory survival (Norton and Wilson, 2002). A surgical approach is also suggested to influence preservation of alveolar ridge dimensions as BG is shown to be of little benefit when used in an open socket surgical approach (Camargo et al., 2000).

Another clinical application of BG, notably a mixture of BG particles and autogenous bone, is in sinus floor augmentation. The mixture has shown comparable histological outcomes to the autogenous bone alone demonstrating active bone remodeling over 6 months on implant surface and complete resorption of the implant in long-term follow-ups. The mixture can be a potential substitute for autogenous bone graft (Tadjoedin et al., 2000, 2002).

2.3 Calcium phosphates

One of the main constituents of bone and teeth that plays a crucial role is calcium phosphate (CPs) material (Vallet-Regí and González-Calbet, 2004; Bizari et al., 2011; Rezvannia et al., 2009; Haghbin-Nazarpak et al., 2010, 2011; Zamanian et al., 2010). As early as 1920, biomaterials such as CPs were proposed for fracture treatment in response to tissue damage (Habraken et al., 2016; Albee, 1920). Research on the biomedical applications of CPs boomed in the 1970s; studies found that CPs possessed broad capabilities in orthopedic and dental applications (Zaner and Yukna, 1984; Klein et al., 1985; Masaeli et al., 2016; Haghbin-Nazarpak et al., 2011; Masaeli et al., 2015; Bizari et al., 2016; Khoshroo et al., 2016; Raz et al., 2014b). Potential applications include thin coats on metallic implants, aiding implant fixation into bone (Surmenev et al., 2014) and sintering CP to be used as synthetic bone graft substitutes (LeGeros, 2002). The utilization of CPs has resulted in the immense success of biomedical applications, such as increasing the clinical survival rate of the femoral component of total hip implants (Havelin et al., 2000), reducing the risk of pin loosening for external fixators (Moroni et al., 2002), and allowing earlier weight bearing after tibia plateau fractures (Larsson and Bauer, 2002). In some cases, CPs have even been found to be superior to autografts in terms of their application process on human tissue (Russell et al., 2008). Nevertheless, all these achievements have become somewhat surpassed by the advances of polymers in biomedical applications. Polymers have multiple diverse qualities upon examining their control of composition and related properties (e.g., copolymers, supramolecular self-assemblies), applicable processing techniques (e.g., additive manufacturing), and functionalization possibilities (e.g., surface micro- and nanostructuring, and chemical functionalization).

Recent reports have questioned whether CPs are old biomaterials that are functional, but not particularly elegant. Will they be the materials of the future? Even though many natural and synthetic polymers exist and have been used in various biomedical applications, CPs are present in the human body and easy to certify. CP presence in the human body can do wonders due to being a successful, cost-effective, and affordable strategy for treating diseases and regenerating malfunctioning organs and tissues. This treatment is vital for an increasing aging population

in the Western world. CPs meet these requirements; they can be produced in large quantities, are cost-efficient, and are stable on and off the shelf. However, one drawback to CPs is their poor mechanical properties. They can be obstacles for orthopedic and dental applications. In addition, efforts in biomedical research need to be done to advance CP-based strategies, as they show high potential utility as a material of the future (Habraken et al., 2016).

2.3.1 Osteoinductivity of CPs

Osteoinductivity is the ability to differentiate towards the osteoblastic lineage with the recruitment and induction of progenitor and undifferentiated cells (Samavedi et al., 2013). Depending on the materials used, the osteoinductivity of a CP has various characteristics. Cell type, along with the presence of osteogenic supplements, can also have certain influences with the degree of osteoinduction (Nakashima et al., 2002). In order to differentiate towards the osteoblastic lineage, undifferentiated mesenchymal stem cells (MSCs), such as bone marrow MSCs and adipose-derived stem cells, are commonly cultured in the presence of osteogenic supplements. However, osteoblastic cell lines such as MC3T3-E1 and MG63 show a greater degree of osteoinductivity effects than MSCs. Though osteogenic supplements can arguably marginalize the contribution of CPs, many studies report the osteoinductive effect of CPs in their presence. The following sections summarize the effect of CPs on cell differentiation, both in the presence and absence of osteogenic supplements (Samavedi et al., 2013).

2.3.2 Chemical properties of CPs

The osteoinductive abilities of CPs are linked to intrinsic property differences such as solubility, crystallinity, and stoichiometry [e.g., calcium-to-phosphate (Ca/P) ratio]. The section below provides a brief description of these properties for four types of CPs. Additional information regarding the syntheses and properties of these CPs can be found elsewhere (Vallet-Regí and González-Calbet, 2004; Carrodeguas and De Aza, 2011; LeGeros et al., 2003; Combes and Rey, 2010).

2.3.2.1 Hydroxyapatite (HA)

HA is a CP that has shown great potential for bone regeneration due to its composition resembling bone mineral (Yoshikawa and Myoui, 2005; Poursamar et al., 2009; Eslami et al., 2010; Fatehi et al., 2009; Shafiei et al., 2012; Raz et al., 2014a; Hooshmand et al., 2013; Behroozibakhsh et al., 2014; Barandehfard et al., 2016; Heidari et al., 2017; Tahriri et al., 2016; Tahriri and Moztarzadeh, 2014). Among the different types of monophasic CPs, HA has been shown to possess the most stable and least soluble phase properties (Klein et al., 1990; Ducheyne et al., 1993), with a K_{sp} value of around 2.9×10^{-58} over a pH range of ~ 3.5 to ~ 9.7 (Bell et al., 1978). Despite its low solubility, HA surfaces can provide nucleating sites for the precipitation of apatite crystals in culture medium, which is typically

saturated with calcium and phosphate ions (Bohner and Lemaitre, 2009). Moreover, stoichiometric HA ($Ca_5(PO_4)_3OH$) has a Ca/P ratio of 1.67 and has osteoconductive properties instead of osteoinductive (Ogata et al., 2005). However, these properties can be adapted by using ionic substitutions. For example, increasing HA solubility was made possible through the anionic substitution of carbonate for phosphate [and bioactivity (Huang et al., 2008)], whereas the substitution of fluoride for hydroxide increased HA stability. Research also shows that favorable biological effects are possible by cationic substitutions such as magnesium replacing calcium.

2.3.2.2 Tricalcium phosphate

Stoichiometric TCP ($Ca_3(PO_4)_2$) possesses a Ca/P ratio of 1.5 and occurs in two phases, α and β. These phases have identical chemistries, but different crystal structures (Barrère et al., 2006; Khojasteh et al., 2016). Both phases are less stable than HA but more soluble in aqueous environments (Ducheyne et al., 1993): K_{sp} values of $10^{-25.5}$ for α-TCP and $10^{-28.9}$ for β-TCP at 25°C have been reported (Ferna et al., 1999). Along with sharing osteoconductive and osteoinductive properties, β-TCP studies show that it can induce apatite layer precipitation upon being incubated in aqueous ionic solutions due to the low interfacial energy with respect to apatite (Bohner and Lemaitre, 2009). In bone regeneration, however, β-TCP is used more than α-TCP (Samavedi et al., 2013).

2.3.2.3 Amorphous calcium phosphate (ACP)

ACPs belong to a family of CPs. They lack in long-range order (Ter Brugge et al., 2003), well defined stoichiometry and contain impurities in their composite such as Na^+ and Cl^- (Popp et al., 2012). To generate potential for production, calcium and phosphate ions must be rapidly precipitated from aqueous solutions and typically have a wide range of Ca/P ratios (usually between 1.15 and 1.67). However, the pH and the starting ratio of ions in solution depend on synthesis conditions (Zhao et al., 2011). In their amorphous nature, a low Ca/P ratio and high concentration of ionic substitutions (e.g., Na^+, K^+, Mg^{2+}, Cl^-, CO_3^{2-}) confer a high solubility degree on ACPs. K_{sp} values of $10^{-24.8}$ for ACPs possess a Ca/P ratio of 1.5, and $10^{-23.9}$ for ACPs that incorporate small amounts of carbonate have also been reported (Combes and Rey, 2010). ACPs can also release a host of ions that reprecipitate in a more stable form, such as poorly crystalline apatite when they are exposed to aqueous environments (Whited et al., 2006). Furthermore, one of the greatest factors in their osteoconductibility comes from their ability to release calcium phosphate and other ions in aqueous environments (Eanes, 2001; Popp et al., 2012). However, the rapid release of ions from ACPs perturbs the local pH, creating a negative impact on short-term cell attachment/proliferation and long-term viability (Oreffo et al., 1998). However, divalent cations such as Zn and ZrO can lower their dissolution rates and incorporating Zn and Cu can impede their conversion to HA (Skrtic et al., 2002; Okamoto and Hidaka, 1994).

2.3.2.4 Biphasic calcium phosphate (BCP)

BCPs are a two-phase ceramic family. They combine the low solubility and osteoconductivity of apatite with the osteoinductivity of the more soluble phase such as TCP (Bansal et al., 2009). BCPs can be produced physically by mixing HA and TCP, or chemically by sintering calcium-deficient apatites at high temperatures to create a two-phase mixture (Vallet-Regí and González-Calbet, 2004). BCP's chemical properties, including solubility, depend upon the characteristics and relative amounts of the individual phases. Typically, the Ca/P ratios of BCPs also fall between those for pure TCP and HA.

2.3.3 Clinical application of calcium phosphates in oral and maxillofacial tissue regeneration

Calcium phosphate-derived implants have shown to be biocompatible, osteoconductive (Zerbo et al., 2004), and resorbable (Horch et al., 2006). The mentioned characteristics have made them suitable candidates for oral and maxillofacial regeneration. They have been successfully used in regeneration of various types of bone defects such as maxillary sinus floor augmentation (Zerbo et al., 2004; Friedmann et al., 2009; Lee et al., 2008), periodontal (Sculean et al., 2008), and alveolar bone defects (Brkovic et al., 2012). Clinical examples are the use of TCP such as porous b-TCP (Cerasorbs) for sinus floor augmentation (Zerbo et al., 2004), macroporous biphasic calcium phosphate for maxillary sinus augmentation (Lee et al., 2008), and BCP CHA/TCP 60/40 for ridge augmentation and maxillary sinus grafting (Friedmann et al., 2009).

The bone tissue is shown to successfully grow into the structure of calcium phosphate implants in at least 6 months of follow-up, indicative of favorable osteoconductivity. The higher volume of osteoid in defects implanted by calcium phosphates suggests that the material is mainly osteoconductive, especially in the early postoperative stage, and can potentially delay the formation of mature bony structure in implanted groups (Zerbo et al., 2004).

The absence of osteoinductive properties in TCPs is primarily related to their acellular attribute and the lack of growth factors. Combining BCP with bone regeneration promotors such as particulate autogenous bone (Artzi et al., 2008), collagen (Brkovic et al., 2012), and MSCs (Shayesteh et al., 2008) appears to result in accelerated bone formation and a larger percentage of lamellar/mature bone tissue. A noteworthy point in the application of MSCs combined with BCP is that augmented bone structures should be sufficiently stable by themselves as CP implants usually do not provide enough mechanical strength for augmented damaged tissues in the early postoperative stage, at the least. The stability of augmented bone structure should be particularly noted when calcium phosphate implants are used without concurrent autologous bone fragments. The complete bony substitution of an implanted TCP is also influenced by factors other than the used material, for instance by the defect size, the implant size, and an individual's osteogenetic bone potential (Horch et al., 2006). BCP [with greater than 99% crystallinity, consisting

of 60% hydroxyapatite (HA) and 40% b-TCP in particulate form] is also used successfully in combination with EMD for regeneration of intrabony periodontal defects. BCP serves as a slowly absorbable placeholder and prevents gingival recession whereas EMD enhances peridontal regeneration (Sculean et al., 2008). The modifications in nanotopography and chemical composition of a newly developed BCP bone graft material significantly promoted ectopic bone formation in a recent in vivo study further confirming its osteoinductive potential (Miron et al., 2015).

2.4 Conclusions

In recent few years, bioceramics have enhanced the quality of life for many individuals. These ceramic-based biomaterials (bioactive glasses and calcium phosphate-based ceramics) have been effectively utilized for the repair, reconstruction, and replacement of diseased or damaged parts of the body, particularly bone and periodontal hard tissues. Bioactive glasses and calcium phosphates have advanced to become an integral and imperative portion of our present day human services conveyance framework and we have only begun to perceive their maximum regenerative capacity. In the future, microstructure and molecular surface chemistry of different types of bioceramics will be custom-fitted to coordinate the particular biological and metabolic requirements of tissues or disease states.

References

Ahmed, I., Lewis, M., Olsen, I., Knowles, J., 2004. Phosphate glasses for tissue engineering: part 1. Processing and characterisation of a ternary-based $P_2O_5-CaO-Na_2O$ glass system. Biomaterials. 25, 491−499.

Al-Sanabani, J.S., Madfa, A.A., Al-Sanabani, F.A., 2013. Application of calcium phosphate materials in dentistry. Int. J. Biomater. 2013, 1−12.

Albee, F.H., 1920. Studies in bone growth: triple calcium phosphate as a stimulus to osteogenesis. Ann. Surg. 71, 32−39.

Artzi, Z., Weinreb, M., Carmeli, G., Lev-Dor, R., Dard, M., Nemcovsky, C.E., 2008. Histomorphometric assessment of bone formation in sinus augmentation utilizing a combination of autogenous and hydroxyapatite/biphasic tricalcium phosphate graft materials: at 6 and 9 months in humans. Clin. Oral. Implants. Res. 19, 686−692.

Ashuri, M., Moztarzadeh, F., Nezafati, N., Hamedani, A.A., Tahriri, M., 2012. Development of a composite based on hydroxyapatite and magnesium and zinc-containing sol−gel-derived bioactive glass for bone substitute applications. Mater. Sci. Eng.: C. 32, 2330−2339.

Aurégan, J.-C., Bégué, T., 2015. Bioactive glass for long bone infection: a systematic review. Injury. 46, S3−S7.

Baino, F., Novajra, G., Vitale-Brovarone, C., 2014. Bioceramics and scaffolds: a winning combination for tissue engineering. Front. Bioeng. Biotechnol. 3, 202.

Banaszkiewicz, P., Kader, D.F., 2014. Classic Papers in Orthopaedics. Springer Science & Business Media, London.

Bansal, S., Chauhan, V., Sharma, S., Maheshwari, R., Juyal, A., Raghuvanshi, S., 2009. Evaluation of hydroxyapatite and beta-tricalcium phosphate mixed with bone marrow aspirate as a bone graft substitute for posterolateral spinal fusion. Indian J. Orthop. 43, 234.
Barandehfard, F., Rad, M.K., Hosseinnia, A., Khoshroo, K., Tahriri, M., Jazayeri, H., et al., 2016. The addition of synthesized hydroxyapatite and fluorapatite nanoparticles to a glass-ionomer cement for dental restoration and its effects on mechanical properties. Ceramics Int. 42, 17866−17875.
Barrère, F., van Blitterswijk, C.A., de Groot, K., 2006. Bone regeneration: molecular and cellular interactions with calcium phosphate ceramics. Int. J. Nanomedicine. 1, 317.
Behroozibakhsh, M., Shafiei, F., Hooshmand, T., Moztarzadeh, F., Tahriri, M., Bagheri, H., 2014. Effect of a synthetic nanocrystalline-fluorohydroxyapatite on the eroded enamel lesions. Dent. Mater. 30, e117−e118.
Bell, L., Mika, H., Kruger, B., 1978. Synthetic hydroxyapatite-solubility product and stoichiometry of dissolution. Arch. Oral. Biol. 23, 329−336.
Bizari, D., Moztarzadeh, F., Rabiee, M., Tahriri, M., Banafatizadeh, F., Ansari, A., et al., 2011. Development of biphasic hydroxyapatite/dicalcium phosphate dihydrate (DCPD) bone graft using polyurethane foam template: in vitro and in vivo study. Adv. Appl. Ceram. 110, 417−425.
Bizari, D., Rabiee, M., Moztarzadeh, F., Tahriri, M., Alavi, S., Masaeli, R., 2013. Synthesis, characterization and biological evaluation of sol−gel derived nanomaterial in the ternary system 64% SiO_2-31% CaO-5% P_2O_5 as a bioactive glass: in vitro study. Ceramics−Silikáty. 57, 201−209.
Bizari, D., Moztazradeh, F., Fahmy, M., Tahriri, M., Tayebi, L., 2016. Hydroxyapatite/dicalcium phosphate dihydrate composite for dental bone defect repair. Dent. Mater. 32, e56.
Bohner, M., Lemaitre, J., 2009. Can bioactivity be tested in vitro with SBF solution? Biomaterials. 30, 2175−2179.
Brink, M., Turunen, T., Happonen, R.P., Yli-Urpo, A., 1997. Compositional dependence of bioactivity of glasses in the system Na_2O-K_2O-MgO-CaO-B_2O_3-P_2O_5-SiO_2. J. Biomed. Mater. Res. A. 37, 114−121.
Brkovic, B.M., Prasad, H.S., Rohrer, M.D., Konandreas, G., Agrogiannis, G., Antunovic, D., et al., 2012. Beta-tricalcium phosphate/type I collagen cones with or without a barrier membrane in human extraction socket healing: clinical, histologic, histomorphometric, and immunohistochemical evaluation. Clin. Oral. Investig. 16, 581−590.
Brown, R.F., Dwilewicz, A.B., Huang, W.-H., Li, Y., Rahaman, M., Bal, B.S., et al., 2008. Conversion of borate glass to hydroxyapatite and its effect on proliferation of MC3T3-E1 cells. J. Biomed. Mater. Res. A. 88, 392−400.
Camargo, P.M., Lekovic, V., Weinlaender, M., Klokkevold, P.R., Kenney, E.B., Dimitrijevic, B., et al., 2000. Influence of bioactive glass on changes in alveolar process dimensions after exodontia. Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol. Endod. 90, 581−586.
Carrodeguas, R.G., De Aza, S., 2011. α-Tricalcium phosphate: synthesis, properties and biomedical applications. Acta Biomater. 7, 3536−3546.
Chiang, Y.-M. & Jakus, K. Fundamental research needs in ceramics. USA NSF Workshop Report, 1999.
Combes, C., Rey, C., 2010. Amorphous calcium phosphates: synthesis, properties and uses in biomaterials. Acta Biomater. 6, 3362−3378.
Day, D., White, J., Brown, R., Mcmenamin, K., 2003. Transformation of borate glasses into biologically useful materials. Glass Technol. 44, 75−81.
Day, R.M., 2005. Bioactive glass stimulates the secretion of angiogenic growth factors and angiogenesis in vitro. Tiss. Eng. 11, 768−777.

Demir, B., Sengun, D., Berberoglu, A., 2007. Clinical evaluation of platelet-rich plasma and bioactive glass in the treatment of intra-bony defects. J. Clin. Periodontol. 34, 709–715.
Ducheyne, P., Qiu, Q., 1999. Bioactive ceramics: the effect of surface reactivity on bone formation and bone cell function. Biomaterials. 20, 2287–2303.
Ducheyne, P., Radin, S., King, L., 1993. The effect of calcium phosphate ceramic composition and structure on in vitro behavior. I. Dissolution. J. Biomed. Mater. Res. A. 27, 25–34.
Eanes, E.D., 2001. Amorphous calcium phosphate. Octacalcium Phosphate. Karger Publishers.
Eslami, H., Solati-Hashjin, M., Tahriri, M., 2010. Effect of fluorine ion addition on structural, thermal, mechanical, solubility and biocompatibility characteristics of hydroxyapatite nanopowders. Adv. Appl. Ceram.: Struct. Funct. Bioceram. 109, 200.
Fatehi, K., Moztarzadeh, F., Solati-Hashjin, M., Tahriri, M., Rezvannia, M., Saboori, A., 2009. Biomimetic hydroxyapatite coatings deposited onto heat and alkali treated Ti6Al4V surface. Surf. Eng. 25, 583–588.
Ferna, E., Gil, F., Ginebra, M., Driessens, F., Planell, J., Best, S., 1999. Calcium phosphate bone cements for clinical applications. Part I: solution chemistry. J. Mater. Sci.: Mater. Med. 10, 169–176.
Flory, P.J., 1953. Principles of Polymer Chemistry. Cornell University Press, Ithaca.
Franks, K., Abrahams, I., Knowles, J., 2000. Development of soluble glasses for biomedical use Part I: In vitro solubility measurement. J. Mater. Sci.: Mater. Med. 11, 609–614.
Friedmann, A., Dard, M., Kleber, B.M., Bernimoulin, J.P., Bosshardt, D.D., 2009. Ridge augmentation and maxillary sinus grafting with a biphasic calcium phosphate: histologic and histomorphometric observations. Clin. Oral. Implants. Res. 20, 708–714.
Froum, S., Cho, S.-C., Rosenberg, E., Rohrer, M., Tarnow, D., 2002. Histological comparison of healing extraction sockets implanted with bioactive glass or demineralized freeze-dried bone allograft: a pilot study. J. Periodontol. 73, 94–102.
Fu, H., Fu, Q., Zhou, N., Huang, W., Rahaman, M.N., Wang, D., et al., 2009. In vitro evaluation of borate-based bioactive glass scaffolds prepared by a polymer foam replication method. Mater. Sci. Eng.: C. 29, 2275–2281.
Fu, Q., Rahaman, M.N., Fu, H., Liu, X., 2010. Silicate, borosilicate, and borate bioactive glass scaffolds with controllable degradation rate for bone tissue engineering applications. I. Preparation and in vitro degradation. J. Biomed. Mater. Res. A. 95, 164–171.
Greenspan, D.C., 1999. Bioactive glass: mechanism of bone bonding. Tandläkartidningen Årk. 91, 1–32.
Habraken, W., Habibovic, P., Epple, M., Bohner, M., 2016. Calcium phosphates in biomedical applications: materials for the future? Mater. Today. 19, 69–87.
Haghbin-Nazarpak, M., Moztarzadeh, F., Solati-Hashjin, M., Mirhabibi, A.R., Tahriri, M., 2010. Preparation, characterization and gentamicin sulfate release investigation of biphasic injectable calcium phosphate bone cement. Ceramics–Silikáty. 54, 334–340.
Haghbin-Nazarpak, M., Moztarzadeh, F., Solati-Hashjin, M., Tahriri, M., Khoshroo, K., 2011. Injectable and bioresorbable calcium phosphate delivery system with gentamicin sulphate for treatment of bone diseases: in vitro study. Adv. Appl. Ceram. 110, 482–489.
Hamedani, A.A., Moztarzadeh, F., Bizari, D., Ashuri, M., Tahriri, M., 2011. Ion release behavior and apatite-forming ability of sol-gel derived 70S30C bioactive glass with magnesium/zinc substitution. Key Eng. Mater. 1463, 55.
Han, X., Day, D.E., 2007. Reaction of sodium calcium borate glasses to form hydroxyapatite. J. Mater. Sci.: Mater. Med. 18, 1837–1847.
Havelin, L.I., Engesæter, L.B., Espehaug, B., Furnes, O., Lie, S.A., Vollset, S.E., 2000. The Norwegian arthroplasty register: 11 years and 73,000 arthroplasties. Acta Orthop. Scand. 71, 337–353.

Heidari, F., Razavi, M., Ghaedi, M., Forooghi, M., Tahriri, M., Tayebi, L., 2017. Investigation of mechanical properties of natural hydroxyapatite samples prepared by cold isostatic pressing method. J. Alloys Compd. 693, 1150−1156.

Hench, L.L., 1991. Bioceramics: from concept to clinic. J. Am. Ceramic Soc. 74, 1487−1510.

Hench, L.L., 2006. The story of Bioglass®. J. Mater. Sci.: Mater. Med. 17, 967−978.

Hench, L.L., Polak, J.M., 2002. Third-generation biomedical materials. Science. 295, 1014−1017.

Hench, L.L., Splinter, R.J., Allen, W., Greenlee, T., 1971. Bonding mechanisms at the interface of ceramic prosthetic materials. J. Biomed. Mater. Res. 5, 117−141.

Hooshmand, T., Abrishamchian, A., Najafi, F., Mohammadi, M., Najafi, H., Tahriri, M., 2013. Development of sol-gel-derived multi-wall carbon nanotube/hydroxyapatite nanocomposite powders for bone substitution. J. Compos. Mater. 48, 483−489.

Horch, H.H., Sader, R., Pautke, C., Neff, A., Deppe, H., Kolk, A., 2006. Synthetic, pure-phase beta-tricalcium phosphate ceramic granules (Cerasorb) for bone regeneration in the reconstructive surgery of the jaws. Int. J. Oral. Maxillofac. Surg. 35, 708−713.

Hu, S., Chang, J., Liu, M., Ning, C., 2009. Study on antibacterial effect of 45S5 Bioglass®. J. Mater. Sci.: Mater. Med. 20, 281−286.

Huang, J., Best, S., Brooks, R., Rushton, N., Bonfield, W., 2008. In vitro evaluation of nanosized carbonate-substituted hydroxyapatite and its polyhydroxyethylmethacrylate nanocomposite. J. Biomed. Mater. Res. A. 87, 598−607.

Huang, W., Day, D.E., Kittiratanapiboon, K., Rahaman, M.N., 2006a. Kinetics and mechanisms of the conversion of silicate (45S5), borate, and borosilicate glasses to hydroxyapatite in dilute phosphate solutions. J. Mater. Sci.: Mater. Med. 17, 583−596.

Huang, W., Rahaman, M.N., Day, D.E., Li, Y., 2006b. Mechanisms for converting bioactive silicate, borate, and borosilicate glasses to hydroxyapatite in dilute phosphate solution. Phys. Chem. Glasses-European J. Glass Sci. Technol. B. 47, 647−658.

Jia, W.-T., Zhang, X., Luo, S.-H., Liu, X., Huang, W.-H., Rahaman, M.N., et al., 2010. Novel borate glass/chitosan composite as a delivery vehicle for teicoplanin in the treatment of chronic osteomyelitis. Acta Biomater. 6, 812−819.

Kaur, G., Pickrell, G., Sriranganathan, N., Kumar, V., Homa, D., 2015. Review and the state of the art: sol−gel and melt quenched bioactive glasses for tissue engineering. J. Biomed. Mater. Res. B: Appl. Biomater. 104, 1248−1275.

Khojasteh, A., Fahimipour, F., Eslaminejad, M.B., Jafarian, M., Jahangir, S., Bastami, F., et al., 2016. Development of PLGA-coated β-TCP scaffolds containing VEGF for bone tissue engineering. Mater. Sci. Eng.: C. 69, 780−788.

Khoshroo, K., Almela, T., Tahriri, M., Fahimipour, F., Metalwala, Z., Moharamzadeh, K., et al., 2016. 3D-printing of porous calcium phosphate cements for bone tissue engineering. Dent. Mater. 32, e56−e57.

Kinoshita, Y., Maeda, H., 2013. Recent developments of functional scaffolds for craniomaxillofacial bone tissue engineering applications. Sci. World J. 2013, 1−21.

Klein, C.P., de Groot, K., Drissen, A., van der Lubbe, H., 1985. Interaction of biodegradable β-whitlockite ceramics with bone tissue: an in vivo study. Biomaterials. 6, 189−192.

Klein, C.P., de Blieck-Hogemrst, J., Wolket, J., de Groot, K., 1990. Studies of the solubility of different calcium phosphate ceramic particles in vitro. Biomaterials. 11, 509−512.

Klemperer, W., Mainz, V., Ramamurthi, S., Rosenberg, F., Brinker, C., Clark, D., et al., 1988. Better ceramics Through Chemistry III. Materials Research Society, Pittsburgh.

Krishnan, V., Lakshmi, T., 2013. Bioglass: a novel biocompatible innovation. J. Adv. Pharm. Technol. Res. 4, 78.

Kuru, B., Yilmaz, S., Argin, K., Noyan, U., 2006. Enamel matrix derivative alone or in combination with a bioactive glass in wide intrabony defects. Clin. Oral. Investig. 10, 227−234.

Lai, W., Garino, J., Ducheyne, P., 2002. Silicon excretion from bioactive glass implanted in rabbit bone. Biomaterials. 23, 213−217.

Larsson, S., Bauer, T.W., 2002. Use of injectable calcium phosphate cement for fracture fixation: a review. Clin. Orthop. Relat. Res. 395, 23−32.

Lee, J.H., Jung, U.W., Kim, C.S., Choi, S.H., Cho, K.S., 2008. Histologic and clinical evaluation for maxillary sinus augmentation using macroporous biphasic calcium phosphate in human. Clin. Oral. Implants. Res. 19, 767−771.

LeGeros, R., Lin, S., Rohanizadeh, R., Mijares, D., LeGeros, J., 2003. Biphasic calcium phosphate bioceramics: preparation, properties and applications. J. Mater. Sci.: Mater. Med. 14, 201−209.

LeGeros, R.Z., 2002. Properties of osteoconductive biomaterials: calcium phosphates. Clin. Orthop. Relat. Res. 395, 81−98.

Leppäranta, O., Vaahtio, M., Peltola, T., Zhang, D., Hupa, L., Hupa, M., et al., 2008. Antibacterial effect of bioactive glasses on clinically important anaerobic bacteria in vitro. J. Mater. Sci.: Mater. Med. 19, 547−551.

Liu, X., Xie, Z., Zhang, C., Pan, H., Rahaman, M.N., Zhang, X., et al., 2010. Bioactive borate glass scaffolds: in vitro and in vivo evaluation for use as a drug delivery system in the treatment of bone infection. J. Mater. Sci.: Mater. Med. 21, 575−582.

Mackenzie, J.D., Ulrich, D.R., 1988. Ultrastructure Processing of Advanced Ceramics. Wiley, New York.

Marion, N.W., Liang, W., Liang, W., Reilly, G.C., Day, D.E., Rahaman, M.N., et al., 2005. Borate glass supports the in vitro osteogenic differentiation of human mesenchymal stem cells. Mech. Adv. Mater. Struct. 12, 239−246.

Masaeli, R., Kashi, T.J., Dinarvand, R., Tahriri, M., Shahoon, H., Raz, M., et al., 2015. Preparation, characterization and investigation of in vitro and in vivo biological properties of strontium-modified calcium phosphate cement for bone defect repair. J. Dent. Med. 28, 177−188.

Masaeli, R., Kashi, T.S.J., Dinarvand, R., Rakhshan, V., Shahoon, H., Hooshmand, B., et al., 2016. Efficacy of the biomaterials 3wt%-nanostrontium-hydroxyapatite-enhanced calcium phosphate cement (nanoSr-CPC) and nanoSr-CPC-incorporated simvastatin-loaded poly (lactic-*co*-glycolic-acid) microspheres in osteogenesis improvement: an explorative multi-phase experimental in vitro/vivo study. Mater. Sci. Eng.: C. 69, 171−183.

Mengel, R., Schreiber, D., Flores-De-Jacoby, L., 2006. Bioabsorbable membrane and bioactive glass in the treatment of intrabony defects in patients with generalized aggressive periodontitis: results of a 5-year clinical and radiological study. J. Periodontol. 77, 1781−1787.

Miron, R.J., Sculean, A., Shuang, Y., Bosshardt, D.D., Gruber, R., Buser, D., et al., 2015. Osteoinductive potential of a novel biphasic calcium phosphate bone graft in comparison with autographs, xenografts, and DFDBA. Clin. Oral. Implants. Res. 27, 668−675.

Moroni, A., Faldini, C., Rocca, M., Stea, S., Giannini, S., 2002. Improvement of the bone−screw interface strength with hydroxyapatite-coated and titanium-coated AO/ASIF cortical screws. J. Orthop. Trauma. 16, 257−263.

Mozafari, M., Moztarzadeh, F., Rabiee, M., Azami, M., Maleknia, S., Tahriri, M., et al., 2010a. Development of macroporous nanocomposite scaffolds of gelatin/bioactive glass prepared through layer solvent casting combined with lamination technique for bone tissue engineering. Ceramics Int. 36, 2431−2439.

Mozafari, M., Moztarzadeh, F., Rabiee, M., Azami, M., Nezafati, N., Moztarzadeh, Z., et al., 2010b. Development of 3D bioactive nanocomposite scaffolds made from gelatin and nano bioactive glass for biomedical applications. Adv. Compos. Lett. 19, 91−96.

Mozafari, M., Moztarzadeh, F., Tahriri, M., 2010c. Investigation of the physico-chemical reactivity of a mesoporous bioactive $SiO_2-CaO-P_2O_5$ glass in simulated body fluid. J. Non-Crystalline Solids. 356, 1470−1478.

Nakashima, K., Zhou, X., Kunkel, G., Zhang, Z., Deng, J.M., Behringer, R.R., et al., 2002. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell. 108, 17−29.

Norton, M.R., Wilson, J., 2002. Dental implants placed in extraction sites implanted with bioactive glass: human histology and clinical outcome. Int. J. Oral. Maxillofac. Implants. 17, 249−257.

Ogata, K., Imazato, S., Ehara, A., Ebisu, S., Kinomoto, Y., Nakano, T., et al., 2005. Comparison of osteoblast responses to hydroxyapatite and hydroxyapatite/soluble calcium phosphate composites. J. Biomed. Mater. Res. A. 72, 127−135.

Okamoto, Y., Hidaka, S., 1994. Studies on calcium phosphate precipitation: effects of metal ions used in dental materials. J. Biomed. Mater. Res. A. 28, 1403−1410.

Oreffo, R.O., Driessens, F.C., Planell, J.A., Triffitt, J.T., 1998. Effects of novel calcium phosphate cements on human bone marrow fibroblastic cells. Tiss. Eng. 4, 293−303.

Peitl Filho, O., Latorre, G.P., Hench, L., 1996. Effect of crystallization on apatite-layer formation of bioactive glass 45%. J. Biomed. Mater. Res. 30, 509−514.

Popp, J.R., Laflin, K.E., Love, B.J., Goldstein, A.S., 2012. Fabrication and characterization of poly(lactic-*co*-glycolic acid) microsphere/amorphous calcium phosphate scaffolds. J. Tissue. Eng. Regen. Med. 6, 12−20.

Poursamar, S.A., Rabiee, M., Samadikuchaksaraei, A., Tahriri, M., Karimi, M., Azami, M., 2009. Influence of the value of the pH on the preparation of nano hydroxyapatite polyvinyl alcohol composites. J. Ceram. Proc. Res. 10, 679−682.

Rahaman, M.N., Day, D.E., Bal, B.S., Fu, Q., Jung, S.B., Bonewald, L.F., et al., 2011. Bioactive glass in tissue engineering. Acta Biomater. 7, 2355−2373.

Ravarian, R., Moztarzadeh, F., Hashjin, M.S., Rabiee, S., Khoshakhlagh, P., Tahriri, M., 2010. Synthesis, characterization and bioactivity investigation of bioglass/hydroxyapatite composite. Ceramics Int. 36, 291−297.

Raz, M., Moztarzadeh, F., Shokrgozar, M.A., Azami, M., Tahriri, M., 2014a. Development of biomimetic gelatin−chitosan/hydroxyapatite nanocomposite via double diffusion method for biomedical applications. Int. J. Mater. Res. 105, 493−501.

Raz, M., Moztarzadeh, F., Shokrgozar, M.A., Tahriri, M., 2014b. Synthesis of nano calcium phosphate via biomimetic method for bone tissue engineering scaffolds and investigation of its phase transformation in simulated body fluid. Key Engineering Materials. Trans Tech Publications, Zurich, pp. 86−92.

Rezaei, Y., Moztarzadeh, F., Shahabi, S., Tahriri, M., 2014. Synthesis, characterization, and in vitro bioactivity of sol−gel-derived $SiO_2-CaO-P_2O_5-MgO-SrO$ bioactive glass. Synth. React. Inorg. Met.-Org. Chem. 44, 692−701.

Rezvannia, M., Moztarzadeh, F., Tahriri, M., 2009. Formation of hydroxyapatite nanoneedles on the surface of a novel calcium phosphate/blood plasma proteins biocement in simulated body fluid (SBF). J. Ceram. Proc. Res. 10, 669−673.

Rohanová, D., Boccaccini, A.R., Yunos, D.M., Horkavcová, D., Březovská, I., Helebrant, A., 2011. TRIS buffer in simulated body fluid distorts the assessment of glass−ceramic scaffold bioactivity. Acta Biomater. 7, 2623−2630.

Russell, T.A., Leighton, R.K., Group, A.-B.T.P.F.S., 2008. Comparison of autogenous bone graft and endothermic calcium phosphate cement for defect augmentation in tibial plateau fractures. J. Bone Joint Surg. 90, 2057−2061.

Saboori, A., Rabiee, M., Moztarzadeh, F., Sheikhi, M., Tahriri, M., Karimi, M., 2009a. Synthesis, characterization and in vitro bioactivity of sol-gel-derived $SiO_2-CaO-P_2O_5-MgO$ bioglass. Mater. Sci. Eng.: C. 29, 335−340.

Saboori, A., Sheikhi, M., Moztarzadeh, F., Rabiee, M., Hesaraki, S., Tahriri, M., et al., 2009b. Sol−gel preparation, characterisation and in vitro bioactivity of Mg containing bioactive glass. Adv. Appl. Ceram.: Struct. Funct. Bioceram. 108, 155.

Samavedi, S., Whittington, A.R., Goldstein, A.S., 2013. Calcium phosphate ceramics in bone tissue engineering: a review of properties and their influence on cell behavior. Acta Biomater. 9, 8037−8045.

Sculean, A., Barbé, G., Chiantella, G.C., Arweiler, N.B., Berakdar, M., Brecx, M., 2002. Clinical evaluation of an enamel matrix protein derivative combined with a bioactive glass for the treatment of intrabony periodontal defects in humans. J. Periodontol. 73, 401−408.

Sculean, A., Pietruska, M., Schwarz, F., Willershausen, B., Arweiler, N.B., Auschill, T.M., 2005. Healing of human intrabony defects following regenerative periodontal therapy with an enamel matrix protein derivative alone or combined with a bioactive glass. A controlled clinical study. J. Clin. Periodontol. 32, 111−117.

Sculean, A., Windisch, P., Szendroi-Kiss, D., Horvath, A., Rosta, P., Becker, J., et al., 2008. Clinical and histologic evaluation of an enamel matrix derivative combined with a biphasic calcium phosphate for the treatment of human intrabony periodontal defects. J. Periodontol. 79, 1991−1999.

Seyedmomeni, S.S., Naeimi, M., Raz, M., Mohandesi, J.A., Moztarzadeh, F., Baghbani, F., et al., 2016. Synthesis, characterization and biological evaluation of a new sol−gel derived B and Zn-containing bioactive glass: in vitro study. Silicon1−7.

Shafiei, F., Behroozibakhsh, M., Moztarzadeh, F., Haghbin-Nazarpak, M., Tahriri, M., 2012. Nanocrystalline fluorine-substituted hydroxyapatite [Ca5(PO4)3(OH)$1-x$Fx $(0 \leqslant x \leqslant 1)$] for biomedical applications: preparation and characterisation. IET Micro Nano Lett. 7, 109−114.

Shayesteh, Y.S., Khojasteh, A., Soleimani, M., Alikhasi, M., Khoshzaban, A., Ahmadbeigi, N., 2008. Sinus augmentation using human mesenchymal stem cells loaded into a beta-tricalcium phosphate/hydroxyapatite scaffold. Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol. Endod. 106, 203−209.

Sheikh, Z., Najeeb, S., Khurshid, Z., Verma, V., Rashid, H., Glogauer, M., 2015. Biodegradable materials for bone repair and tissue engineering applications. Materials. 8, 5744−5794.

Skrtic, D., Antonucci, J., Eanes, E., Brunworth, R., 2002. Silica-and zirconia-hybridized amorphous calcium phosphate: effect on transformation to hydroxyapatite. J. Biomed. Mater. Res. A. 59, 597−604.

Solgi, S., Khakbiz, M., Shahrezaee, M., Zamanian, A., Tahriri, M., Keshtkari, S., et al., 2015a. Synthesis, characterization and in vitro biological evaluation of sol-gel derived Sr-containing nano bioactive glass. Silicon1−8.

Solgi, S., Shahrezaee, M., Zamanian, A., Jafarzadeh Kashi, T., Raz, M., Khoshroo, K., et al., 2015b. Sol−gel synthesis and characterization of $SiO_2-CaO-P_2O_5-SrO$ bioactive glass: in vitro study. Key Engineering Materials. Trans Tech Publications, pp. 30−35.

Surmenev, R.A., Surmeneva, M.A., Ivanova, A.A., 2014. Significance of calcium phosphate coatings for the enhancement of new bone osteogenesis—a review. Acta Biomater. 10, 557−579.

Tadjoedin, E.S., de Lange, G.L., Holzmann, P.J., Kulper, L., Burger, E.H., 2000. Histological observations on biopsies harvested following sinus floor elevation using a bioactive glass material of narrow size range. Clin. Oral. Implants Res. 11, 334−344.

Tadjoedin, E.S., de Lange, G.L., Lyaruu, D.M., Kuiper, L., Burger, E.H., 2002. High concentrations of bioactive glass material (BioGran) vs. autogenous bone for sinus floor elevation. Clin. Oral. Implants Res. 13, 428−436.

Tahriri, M., Moztarzadeh, F., 2014. Preparation, characterization, and in vitro biological evaluation of PLGA/nano-fluorohydroxyapatite (FHA) microsphere-sintered scaffolds for biomedical applications. Appl. Biochem. Biotechnol. 172, 2465−2479.

Tahriri, M., Moztarzadeh, F., Hresko, K., Khoshroo, K., Tayebi, L., 2016. Biodegradation properties of PLGA/nano-fluorhydroxyapatite composite microsphere-sintered scaffolds. Dent. Mater. 32, e49−e50.

Ter Brugge, P.J., Wolke, J.G., Jansen, J.A., 2003. Effect of calcium phosphate coating composition and crystallinity on the response of osteogenic cells in vitro. Clin. Oral. Implants Res. 14, 472−480.

Touri, R., Moztarzadeh, F., Sadeghian, Z., Bizari, D., Tahriri, M., Mozafari, M., 2013. The use of carbon nanotubes to reinforce 45S5 bioglass-based scaffolds for tissue engineering applications. BioMed Res. Int. 2013, 1−8.

Uo, M., Mizuno, M., Kuboki, Y., Makishima, A., Watari, F., 1998. Properties and cytotoxicity of water soluble $Na_2O-CaO-P_2O_5$ glasses. Biomaterials. 19, 2277−2284.

Vallet-Regí, M., 2001. Ceramics for medical applications. J. Chem. Soc., Dalton Trans.97−108.

Vallet-Regí, M., González-Calbet, J.M., 2004. Calcium phosphates as substitution of bone tissues. Prog. Solid State Chem. 32, 1−31.

West, J., Hench, L., 1990. The sol−gel process. Chem. Rev. 90, 33−72.

Whited, B.M., Skrtic, D., Love, B.J., Goldstein, A.S., 2006. Osteoblast response to zirconia-hybridized pyrophosphate-stabilized amorphous calcium phosphate. J. Biomed. Mater. Res. A. 76, 596−604.

Wilson, J., Pigott, G., Schoen, F., Hench, L., 1981. Toxicology and biocompatibility of bioglasses. J. Biomed. Mater. Res. A. 15, 805−817.

Yao, A., Wang, D., Huang, W., Fu, Q., Rahaman, M.N., Day, D.E., 2007. In vitro bioactive characteristics of borate-based glasses with controllable degradation behavior. J. Am. Ceramic Soc. 90, 303−306.

Yoshikawa, H., Myoui, A., 2005. Bone tissue engineering with porous hydroxyapatite ceramics. J. Artif. Organs. 8, 131−136.

Zamanian, A., Moztarzadeh, F., Kordestani, S., Hesaraki, S., Tahriri, M., 2010. Novel calcium hydroxide/nanohydroxyapatite composites for dental applications: in vitro study. Adv. Appl. Ceram. 109, 440−444.

Zaner, D.J., Yukna, R.A., 1984. Particle size of periodontal bone grafting materials. J. Periodontol. 55, 406−409.

Zerbo, I.R., Zijderveld, S.A., de Boer, A., Bronckers, A.L., de Lange, G., Ten Bruggenkate, C.M., et al., 2004. Histomorphometry of human sinus floor augmentation using a porous beta-tricalcium phosphate: a prospective study. Clin. Oral. Implants Res. 15, 724−732.

Zhang, X., Jia, W., Gu, Y., Xiao, W., Liu, X., Wang, D., et al., 2010. Teicoplanin-loaded borate bioactive glass implants for treating chronic bone infection in a rabbit tibia osteomyelitis model. Biomaterials. 31, 5865−5874.

Zhao, J., Liu, Y., Sun, W.-B., Zhang, H., 2011. Amorphous calcium phosphate and its application in dentistry. Chem. Cent. J. 5, 40.

Polymers for oral and dental tissue engineering

3

Nasim Salehi-Nik[1], *Maryam Rezai Rad*[1], *Pantea Nazeman*[1] *and Arash Khojasteh*[1,2]
[1]Shahid Beheshti University of Medical Sciences, Tehran, Iran, [2]University of Antwerp, Antwerp, Belgium

3.1 Introduction

The concept of tissue engineering and application of scaffolds in combination with cells and bioactive molecules have been proposed as a promising approach for regeneration in craniofacial region (Zuk, 2008; Weigel et al., 2006). A variety of materials including metals, ceramics, polymers, and their combination have been used for the replacement and repair of damaged tissues (Patil et al., 2013; Jafari et al., 2015). Among them, polymers have received a great attention due to easy control over their biocompatibility, biodegradability, and processability accordant with the tissue specificities (Kim et al., 2000; Agrawal and Ray, 2001). For example, biodegradability can be modified by adjusting molecular design and pore size (Hollister, 2005; Ma and Choi, 2001), and physicochemical properties can be altered by surface modification to control biological responses.

Moreover, a variety of fabrication techniques have been used for preparing polymeric scaffolds (Tabatabaei et al., 2012; Jafari et al., 2015). Recently the utilization of computer-aided design (CAD) and three-dimensional (3-D) printing in fabricating scaffolds precisely compatible with defect geometry has revolutionized regeneration in craniofacial regions. Also, rapid prototyping technology with widespread availability of high-resolution medical imaging is feasible to generate contoured 3-D prostheses for craniofacial reconstruction (Bose et al., 2012; Tevlin et al., 2014). The purpose of this chapter is to review an approach for engineering polymeric scaffolds for craniofacial regeneration that fulfills the basic requirements of this complex site. In particular, it provides information regarding different types of polymeric materials, fabrication techniques, and parameters which should be considered for scaffold design.

3.2 Different types of polymeric scaffolds

Polymeric materials are classified into two general categories: synthetic and naturally derived polymers (Table 3.1). The main biodegradable synthetic polymers

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00003-7
© 2017 Elsevier Ltd. All rights reserved.

Table 3.1 Naturally derived and synthetic polymers used in scaffold fabrication

Naturally derived polymers
Matrigel: Matrigel is a well-known extract from the Engelbreth−Holm−Swarm sarcoma that is rich in basement membrane components. It is widely used in basic science for the evaluation of cell−matrix interactions and increasingly in tissue engineering, particularly in areas such as nerves, adipose tissue, and skeletal muscle (Kleinman and Martin, 2005)
Collagen: Collagen is synthesized by several cell types and used in various formats such as gels, sponges, or sheets as cell scaffolds for the engineering of tissues. The mechanical strength of collagen scaffolds and the rate of absorption have been of concern, resulting in the use of cross-linking agents to change the thermal, and mechanical properties of collagen. Twenty-seven types of collagens have been identified so far, but collagen type I is the most abundant and the most investigated for biomedical applications (Yang et al., 2004; Rosso et al., 2005)
Fibronectin: Fibronectin is a glycoprotein which exists outside cells and on the cell surface. This protein associates with the other proteins of the ECM like fibrinogen, collagen, glycosaminoglycans and with suitable receptors which are in the cell membrane. Fibronectin is composed of tandem repeats of three distinct types (I, II, and III) of individually folded modules (Ebner et al., 2006). Studies looking at the addition of fibronectin to tissue engineering scaffolds suggest that fibronectin may be effective in scaffold vascularization (Hall et al., 2001)
Fibrin and fibrinogen: Other ECM components used as scaffolds include fibrin and fibrinogen. Fibrin consists of the blood proteins fibrinogen and thrombin, produced naturally in the body following injury to establish hemostasis and enhance wound healing. Because of their biocompatibility, biodegradability, easy preparation, and manipulation, fibrin scaffolds have been used for multiple purposes (e.g., filling in bone cavities, vascular graft, and repairing injuries to urinary tract, liver, and lung) and are also available as combinations with other polymers such as fibrin−poly ethylene glycol (PEG) blend (Galler et al., 2011)
Chitosan: Chitosan is a polymer derived from the deacetylation of chitin, the major component of crustacean exoskeletons. Chitosan has properties that make it appropriate for wound dressing, drug delivery, and tissue engineering applications. One of the most important characteristics of chitosan for tissue engineering applications is its ability to be shaped into various structures such as microspheres, paste, membranes, sponges, fibers, and porous scaffolds. It is a potent wound-healing accelerator, possesses immunological activity, produces cytokines, and inhibits infection (Horst et al., 2012; Muzzarelli et al., 1993; Yin et al., 2003)
Alginate: Alginate is linear polysaccharide obtained from algae and requires extensive purification to avoid immune responses after implantation. The advantages of alginate are its biocompatibility, low toxicity, and slow gelling time (20−60 min), depending on the concentration and temperature. Disadvantages of the material are poor cell adhesion, inability to control its degradation rate in vivo, mechanical weakness, and its low viscoelasticity, although this can be enhanced by increased cross-linking or addition of other substances such as hydroxyapatite (HA). Several studies using alginate and alginate/HA mixtures have been performed in bone and cartilage tissue engineering (Yuan et al., 2011)

(*Continued*)

Table 3.1 **(Continued)**

Agarose: Agarose is linear polysaccharide obtained from seaweed and is well known for its use in nucleic acid electrophoresis, but it is also useful for cell encapsulation. It has been used in neuronal and cartilage tissue engineering, as well as in composites for engineering of bone (Khanarian et al., 2012) and cornea (Alaminos et al., 2006) with HA and fibrin, respectively
Synthetic polymers
Polyglycolic acid (PGA): PGA is a FDA-approved synthetic biomaterial with a variety of tissue engineering applications, including regeneration of cartilage, bone, tendon, muscle, and skin. Despite such adaptability, its mechanical properties are not ideal for the precision bone reconstruction necessary for craniofacial defect repair because of being soft and inability to maintain shape. PGA is insoluble in water, and glycolic acid is the final degradation product leading to local acidosis and potential tissue damage (Gunatillake and Adhikari, 2003; Tollemar et al., 2016) **Polylactic acid (PLA)**: PLA is another biodegradable aliphatic polyester, more hydrophobic than PGA and more resistant to hydrolysis. There are two racemic isoforms: poly-L-lactic acid (PLLA) and poly-D-lactic acid (PDLA). The racemic mixture can be termed poly-D,L-lactic acid (PDLLA) or simply PLA, without indication of which chiral form is present (Horst et al., 2012). PLA has several properties conducive to tissue engineering, including controllable biodegradation rate, biocompatibility, and good mechanical strength. It has been applied clinically for fabrication of resorbable sutures and bone fixation devices in fracture healing, as a drug delivery carrier, and as a scaffold for cartilage and nerve regeneration. However, its application as a scaffold biomaterial for craniofacial bone regeneration is limited by its poor osteoinductive properties (Neumann and Kevenhoerster, 2009). It degrades into lactic acid, which may be locally toxic to tissues (Gunatillake and Adhikari, 2003) **Polyglycerol sebacic acid (PGS)**: PGS, also called biorubber, is a tough, biodegradable elastomer made from biocompatible monomers. It has good mechanical properties, rubber like elasticity, surface erosion biodegradation, and in vitro and in vivo biocompatibility (Sundback et al., 2005) **Polypropylene fumarate (PPF)**: PPF is a synthetic, unsaturated, linear polyester polymer that is biodegradable, biocompatible, osteoconductive, injectable, and sufficiently strong for craniofacial bone tissue engineering. It generally requires a small monomer accelerating agent, such as N-vinylpyrrolidone, in order to cross-link as an injectable polymer. A two-phase PPF cement incorporating cross-linked microparticles to increase strength and decrease setting temperature is developed. This PPF-based system has improved injectability, setting temperature, and setting time over polymethyl methacrylate (PMMA) bone cement and is believed to be beneficial for application in craniofacial bone regeneration (Henslee et al., 2012) **Poly-ε-caprolactone (PCL)**: PCL, an aliphatic polyester, is biocompatible, highly flexible and has a controllable biodegradation rate due to alterable substituent molecular weight (Kweon et al., 2003). This polymer is generally used in pharmaceutical products and wound dressings. PCL degrades by hydrolysis of its ester linkages in physiological conditions (e.g., in the human body) in a long time period, and therefore, has been in the spotlight for using as an implantable biomaterial in long term applications (Schnell et al., 2007). PCL scaffolds have been used for bone tissue engineering, either alone or combined with HA (Neumann and Kevenhoerster, 2009)

(*Continued*)

Table 3.1 (Continued)

Polyamide (PA): PA is a synthetic polymeric collagen analog that has significant strength as well as biocompatibility. These properties have made PA a promising partner for bioceramics in osteoconductive composite scaffolds (Tollemar et al., 2016)
Polyethylene glycol (PEG): PEG, also known as polyethylene oxide or polyoxyethylene, is the most commercially important polyethers, which refers to an oligomer or polymer of ethylene oxide resistant to protein adsorption and cell adhesion. These characteristics decrease the immune response associated with implantation. This polymer can also help to seal cell membranes after injury, making it useful for avoiding cell death. Hydrophilic PEG hydrogels can be made through a variety of cross-linking schemes to develop scaffolds with different degradation as well as release rates. Further chemistry can be used to modify these gels to add sites for cell adhesion or ECM molecules to allow cells to infiltrate into these scaffolds, extending their potential applications in regenerative medicine (Horst et al., 2012)

include polyesters, polyanhydride, polycaprolactone, polycarbonate, polyfumarate, and polyorthoester (Burg et al., 2000; Langer, 2000). The polyesters such as poly (glycolic acid) (PGA), poly (lactic acid) (PLA), and their copolymer of poly [lactic-*co*-(glycolic acid)] (PLGA) are the most commonly used scaffolds in tissue engineering. The naturally derived polymers include proteins of natural extracellular matrices (ECM) such as collagen and glycosaminoglycan, alginate, chitosan, matrigel, and polypeptides (Suh and Matthew, 2000; Jafari et al., 2015). Each group of synthetic and naturally derived polymers has several advantages and disadvantages in the field of scaffold fabrication as demonstrated in Table 3.2.

3.2.1 Synthetic polymers

The use of synthetic biodegradable polymers in oral and dental tissue engineering is well described (Ahmad et al., 2008). Currently, the most common synthetic absorbable polymers available for oral and maxillofacial applications include the poly-ε-caprolactone (PCL), PGA, PLA, and their copolymer PLGA (Yoshimoto et al., 2003; Jafari et al., 2015). Resorbable membranes made of PLA and PLGA have been successfully used as barriers in guided tissue regeneration in treatment of periodontal diseases (Christgau et al., 1998) and craniofacial defect reconstruction (Ahmad et al., 2008).

In an effort to engineer the pulp tissue in vitro, human pulp fibroblasts were cultured on PGA fibers for 45–60 days. The pulp-like tissue was observed on the PGA scaffolds, which was the proof of principle that pulp cells can proliferate and deposit a matrix on suitable synthetic polymers in vitro (Mooney et al., 1996c). PGA scaffolds were also used with human dental pulp and the upregulation of type I collagen, fibronectin, several bone morphogenetic proteins (BMPs) and their receptors were observed, suggesting the potential of this scaffold to promote differentiation of human dental pulp cells (hDPSCs) (Buurma et al., 1999).

Table 3.2 Advantages and disadvantages of naturally derived and synthetic polymers used in scaffold fabrication

	Advantages	Disadvantages
Naturally derived polymers	• Biodegradable • Possess known cell-binding sites that support cell attachment and proliferation • Do not lead to immunogenic response • Do not involve the use of harsh chemicals during processing	• Poor mechanical strength • High speed of degradation • Limited ability to tailor for specific properties • Lack of control over the pore size and mechanical properties of the scaffold • Exist in finite supply • Expensive
Synthetic polymers	• Easily formed into desired scaffold architectures with relatively good mechanical strength • Controllable degradability by manipulating the crystallinity, molecular weight, and copolymer ratio • Exist in adequate supply	• Difficulty in 3-D fabrication (specifically, 3-D printing) • Uncontrollable shrinkage • Questionable cell−polymer interactions (lack cell recognition signals) • Possible local toxicity resulting from acidic degradation products

The capability of PGA scaffolds seeded with tooth germs from 6-month-old minipigs to generate dental structure was compared with β-tricalcium phosphate (β-TCP), fibrin, and collagen scaffolds (Ohara et al., 2010). The results demonstrated that in fibrin and collagen gels, the epithelial structure was maintained in porcine third molar tooth bud which resembled tooth buds, whereas on PGA and β-TCP, the implanted tooth buds produced more dentin-like structure.

Khojasteh et al. compared adhesion, proliferation, and differentiation of hDPSCs on poly-L-lactic acid (PLLA) with SureOss (Allograft), Cerabone (Xenograft), and OSTEON/Collagen (Composite). They demonstrated that hDPSCs seeded on PLLA scaffold demonstrated the highest cell proliferation, attachment, and alkaline phosphatase (ALP) activity (Khojasteh et al., 2015), suggesting that PLLA scaffold is a suitable scaffold for tooth regeneration.

It is demonstrated that proliferation rate of periodontal cells improves when exposed to organized PLGA nanofibers (Shang et al., 2010). PLGA scaffolds with two different pore sizes (150−180 μm and 180−300 μm) were seeded with DPSCs and transplanted subcutaneously in rabbits for 2 and 6 weeks and formation of osteo-dentin structures were observed with these scaffolds (El-Backly et al., 2008). Implantation of biodegradable tooth-shaped PLGA scaffolds seeded with dissociated porcine tooth buds successfully produced distinguishable tooth structures. However, the size of bioengineered tooth was very small and did not conform to the shape and size of the scaffolds (Young et al., 2002). Similar results were

obtained by transplanting both PGA and PLGA scaffolds loaded by rat tooth bud cells in the rat jaw for 12 weeks. 3-D PLGA scaffolds have been fabricated with heterogeneous pore sizes (small, 20−200 μm diameter, and large, 1−2 mm diameter) and seeded with mesenchymal stem cells (MSCs) harvested from the ilium of a minipig. After incubation in a bioreactor for 10 days, constructs were implanted into mandibular defects of the same minipig. Histological examination at 8th week demonstrated bone regeneration and bridging of the defects (Abukawa et al., 2004). However, unfavorable mechanical properties of PLGA and difficulty in use of 3-D printing for manufacturing scaffolds have limited use of PLGA for bone regeneration (Abukawa et al., 2006).

PCL scaffolds were used for the regeneration of various mineralized tissues such as bone, cartilage, and dentin (Williams et al., 2005; Yang et al., 2010). They promote adhesion, proliferation, and odontoblastic differentiation. Incorporation of HA into PCL scaffolds enhances odontoblastic differentiation of human postnatal DPSCs (Yang et al., 2010). Also, PCL scaffolds seeded with MSCs were used to repair cranial defects in different animal models (Bidic et al., 2003; Rezai-Rad et al., 2015).

3.2.2 Naturally derived polymers

Polymers of natural origin, such as collagen, alginate, and chitosan, have been used for scaffold fabrication due to their similarity to ECM as well as their biocompatibility and biodegradability (Jafari et al., 2015). Natural polymers seeded with chondrocytes were used to engineer a human ear, temporomandibular joint disc, and meniscal-shaped constructs (Weng et al., 2001). Gelatin sponges loaded with MSCs were also used for repair of calvarial defects in mice (Krebsbach et al., 1998).

Alginate has been widely used in tooth engineering for delivery of cells and/or growth factors. Alginate hydrogel with either transforming growth factor beta 1 (TGF-β1) or acid treatment was applied to slices of human teeth with vital dentin−pulp complex tissues and dentin matrix secretion and formation of new odontoblast-like cells was observed in tooth slices (Dobie et al., 2002).

Collagens, particularly type I collagen, are the main component of dentin and bone. They provide a 3-D culture environment for various types of cells, including DPSCs (Kim et al., 2009). It was demonstrated that collagen sponge was more potent in tooth production than synthetic scaffold materials, e.g., PLGA mesh (Sumita et al., 2006). Compared with other natural scaffolds including gelatin and chitosan, type I and III collagen gels seeded with DPSCs exhibited a higher degree of odontoblastic differentiation as shown by ALP activity and expression of osteocalcin, dentin sialophosphoprotein (DSPP), and dentin matrix protein 1 (DMP1) (Kim et al., 2009; Mizuno et al., 2003; Yamauchi et al., 2011). Collagen gel has been used alone or in combination with growth factors and other scaffold materials such as chitosan (Yang et al., 2012b).

Seeding human salivary gland epithelial cells on polyglutamic acid polymer scaffold has demonstrated functional, differentiated salivary units of acini and ducts

in a 3-D construct, with the production of secretory granules and expression of aquaporin 5 protein (Joraku et al., 2007).

Other polymers have been also employed to engineer oral mucosa, e.g., silk fibroin reduces wound contraction (Ge et al., 2012) and nanofibrous elastin-like recombinant polymer collagen improved the self-renewal potential of epithelial cells after grafting (Kinikoglu et al., 2011; Golinski et al., 2011). As a potential scaffold for skin or mucosal grafting procedures, plastic compressed collagen has been widely investigated (Abou Neel et al., 2013; Moharamzadeh et al., 2012).

3.2.3 Composite scaffolds

Composite materials are composed of two or more biomaterials, in the form of copolymers, polymer–polymer blends, or polymer–ceramic composites (Hyun et al., 2013). Developing a copolymer system potentially offers the best qualities of each material, e.g., balancing glass transition temperature and degradation potential (Amini et al., 2012; Zhang et al., 2014). For instance, PGA and PLA alone are not suitable scaffolds for bone tissue engineering, but their respective softness and low osteoinductivity have been partially addressed by combining them to form a PLGA composite scaffold (Mooney et al., 1996b). No linear relationship exists between the ratio of glycolic acid to lactic acid and the physicomechanical properties of them regarding copolymers. PLGA promotes osteoblast attachment, growth, and differentiation both in vitro and in vivo and its degradation rate could be adjusted by changing the ratio of PGA to PLA (Gentile et al., 2014; Liu and Ma, 2004). PLGA is commonly combined with ceramics, bioglass, or other osteoconductive materials for bone tissue engineering (Gentile et al., 2014; Pan and Ding, 2012). Studies comparing pure PLGA with composite scaffolds of PLGA/HA or PLGA/TCP found that hDPSCs had the greatest proliferation on composite scaffolds of PLGA/TCP and were able to form mineralized structures. Furthermore, after 4–5 weeks, the rat tooth bud cells on the PLGA/TCP scaffold expressed dentin sialoprotein and generated dentin- and pulp-like tissues (Zheng et al., 2011). The dental pulp-derived cells were seeded on mixtures of PGA fiber mesh scaffolds with porous or nonporous HA/β-TCP and implanted subcutaneously for 6 weeks. Newly formed hard tissues were observed in all samples but dentin-like structure with expression of dentin sialoprotein, collagen type I, osteonectin, and bone sialoprotein was only evident in the PGA-cell implants with porous HA/β-TCP (Tonomura et al., 2010).

Polypropylene fumarate (PPF) has been used as a copolymer with PCL as a scaffold for osteoblastic differentiation and maturation in vitro (Becker et al., 2015). The setting time, setting temperature, mechanical strength, and other physical properties of PPF–PCL copolymer can be tuned by modifying substituent molecular weight and relative proportion of PPF and PCL (Becker et al., 2015; Yan et al., 2011). Chemical structure of PPF–PCL enables HA incorporation which enhances osteoblast progenitor cell adhesion and proliferation (Zaky and Cancedda, 2009).

Polyamide (PA) is a synthetic polymeric collagen analog with significant strength and biocompatibility. Given its mechanical strength, PA has been used in

combination with HA to compensate for HA brittleness and fatigue. PA scaffold in combination with HA nanoparticles loaded with BMP-7-transduced MSCs has demonstrated promising results in repair of rabbit mandibular defects (Li et al., 2010).

Sodium hyaluronate/chitosan complex has been demonstrated to be compatible with MSCs (Coimbra et al., 2011a), provides appropriate osteoblast attachment and noncytotoxicity of the degradation by-products of the scaffold were observed in application of chitosan/pectin scaffolds in bone regeneration (Coimbra et al., 2011b). Loading DPSCs on chitosan/collagen scaffolds impregnated with BMP-7 has demonstrated high expression of DSPP and DMP1 (Yang et al., 2012b). Incorporating fibrin with other polymers such as polyethylene glycol (PEG) enhanced material handling. The PEGylated fibrin scaffold is injectable, tunable, degradable, and compatible with dental stem cells. It has osteoinductive and odontoinductive properties and allows the formation of dentin-like collagenous matrix and vascularized pulp-like structure after transplantation in vivo (Yang et al., 2012a).

In addition, using a combination of natural and synthetic polymers has compensated the shortcomings of individual materials and has enhanced mechanical strength and cell−scaffold interactions at the same time. For example, the in vivo degradation of collagen is too fast that impairs regeneration of large defects. Therefore, multilayered membranes with a layer of flexible synthetic polymer (e.g., polylactide-*co*-glycolide dimethacrylate) surrounded with two layers of natural polymers (e.g., collagen) have been developed. Flexibility of synthetic polymers enhances handling, adaptation, and degradation rate of scaffolds. On the other hand, collagen part of the scaffold provides an excellent biocompatibility and enhances cellular response (Abou Neel et al., 2014).

3.2.4 Biomimetic scaffolds

Recently, efforts are made to enhance structural design and surface properties of scaffolds. Various modifications such as addition of bioactive molecules, e.g. ECM-like molecules, nanoparticles, and growth factors, can enhance attachment and proliferation of cells on the scaffold. These "biomimetic" or "smart" scaffolds improve cell attachment, proliferation, and differentiation (Motamedian et al., 2015).

3.2.4.1 Scaffolds with improved hydrophilicity

Due to hydrophobic properties of biodegradable synthetic polymers, uniform distribution of cells through them is challenging and in this case, wettability of the polymer plays an important role (Kim et al., 2012). Introducing hydrophilic PEG into the hydrophobic polymer network (Gao et al., 1998), or coating polymer surface with ECM proteins, e.g., collagen, gelatin, and fibronectin, improves wettability of synthetic polymers which enhances cell seeding efficiency (Patil et al., 2013). It is demonstrated that selective functionalization of degradable synthetic scaffolds with chitosan and/or laminin-111 exerts chemical signals that enhance epithelial cell

proliferation and apicobasal polarity, which are an integral part of directional secretion of salivary gland secretary cells (Cantara et al., 2012).

3.2.4.2 Scaffolds with improved bioactivity

Efficient administration of 3-D scaffolds in tissue repair is influenced by their bioactivity (Zuk, 2008). For example, in bone tissue engineering, 3-D scaffolds need to be osteoconductive to enable adherence, migration and differentiation of osteoprogenitors as well as new bone matrix synthesis (Patil et al., 2013). Although scaffolds such as PLGA or PLA provide a biodegradable platform for stem cell adhesion and differentiation, they have limited bioactivity (Zuk, 2008). It is suggested that incorporating HA into these scaffolds enhances their bioactivity (Bakos et al., 1999) by attracting osteoblasts, improving mechanical properties (Wei and Ma, 2004), and reducing adverse effects associated with the synthetic polymer degradation (Smith et al., 2009).

Since all interactions with biological components occur at the pore surface, it is recommended to coat the polymer surface with HA to enhance surface characteristics. A novel technology known as biomimetic apatite coatings includes soaking scaffolds in ionic solutions similar to blood plasma, which enables growth of apatite crystals onto its pore surfaces (Boskey, 2003; Kokubo et al., 2004; Smith et al., 2009). Murine adipose-derived stem cells seeded onto biomimetic apatite-coated PLGA scaffolds were found to heal critical-sized cranial defects without any exogenous growth factors (Cowan et al., 2004; Zuk, 2008). To control degradability and enhance osteogenic potential of collagen membranes, immobilization of HA nanoparticles (Song et al., 2007), ALP (Oortgiesen et al., 2012), or bioactive glass (Yadav et al., 2011) on collagen membranes has been studied. HA is also added to collagen to mimic the unique structure of collagen and HA in natural bone. Addition of HA to collagen has demonstrated improved stiffness and interconnectivity in a critical-sized rat calvarial defect (Friedman et al., 1998). Similarly, high bioconductivity of calcium phosphates (CPC) has put them in the spotlight. In the first study of its kind to investigate the addition of CPCs to collagen, it has been demonstrated that the number of human umbilical cord stem cells increased on CPC-containing scaffolds (Thein-Han and Xu, 2011). Those composite scaffolds also showed enhanced osteogenic differentiation (as evidenced by increased levels of ALP, collagen I, and Runx2 gene expression), mineralization, and ECM development compared with pure CPC or collagen (Thein-Han and Xu, 2011). Khojasteh et al. have shown that PCL−TCP composite scaffolds seeded with MSCs enhance early revascularization and accelerate bone regeneration in canine mandible (Khojasteh et al., 2013).

One of the potential benefits of using implant coatings is that the materials may be used as a drug delivery system for growth factors and osteogenic supplements, and therefore assist in developing smart scaffolds (Abukawa et al., 2006). The combination of BMP-4 and release of vascular endothelial growth factor (VEGF) from PLGA scaffold is reported to enhance bone formation (Huang et al., 2005; Simmons et al., 2004). It is also demonstrated that sustained delivery of VEGF enhances

vascularization at the location of transplanted cells, which contributes to their survival (Smith et al., 2004). The transplanted cells, therefore, subsequently proliferate and produce bone matrix. Kim and colleagues designed a biodegradable PLGA scaffold that released osteogenic media containing dexamethasone and ascorbic acid (Kim et al., 2005). These smart materials may revolutionize tissue engineering research, since controlled release of biochemical and growth factors enhance cell penetration, proliferation, differentiation, and bone matrix production and improve vascularization of the grafts (Abukawa et al., 2006).

3.3 Methods for scaffold fabrication

3.3.1 Conventional scaffold fabrication techniques

Several techniques have been developed to process synthetic and natural scaffold materials into porous scaffolds (Tabatabaei et al., 2012) (Table 3.3). However, conventional techniques are not effective methods for controlling pore size, geometry, spatial distribution, and creating internal channels. Solvent-casting and particulate-leaching techniques may not be reliable for creating interconnective pores since it is dependent on the contact of adjacent salt particles (Hutmacher, 2001). Moreover, only thin scaffolds can be produced due to difficulty in removing salt particles deep in the matrix. In gas foaming technique, it is estimated that only 10%−30% of the pores are interconnected (Mooney et al., 1996a). Cytotoxicity of organic solvents frequently used in these conventional methods, such as chloroform and methylene chloride, is the major drawback of these techniques.

Due to pivotal role of oxygen and nutrient delivery and waste removal from the tissues, incorporating an artificial vascular system into the scaffolds is of outmost importance, hence efforts are being made to optimize scaffolds in this regard (Sachlos and Czernuszka, 2003).

3.3.2 Recent Advances in Complex 3-D Scaffold Development

As craniofacial defects and anatomical structures vary among individuals, manufacturing specific personal scaffolds may be more effective in regenerating defects with complex geometry (Weigel et al., 2006). Ideally, craniofacial scaffolds should have several properties as follows: (1) precisely resembling the complex 3-D anatomic defect, (2) porous structure to effectively deliver bioactive agents (e.g., recombinant proteins), and (3) appropriate mechanical properties to bear forces up to defect regeneration (Hollister, 2005; Hollister et al., 2005). Since both mechanical properties and mass transport depend on 3-D scaffold architecture, computerized designing techniques may be effective in predicting and optimizing a microstructure to achieve the desired balance (Hollister, 2005). In addition, increased design flexibility requires the capability to use materials with a wide range of properties. Conventional fabrication techniques cannot precisely fabricate a 3-D scaffold with an exactly predefined shape (Hollister et al., 2005). Therefore,

Table 3.3 Conventional methods of scaffold fabrication

Solvent-casting particulate-leaching
This technique involves producing a solution of polymer in solvent and adding salt particles with specific dimension to produce a uniform suspension. The solvent is allowed to evaporate leaving a polymer matrix with salt particles within. The composite is then placed in water to leach the salts and produce a porous structure (Sachlos and Czernuszka, 2003; Tabatabaei et al., 2012)
Gas foaming
Polymer is saturated with carbon dioxide (CO_2) at high pressures (Mooney et al., 1996a; Tabatabaei et al., 2012). The solubility of the gas in the polymer is then decreased rapidly by bringing the CO_2 pressure back to atmospheric level. This leads to nucleation and growth of gas bubbles, with sizes ranging between 100−500 μm in the polymer (Sachlos and Czernuszka, 2003). The advantages of this method are low cost of CO_2, its availability, and no need for removal before cell seeding. The gas is dissolved in the solid polymer or polymer melt at high temperature and high pressure, e.g., for poly(lactide-*co*-glycolide)acid (PLGA) 35−40 °C, 10−20 MPa, for poly(ε-caprolactone) (PCL) 70−90 °C, 7−32 MPa, and for cyclic olefin copolymer (COC) 100−180 °C and 30 MPa. It has been established that the foams possess closed cells with the pore size of 30−700 μm, however, they have limited interconnectivity and therefore their application in the area of tissue engineering is hampered. Thus foaming processes are often associated with leaching methods to increase the porosity of the resulting scaffold (Nam et al., 2000; Weigel et al., 2006)
Phase separation
Polymer is dissolved in molten phenol or naphthalene. The temperature is then lowered to produce a liquid−liquid phase separation and quenched to form a two-phase solid. The solvent is removed by sublimation to give a porous scaffold with bioactive molecules incorporated in the structure (Sachlos and Czernuszka, 2003; Tabatabaei et al., 2012). This method can be used to prepare nanofibers and the average pore size that can be realized by this process can be varied only from the nanometer to the few micrometer range. However, larger pores are required for tissue engineering, e.g., for skin (20−150 μm) or bone scaffolds (40−400 μm), which cannot be achieved with this technique unless it is combined with other procedures such as salt leaching (Roychowdhury and Kumar, 2006)
Melt molding
This process involves filling a mold with polymer powder and water-soluble particles such as gelatin or salt porogen, and then heating the mold above the glass transition temperature of polymer while applying pressure to the mixture. This treatment causes the polymer particles to bond together. Once the mold is removed, the gelatin component is leached out by immersing in water and the scaffold is then dried (Amoabediny et al., 2011; Sachlos and Czernuszka, 2003)
Freeze drying
Synthetic polymers are dissolved in a solvent. The resultant solution is then frozen and freeze-dried to develop porous matrices. Freezing the dispersion or solution results in the formation of ice crystals that force and aggregate the polymer molecules

(*Continued*)

Table 3.3 (Continued)

into the interstitial spaces. The ice crystals are then removed by freeze drying (Tabatabaei et al., 2012). The pore size can be controlled by freezing rate and pH; a fast freezing rate produces smaller pores (Sachlos and Czernuszka, 2003). Unidirectional solidification has been used to create a homogenous 3-D-pore structure (Schoof et al., 2001). In addition to synthetic polymers, some of the natural polymers such as collagen, chitin, and alginate scaffolds are also fabricated by using freezing-drying (Sachlos and Czernuszka, 2003)
Electrospinning
Electrospinning is an established process capable of fabricating ultrafine fibers, in the micrometer or nanometer scale with a morphologic similarity to the extracellular matrix ECM of natural tissue (Li et al., 2014; Petrovic et al., 2012; Tabatabaei et al., 2012). In this technique, polymers are dissolved into a proper solvent or melted before being subjected to a very high voltage operated between a metallic nozzle of a syringe and a metallic collector. Nanofibers are formed by narrowing of the ejected jet stream as it undergoes increasing surface charge density due to the evaporation of the solvent. This method can be used to produce thin 2-D sheets, while 3-D nanofibrous scaffolds have been fabricated by layering these 2-D sheets or by combining electrospinning with 3-D printing (Moroni et al., 2008). Various polymer nanofibers can be deposited together into one multilayer structure by electrospinning of different polymer solutions layer by layer. If different polymer solutions are ejected by several orifices simultaneously, a mat consisting of a composition of different nanofibers is realizable. The nanofiber-based scaffolds possess a wide range of pore size distribution, high porosity, and a high surface area to volume ratio, which are favorable parameters for cell attachment, growth, and proliferation. Electrospun porous, nanofibrous scaffolds have supported various stem cells and differentiated cells to regenerate several hard and soft tissues (Li et al., 2014). For example, bone formation occurs on a structured collagen matrix with fiber bundle diameter varying from 50 to 500 nm, therefore nanofibrous scaffolds appear to provide better cellular attachment, increased differentiation of osteoblastic cells, and enhanced mineral deposition compared to solid-walled scaffolds (Woo et al., 2007), as they have architectural, functional, and morphologic similarities to collagen fibrils. Good attachment and proliferation of periodontal ligament cells on electrospun PLGA (Inanc et al., 2009) and electrospun gelatin (Zhang et al., 2009) scaffolds, as well as periodontal ligament cell's osteogenic differentiation potential (Inanc et al., 2009) have been shown. Furthermore, human periodontal ligament cells cultured on self-assembled peptide nanofibrous scaffolds promoted deposition of the main periodontal ligament ECM components, collagen type I and type III (Kumada and Zhang, 2010).
Self-assembly systems
Molecular self-assembly is also an approach for fabrication of scaffolds for tissue engineering especially nerve tissue engineering or cartilage repair. Molecular self-assembly uses noncovalent bonds such as hydrogen bonds, van der Waals interactions, electrostatic and hydrophobic interactions for fabricating supramolecular architectures. Limitations in self-assembly methods are related to difficulties in forming macropores and limited mechanical properties (Smith et al., 2009)

based on computer-aided design and computer-aided manufacturing (CAD/CAM) systems (Hollister et al., 2002; Hutmacher et al., 2004), new methods such as solid free-form fabrication (SFF) (Hollister, 2005) and rapid prototyping (RP) technologies have been developed (Xu et al., 2010; Yeong et al., 2004) to create patient-specific, anatomically shaped scaffolds.

RP and SFF are common terms for a group of fabrication techniques that use a layer by layer additive process directly from a CAD data set (Chua et al., 1998; Hollister et al., 2005). Starting from the bottom and building layers upward, each newly formed layer adheres to the previous segment. Each layer corresponds to a cross-sectional division. Postprocessing may be required to remove temporary support structures. Furthermore, data from computerized tomography (CT) or magnetic resonance imaging (MRI) medical scans can be used for creating a customized CAD model with precise architecture to correct the damaged site (Sachlos and Czernuszka, 2003). The SFF techniques are used either to directly build the biomaterial scaffold or to build a mold into which a biomaterial is cast known as indirect SFF (Taboas et al., 2003; Yeong et al., 2004). In contrast to conventional fabrication methods, SFF techniques can precisely fabricate a scaffold with patient-specific macrostructure (3-D shape) and microstructure (pore size, porosity and interconnected channels) for ideal mass transport of oxygen and nutrients throughout the scaffold and vascular in-growth (Abukawa et al., 2006; Sachlos and Czernuszka, 2003).

Furthermore, the cells can be printed on the surface (Mironov et al., 2003) and thus it is feasible to incorporate living biological substances into the prefabricated layer before the final assembly. However, this method is restricted in the type of materials and resolution due to the small final size of the scaffold (Cheah et al., 2004; Weigel et al., 2006). It is demonstrated that SFF can produce highly porous structures resembling human trabecular bone by administrating homogenization-based topology optimization algorithm (Lin et al., 2004). The methods that use SSF technologies to fabricate tissue engineering scaffolds include: (1) three-dimensional printing (3-DP), (2) selective laser sintering (SLS), (3) stereolithography (SLA), and (4) fused deposition modeling (FDM) (Amoabediny et al., 2011).

3.3.2.1 3-D Printing (3-DP)

Controlled 3-D structures can be fabricated through additive manufacturing techniques, also known as 3-DP. Additive manufacturing techniques can be basically classified as follows: extrusion (deformation + solidification), polymerization, laser-assisted sintering, and direct writing-based processes. For example, with extrusion 3-DP, a biomaterial (e.g., polycaprolactone filament) can be melted and extruded in a computer-controlled pattern to construct scaffolds, laying down layers on each other to create patient-specific customized scaffolds (Hollister, 2005). In inkjet 3-DP technique, the scaffold is built by spreading a layer of polymer powder repetitively and selectively joining the powder in the layer by a binder material. After drying of the binder, the nonjoined powder is removed by an air jet flow and the final scaffold is retrieved. Scaffolds with various architectural qualities can be produced by using multiple feeds with various cells on each layer to closely mimic the anatomic features

of a tissue or organ. This technique in porous PLGA/TCP composite scaffolds has demonstrated in vitro bone formation (Sherwood et al., 2002). The resolution of 3-DP is influenced by the powder particle size, the nozzle size, and degree of control over the position of controller that defines print-head movement. Significant point in this procedure is pretreatment of the power particles to achieve the desired size (80−250 μm) that can flow well through the nozzle and has acceptable interaction with the liquid binder. In addition, an appropriate binder for different base materials must be utilized. A preferred solvent with regard to biocompatibility is water which is suitable for natural biopolymers, such as gelatin or collagen, but organic solvent and binders might be harmful to the human body and their complete removal may be challenging (Weigel et al., 2006).

3.3.2.2 Selective laser sintering (SLS)

This technique uses deflected laser beams such as infrared and CO_2 laser to sinter thin layers of powdered materials to prepare solid 3-D scaffolds (Weigel et al., 2006). The powder surface is selectively scanned by laser beam. This interaction elevates the powder temperature just beyond the glass transition temperature but below the melting temperature of the material and subsequently the adjacent powder particles are fused. New layers of powder are deposited by a roller, building a new sintered layer above the previous one. The laser beam diameter or powder particle size limits the dimension of generated scaffold structures. The suitable materials for SLS must be available as a powder and demonstrate suitable melting and welding properties (Weigel et al., 2006; Williams et al., 2005). In addition, it is usually difficult to construct structures with sharp corners or clear boundaries with this method.

3.3.2.3 Stereolithography (SLA)

The process involves selective polymerization of a liquid photocurable monomer by an ultraviolet laser beam. In this technique, the laser beam is directed onto selected regions of a layer of liquid polymer causing solidification in the exposed areas. Following construction of the first layer, the elevator holding the model is lowered into the vat (z-axis control) to expose a new polymer layer to the laser beam for the chain reaction. This procedure is repeated several times to generate 3-D scaffolds. Accordingly the suitable material for SLA must be reactive to light to initiate the chemical reaction (Sachlos and Czernuszka, 2003; Weigel et al., 2006).

3.3.2.4 Fused Deposition Modeling (FDM)

In FDM, a moving nozzle is used to extrude fibers of polymeric material that deposit layer by layer to fabricate the model. The machine first builds a layer of fibers with a well-defined distance. On the top, fiber layers are deposited and form a 3-D scaffold with an exact porous morphology and 100% interconnectivity. The material needs to have a suitable thermoplastic behavior. Therefore, meltable polymers

such as the slow biodegrading PCL or the nonbiodegradable acrylonitrile butadiene styrene or polypropylene are used frequently. Successful use of biodegradable PLGA or PLA in FDM is rarely reported (Sachlos and Czernuszka, 2003; Weigel et al., 2006).

3.4 Conclusion

Fabrication of an ideal scaffold with desirable properties for tissue engineering is a challenging task. A variety of characteristic parameters, such as degradation rate, mechanical strength, porosity, pore size, pore microstructures, surface chemistry, and topography, should be carefully considered and controlled. Several studies suggest that the combination of biodegradable polymers with other materials may lead to emergence of new perspectives for biomedical applications with significant properties. Moreover, new horizon is emerging in tissue engineering by using novel fabrication techniques. Due to multidisciplinary nature of tissue engineering, further progress may be achieved by interaction among clinicians, scientists, and engineers.

References

Abou Neel, E.A., Bozec, L., Knowles, J.C., Syed, O., Mudera, V., Day, R., et al., 2013. Collagen—emerging collagen based therapies hit the patient. Adv Drug Deliv Rev. 65, 429−456.

Abou Neel, E.A., Chrzanowski, W., Salih, V.M., Kim, H.W., Knowles, J.C., 2014. Tissue engineering in dentistry. J Dent. 42, 915−928.

Abukawa, H., Papadaki, M., Abulikemu, M., Leaf, J., Vacanti, J.P., Kaban, L.B., et al., 2006. The engineering of craniofacial tissues in the laboratory: a review of biomaterials for scaffolds and implant coatings. Dent Clin North Am. 50, 205−216, viii.

Abukawa, H., Shin, M., Williams, W.B., Vacanti, J.P., Kaban, L.B., Troulis, M.J., 2004. Reconstruction of mandibular defects with autologous tissue-engineered bone. J Oral Maxillofac Surg. 62, 601−606.

Agrawal, C.M., Ray, R.B., 2001. Biodegradable polymeric scaffolds for musculoskeletal tissue engineering. J Biomed Mater Res. 55, 141−150.

Ahmad, N., Lyles, J., Panchal, J., Deschamps-Braly, J., 2008. Outcomes and complications based on experience with resorbable plates in pediatric craniosynostosis patients. J Craniofac Surg. 19, 855−860.

Alaminos, M., Del Carmen Sanchez-Quevedo, M., Munoz-Avila, J.I., Serrano, D., Medialdea, S., Carreras, I., et al., 2006. Construction of a complete rabbit cornea substitute using a fibrin-agarose scaffold. Invest Ophthalmol Vis Sci. 47, 3311−3317.

Amini, A.R., Laurencin, C.T., Nukavarapu, S.P., 2012. Bone tissue engineering: recent advances and challenges. Crit Rev Biomed Eng. 40, 363−408.

Amoabediny, G., Heli, B., Salehi-Nik, N., 2011. The Role of Biodegradable Engineered Scaffold in Tissue Engineering. INTECH Open Access Publisher.

Bakos, D., Soldan, M., Hernandez-Fuentes, I., 1999. Hydroxyapatite−collagen−hyaluronic acid composite. Biomaterials. 20, 191−195.

Becker, J., Lu, L., Runge, M.B., Zeng, H., Yaszemski, M.J., Dadsetan, M., 2015. Nanocomposite bone scaffolds based on biodegradable polymers and hydroxyapatite. J Biomed Mater Res A. 103, 2549–2557.

Bidic, S.M., Calvert, J.W., Marra, K., Kumta, P., Campbell, P., Mitchell, R., et al., 2003. Rabbit calvarial wound healing by means of seeded Caprotite scaffolds. J Dent Res. 82, 131–135.

Bose, S., Roy, M., Bandyopadhyay, A., 2012. Recent advances in bone tissue engineering scaffolds. Trends Biotechnol. 30, 546–554.

Boskey, A.L., 2003. Biomineralization: an overview. Connect Tissue Res. 44 (Suppl. 1), 5–9.

Burg, K.J., Porter, S., Kellam, J.F., 2000. Biomaterial developments for bone tissue engineering. Biomaterials. 21, 2347–2359.

Buurma, B., Gu, K., Rutherford, R.B., 1999. Transplantation of human pulpal and gingival fibroblasts attached to synthetic scaffolds. Eur J Oral Sci. 107, 282–289.

Cantara, S.I., Soscia, D.A., Sequeira, S.J., Jean-Gilles, R.P., Castracane, J., Larsen, M., 2012. Selective functionalization of nanofiber scaffolds to regulate salivary gland epithelial cell proliferation and polarity. Biomaterials. 33, 8372–8382.

Cheah, C.M., Chua, C.K., Leong, K.F., Cheong, C.H., Naing, M.W., 2004. Automatic algorithm for generating complex polyhedral scaffold structures for tissue engineering. Tissue Eng. 10, 595–610.

Christgau, M., Bader, N., Schmalz, G., Hiller, K.A., Wenzel, A., 1998. GTR therapy of intrabony defects using 2 different bioresorbable membranes: 12-month results. J Clin Periodontol. 25, 499–509.

Chua, C., Chou, S., Wong, T., 1998. A study of the state-of-the-art rapid prototyping technologies. Int. J. Adv. Manuf. Technol. 14, 146–152.

Coimbra, P., Alves, P., Valente, T.A., Santos, R., Correia, I.J., Ferreira, P., 2011a. Sodium hyaluronate/chitosan polyelectrolyte complex scaffolds for dental pulp regeneration: synthesis and characterization. Int J Biol Macromol. 49, 573–579.

Coimbra, P., Ferreira, P., De Sousa, H.C., Batista, P., Rodrigues, M.A., Correia, I.J., et al., 2011b. Preparation and chemical and biological characterization of a pectin/chitosan polyelectrolyte complex scaffold for possible bone tissue engineering applications. Int J Biol Macromol. 48, 112–118.

Cowan, C.M., Shi, Y.Y., Aalami, O.O., Chou, Y.F., Mari, C., Thomas, R., et al., 2004. Adipose-derived adult stromal cells heal critical-size mouse calvarial defects. Nat Biotechnol. 22, 560–567.

Dobie, K., Smith, G., Sloan, A.J., Smith, A.J., 2002. Effects of alginate hydrogels and TGF-beta 1 on human dental pulp repair in vitro. Connect Tissue Res. 43, 387–390.

Ebner, R., Lackner, J., Waldhauser, W., Major, R., Czarnowska, E., Kustosz, R., et al., 2006. Biocompatibile TiN-based novel nanocrystalline films. Tech. Sci.54.

El-Backly, R.M., Massoud, A.G., El-Badry, A.M., Sherif, R.A., Marei, M.K., 2008. Regeneration of dentine/pulp-like tissue using a dental pulp stem cell/poly(lactic-*co*-glycolic) acid scaffold construct in New Zealand white rabbits. Aust Endod J. 34, 52–67.

Friedman, C.D., Costantino, P.D., Takagi, S., Chow, L.C., 1998. BoneSource hydroxyapatite cement: a novel biomaterial for craniofacial skeletal tissue engineering and reconstruction. J Biomed Mater Res. 43, 428–432.

Galler, K.M., Cavender, A.C., Koeklue, U., Suggs, L.J., Schmalz, G., D'souza, R.N., 2011. Bioengineering of dental stem cells in a PEGylated fibrin gel. Regen Med. 6, 191–200.

Gao, J., Niklason, L., Langer, R., 1998. Surface hydrolysis of poly(glycolic acid) meshes increases the seeding density of vascular smooth muscle cells. J Biomed Mater Res. 42, 417–424.

Ge, Z., Yang, Q., Xiang, X., Liu, K.Z., 2012. Assessment of silk fibroin for the repair of buccal mucosa in a rat model. Int J Oral Maxillofac Surg. 41, 673−680.
Gentile, P., Chiono, V., Carmagnola, I., Hatton, P.V., 2014. An overview of poly(lactic-*co*-glycolic) acid (PLGA)-based biomaterials for bone tissue engineering. Int J Mol Sci. 15, 3640−3659.
Golinski, P.A., Groger, S., Herrmann, J.M., Bernd, A., Meyle, J., 2011. Oral mucosa model based on a collagen−elastin matrix. J Periodontal Res. 46, 704−711.
Gunatillake, P.A., Adhikari, R., 2003. Biodegradable synthetic polymers for tissue engineering. Eur Cell Mater. 5, 1−16, discussion 16.
Hall, H., Baechi, T., Hubbell, J.A., 2001. Molecular properties of fibrin-based matrices for promotion of angiogenesis in vitro. Microvasc Res. 62, 315−326.
Henslee, A.M., Gwak, D.H., Mikos, A.G., Kasper, F.K., 2012. Development of a biodegradable bone cement for craniofacial applications. J Biomed Mater Res A. 100, 2252−2259.
Hollister, S.J., 2005. Porous scaffold design for tissue engineering. Nat Mater. 4, 518−524.
Hollister, S.J., Lin, C.Y., Saito, E., Lin, C.Y., Schek, R.D., Taboas, J.M., et al., 2005. Engineering craniofacial scaffolds. Orthod Craniofac Res. 8, 162−173.
Hollister, S.J., Maddox, R.D., Taboas, J.M., 2002. Optimal design and fabrication of scaffolds to mimic tissue properties and satisfy biological constraints. Biomaterials. 23, 4095−4103.
Horst, O.V., Chavez, M.G., Jheon, A.H., Desai, T., Klein, O.D., 2012. Stem cell and biomaterials research in dental tissue engineering and regeneration. Dent Clin North Am. 56, 495−520.
Huang, Y.C., Kaigler, D., Rice, K.G., Krebsbach, P.H., Mooney, D.J., 2005. Combined angiogenic and osteogenic factor delivery enhances bone marrow stromal cell-driven bone regeneration. J Bone Miner Res. 20, 848−857.
Hutmacher, D.W., 2001. Scaffold design and fabrication technologies for engineering tissues—state of the art and future perspectives. J Biomater Sci Polym Ed. 12, 107−124.
Hutmacher, D.W., Sittinger, M., Risbud, M.V., 2004. Scaffold-based tissue engineering: rationale for computer-aided design and solid free-form fabrication systems. Trends Biotechnol. 22, 354−362.
Hyun, J.S., Montoro, D.T., Lo, D.D., Flynn, R.A., Wong, V., Chung, M.T., et al., 2013. The seed and the soil: optimizing stem cells and their environment for tissue regeneration. Ann Plast Surg. 70, 235−239.
Inanc, B., Arslan, Y.E., Seker, S., Elcin, A.E., Elcin, Y.M., 2009. Periodontal ligament cellular structures engineered with electrospun poly(DL-lactide-*co*-glycolide) nanofibrous membrane scaffolds. J Biomed Mater Res A. 90, 186−195.
Jafari, M., Paknejad, Z., Rad, M.R., Motamedian, S.R., Eghbal, M.J., Nadjmi, N., et al., 2015. Polymeric scaffolds in tissue engineering: a literature review. J Biomed Mater Res B Appl Biomater.
Joraku, A., Sullivan, C.A., Yoo, J., Atala, A., 2007. In-vitro reconstitution of three-dimensional human salivary gland tissue structures. Differentiation. 75, 318−324.
Khanarian, N.T., Haney, N.M., Burga, R.A., Lu, H.H., 2012. A functional agarose-hydroxyapatite scaffold for osteochondral interface regeneration. Biomaterials. 33, 5247−5258.
Khojasteh, A., Behnia, H., Hosseini, F.S., Dehghan, M.M., Abbasnia, P., Abbas, F.M., 2013. The effect of PCL−TCP scaffold loaded with mesenchymal stem cells on vertical bone augmentation in dog mandible: a preliminary report. J Biomed Mater Res B Appl Biomater. 101, 848−854.

Khojasteh, A., Motamedian, S.R., Rad, M.R., Shahriari, M.H., Nadjmi, N., 2015. Polymeric vs hydroxyapatite-based scaffolds on dental pulp stem cell proliferation and differentiation. World J Stem Cells. 7, 1215−1221.

Kim, B.S., Baez, C.E., Atala, A., 2000. Biomaterials for tissue engineering. World J Urol. 18, 2−9.

Kim, H., Suh, H., Jo, S.A., Kim, H.W., Lee, J.M., Kim, E.H., et al., 2005. In vivo bone formation by human marrow stromal cells in biodegradable scaffolds that release dexamethasone and ascorbate-2-phosphate. Biochem Biophys Res Commun. 332, 1053−1060.

Kim, N.R., Lee, D.H., Chung, P.H., Yang, H.C., 2009. Distinct differentiation properties of human dental pulp cells on collagen, gelatin, and chitosan scaffolds. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 108, e94-100.

Kim, T.G., Shin, H., Lim, D.W., 2012. Biomimetic scaffolds for tissue engineering. Adv Funct Mater. 22, 2446−2468.

Kinikoglu, B., Rodriguez-Cabello, J.C., Damour, O., Hasirci, V., 2011. The influence of elastin-like recombinant polymer on the self-renewing potential of a 3D tissue equivalent derived from human lamina propria fibroblasts and oral epithelial cells. Biomaterials. 32, 5756−5764.

Kleinman, H.K., Martin, G.R., 2005. Matrigel: basement membrane matrix with biological activity. Semin Cancer Biol. 15, 378−386.

Kokubo, T., Hanakawa, M., Kawashita, M., Minoda, M., Beppu, T., Miyamoto, T., et al., 2004. Apatite formation on non-woven fabric of carboxymethylated chitin in SBF. Biomaterials. 25, 4485−4488.

Krebsbach, P.H., Mankani, M.H., Satomura, K., Kuznetsov, S.A., Robey, P.G., 1998. Repair of craniotomy defects using bone marrow stromal cells. Transplantation. 66, 1272−1278.

Kumada, Y., Zhang, S., 2010. Significant type I and type III collagen production from human periodontal ligament fibroblasts in 3D peptide scaffolds without extra growth factors. PLoS ONE. 5, e10305.

Kweon, H., Yoo, M.K., Park, I.K., Kim, T.H., Lee, H.C., Lee, H.S., et al., 2003. A novel degradable polycaprolactone networks for tissue engineering. Biomaterials. 24, 801−808.

Langer, R., 2000. Biomaterials in drug delivery and tissue engineering: one laboratory's experience. Acc Chem Res. 33, 94−101.

Li, G., Zhang, T., Li, M., Fu, N., Fu, Y., Ba, K., et al., 2014. Electrospun fibers for dental and craniofacial applications. Curr Stem Cell Res Ther. 9, 187−195.

Li, J., Li, Y., Ma, S., Gao, Y., Zuo, Y., Hu, J., 2010. Enhancement of bone formation by BMP-7 transduced MSCs on biomimetic nano-hydroxyapatite/polyamide composite scaffolds in repair of mandibular defects. J Biomed Mater Res A. 95, 973−981.

Lin, C.Y., Kikuchi, N., Hollister, S.J., 2004. A novel method for biomaterial scaffold internal architecture design to match bone elastic properties with desired porosity. J Biomech. 37, 623−636.

Liu, X., Ma, P.X., 2004. Polymeric scaffolds for bone tissue engineering. Ann Biomed Eng. 32, 477−486.

Ma, P.X., Choi, J.W., 2001. Biodegradable polymer scaffolds with well-defined interconnected spherical pore network. Tissue Eng. 7, 23−33.

Mironov, V., Boland, T., Trusk, T., Forgacs, G., Markwald, R.R., 2003. Organ printing: computer-aided jet-based 3D tissue engineering. Trends Biotechnol. 21, 157−161.

Mizuno, M., Miyamoto, T., Wada, K., Watatani, S., Zhang, G.X., 2003. Type I collagen regulated dentin matrix protein-1 (Dmp-1) and osteocalcin (OCN) gene expression of rat dental pulp cells. J Cell Biochem. 88, 1112−1119.

Moharamzadeh, K., Colley, H., Murdoch, C., Hearnden, V., Chai, W.L., Brook, I.M., et al., 2012. Tissue-engineered oral mucosa. J Dent Res. 91, 642–650.
Mooney, D.J., Baldwin, D.F., Suh, N.P., Vacanti, J.P., Langer, R., 1996a. Novel approach to fabricate porous sponges of poly(D,L-lactic-*co*-glycolic acid) without the use of organic solvents. Biomaterials. 17, 1417–1422.
Mooney, D.J., Mazzoni, C.L., Breuer, C., Mcnamara, K., Hern, D., Vacanti, J.P., et al., 1996b. Stabilized polyglycolic acid fibre-based tubes for tissue engineering. Biomaterials. 17, 115–124.
Mooney, D.J., Powell, C., Piana, J., Rutherford, B., 1996c. Engineering dental pulp-like tissue in vitro. Biotechnol Prog. 12, 865–868.
Moroni, L., De Wijn, J.R., van Blitterswijk, C.A., 2008. Integrating novel technologies to fabricate smart scaffolds. J Biomater Sci Polym Ed. 19, 543–572.
Motamedian, S.R., Hosseinpour, S., Ahsaie, M.G., Khojasteh, A., 2015. Smart scaffolds in bone tissue engineering: a systematic review of literature. World J Stem Cells. 7, 657–668.
Muzzarelli, R.A., Biagini, G., Bellardini, M., Simonelli, L., Castaldini, C., Fratto, G., 1993. Osteoconduction exerted by methylpyrrolidinone chitosan used in dental surgery. Biomaterials. 14, 39–43.
Nam, Y.S., Yoon, J.J., Park, T.G., 2000. A novel fabrication method of macroporous biodegradable polymer scaffolds using gas foaming salt as a porogen additive. J Biomed Mater Res. 53, 1–7.
Neumann, A., Kevenhoerster, K., 2009. Biomaterials for craniofacial reconstruction. GMS Curr Top Otorhinolaryngol Head Neck Surg. 8, Doc08.
Ohara, T., Itaya, T., Usami, K., Ando, Y., Sakurai, H., Honda, M.J., et al., 2010. Evaluation of scaffold materials for tooth tissue engineering. J Biomed Mater Res A. 94, 800–805.
Oortgiesen, D.A., Plachokova, A.S., Geenen, C., Meijer, G.J., Walboomers, X.F., Den Beucken, J.J., et al., 2012. Alkaline phosphatase immobilization onto Bio-Gide® and Bio-Oss® for periodontal and bone regeneration. J Clin Periodontol. 39, 546–555.
Pan, Z., Ding, J., 2012. Poly (lactide-*co*-glycolide) porous scaffolds for tissue engineering and regenerative medicine. Interface Focus. 2, 366–377.
Patil, A.S., Merchant, Y., Nagarajan, P., 2013. Tissue engineering of craniofacial tissues–a review. J Regen Med Tissue Eng. 2, 6.
Petrovic, V., Zivkovic, P., Petrovic, D., Stefanovic, V., 2012. Craniofacial bone tissue engineering. Oral Surg Oral Med Oral Pathol Oral Radiol. 114, e1–e9.
Rezai-Rad, M., Bova, J.F., Orooji, M., Pepping, J., Qureshi, A., Del Piero, F., et al., 2015. Evaluation of bone regeneration potential of dental follicle stem cells for treatment of craniofacial defects. Cytotherapy. 17, 1572–1581.
Rosso, F., Marino, G., Giordano, A., Barbarisi, M., Parmeggiani, D., Barbarisi, A., 2005. Smart materials as scaffolds for tissue engineering. J Cell Physiol. 203, 465–470.
Roychowdhury, P., Kumar, V., 2006. Fabrication and evaluation of porous 2,3-dialdehydecellulose membrane as a potential biodegradable tissue-engineering scaffold. J Biomed Mater Res A. 76, 300–309.
Sachlos, E., Czernuszka, J.T., 2003. Making tissue engineering scaffolds work. Review: the application of solid freeform fabrication technology to the production of tissue engineering scaffolds. Eur Cell Mater. 5, 29–39, discussion 39–40.
Schnell, E., Klinkhammer, K., Balzer, S., Brook, G., Klee, D., Dalton, P., et al., 2007. Guidance of glial cell migration and axonal growth on electrospun nanofibers of poly-epsilon-caprolactone and a collagen/poly-epsilon-caprolactone blend. Biomaterials. 28, 3012–3025.

Schoof, H., Apel, J., Heschel, I., Rau, G., 2001. Control of pore structure and size in freeze-dried collagen sponges. J Biomed Mater Res. 58, 352–357.
Shang, S., Yang, F., Cheng, X., Walboomers, X.F., Jansen, J.A., 2010. The effect of electrospun fibre alignment on the behaviour of rat periodontal ligament cells. Eur Cell Mater. 19, 180–192.
Sherwood, J.K., Riley, S.L., Palazzolo, R., Brown, S.C., Monkhouse, D.C., Coates, M., et al., 2002. A three-dimensional osteochondral composite scaffold for articular cartilage repair. Biomaterials. 23, 4739–4751.
Simmons, C.A., Alsberg, E., Hsiong, S., Kim, W.J., Mooney, D.J., 2004. Dual growth factor delivery and controlled scaffold degradation enhance in vivo bone formation by transplanted bone marrow stromal cells. Bone. 35, 562–569.
Smith, I.O., Liu, X.H., Smith, L.A., Ma, P.X., 2009. Nanostructured polymer scaffolds for tissue engineering and regenerative medicine. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 1, 226–236.
Smith, M.K., Peters, M.C., Richardson, T.P., Garbern, J.C., Mooney, D.J., 2004. Locally enhanced angiogenesis promotes transplanted cell survival. Tissue Eng. 10, 63–71.
Song, J.H., Kim, H.E., Kim, H.W., 2007. Collagen-apatite nanocomposite membranes for guided bone regeneration. J Biomed Mater Res B Appl Biomater. 83, 248–257.
Suh, J.K., Matthew, H.W., 2000. Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review. Biomaterials. 21, 2589–2598.
Sumita, Y., Honda, M.J., Ohara, T., Tsuchiya, S., Sagara, H., Kagami, H., et al., 2006. Performance of collagen sponge as a 3-D scaffold for tooth-tissue engineering. Biomaterials. 27, 3238–3248.
Sundback, C.A., Shyu, J.Y., Wang, Y., Faquin, W.C., Langer, R.S., Vacanti, J.P., et al., 2005. Biocompatibility analysis of poly(glycerol sebacate) as a nerve guide material. Biomaterials. 26, 5454–5464.
Tabatabaei, F.S., Motamedian, S.R., Gholipour, F., Khosraviani, K., Khojasteh, A., 2012. Craniomaxillofacial bone engineering by scaffolds loaded with stem cells: a systematic review. JDS. 30, 113–130.
Taboas, J.M., Maddox, R.D., Krebsbach, P.H., Hollister, S.J., 2003. Indirect solid free form fabrication of local and global porous, biomimetic and composite 3D polymer-ceramic scaffolds. Biomaterials. 24, 181–194.
Tevlin, R., Mcardle, A., Atashroo, D., Walmsley, G.G., Senarath-Yapa, K., Zielins, E.R., et al., 2014. Biomaterials for craniofacial bone engineering. J Dent Res. 93, 1187–1195.
Thein-Han, W., Xu, H.H., 2011. Collagen-calcium phosphate cement scaffolds seeded with umbilical cord stem cells for bone tissue engineering. Tissue Eng Part A. 17, 2943–2954.
Tollemar, V., Collier, Z.J., Mohammed, M.K., Lee, M.J., Ameer, G.A., Reid, R.R., 2016. Stem cells, growth factors and scaffolds in craniofacial regenerative medicine. Genes Dis. 3, 56–71.
Tonomura, A., Mizuno, D., Hisada, A., Kuno, N., Ando, Y., Sumita, Y., et al., 2010. Differential effect of scaffold shape on dentin regeneration. Ann Biomed Eng. 38, 1664–1671.
Wei, G., Ma, P.X., 2004. Structure and properties of nano-hydroxyapatite/polymer composite scaffolds for bone tissue engineering. Biomaterials. 25, 4749–4757.
Weigel, T., Schinkel, G., Lendlein, A., 2006. Design and preparation of polymeric scaffolds for tissue engineering. Expert Rev Med Devices. 3, 835–851.
Weng, Y., Cao, Y., Silva, C.A., Vacanti, M.P., Vacanti, C.A., 2001. Tissue-engineered composites of bone and cartilage for mandible condylar reconstruction. J Oral Maxillofac Surg. 59, 185–190.

Williams, J.M., Adewunmi, A., Schek, R.M., Flanagan, C.L., Krebsbach, P.H., Feinberg, S.E., et al., 2005. Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering. Biomaterials. 26, 4817−4827.

Woo, K.M., Jun, J.H., Chen, V.J., Seo, J., Baek, J.H., Ryoo, H.M., et al., 2007. Nano-fibrous scaffolding promotes osteoblast differentiation and biomineralization. Biomaterials. 28, 335−343.

Xu, H., Han, D., Dong, J.S., Shen, G.X., Chai, G., Yu, Z.Y., et al., 2010. Rapid prototyped PGA/PLA scaffolds in the reconstruction of mandibular condyle bone defects. Int J Med Robot. 6, 66−72.

Yadav, V.S., Narula, S.C., Sharma, R.K., Tewari, S., Yadav, R., 2011. Clinical evaluation of guided tissue regeneration combined with autogenous bone or autogenous bone mixed with bioactive glass in intrabony defects. J Oral Sci. 53, 481−488.

Yamauchi, N., Yamauchi, S., Nagaoka, H., Duggan, D., Zhong, S., Lee, S.M., et al., 2011. Tissue engineering strategies for immature teeth with apical periodontitis. J Endod. 37, 390−397.

Yan, J., Li, J., Runge, M.B., Dadsetan, M., Chen, Q., Lu, L., et al., 2011. Cross-linking characteristics and mechanical properties of an injectable biomaterial composed of polypropylene fumarate and polycaprolactone co-polymer. J Biomater Sci Polym Ed. 22, 489−504.

Yang, C., Hillas, P.J., Baez, J.A., Nokelainen, M., Balan, J., Tang, J., et al., 2004. The application of recombinant human collagen in tissue engineering. BioDrugs. 18, 103−119.

Yang, K.C., Wang, C.H., Chang, H.H., Chan, W.P., Chi, C.H., Kuo, T.F., 2012a. Fibrin glue mixed with platelet-rich fibrin as a scaffold seeded with dental bud cells for tooth regeneration. J Tissue Eng Regen Med. 6, 777−785.

Yang, X., Han, G., Pang, X., Fan, M., 2012b. Chitosan/collagen scaffold containing bone morphogenetic protein-7 DNA supports dental pulp stem cell differentiation in vitro and in vivo. J Biomed Mater Res A.

Yang, X., Yang, F., Walboomers, X.F., Bian, Z., Fan, M., Jansen, J.A., 2010. The performance of dental pulp stem cells on nanofibrous PCL/gelatin/nHA scaffolds, J Biomed Mater Res A, 93. pp. 247−257.

Yeong, W.Y., Chua, C.K., Leong, K.F., Chandrasekaran, M., 2004. Rapid prototyping in tissue engineering: challenges and potential. Trends Biotechnol. 22, 643−652.

Yin, Y., Ye, F., Cui, J., Zhang, F., Li, X., Yao, K., 2003. Preparation and characterization of macroporous chitosan-gelatin/beta-tricalcium phosphate composite scaffolds for bone tissue engineering. J Biomed Mater Res A. 67, 844−855.

Yoshimoto, H., Shin, Y.M., Terai, H., Vacanti, J.P., 2003. A biodegradable nanofiber scaffold by electrospinning and its potential for bone tissue engineering. Biomaterials. 24, 2077−2082.

Young, C.S., Terada, S., Vacanti, J.P., Honda, M., Bartlett, J.D., Yelick, P.C., 2002. Tissue engineering of complex tooth structures on biodegradable polymer scaffolds. J Dent Res. 81, 695−700.

Yuan, Z., Nie, H., Wang, S., Lee, C.H., Li, A., Fu, S.Y., et al., 2011. Biomaterial selection for tooth regeneration. Tissue Eng Part B Rev. 17, 373−388.

Zaky, S.H., Cancedda, R., 2009. Engineering craniofacial structures: facing the challenge. J Dent Res. 88, 1077−1091.

Zhang, S., Huang, Y., Yang, X., Mei, F., Ma, Q., Chen, G., et al., 2009. Gelatin nanofibrous membrane fabricated by electrospinning of aqueous gelatin solution for guided tissue regeneration. J Biomed Mater Res A. 90, 671−679.

Zhang, X., Chang, W., Lee, P., Wang, Y., Yang, M., Li, J., et al., 2014. Polymer-ceramic spiral structured scaffolds for bone tissue engineering: effect of hydroxyapatite composition on human fetal osteoblasts. PLoS One. 9, e85871.

Zheng, L., Yang, F., Shen, H., Hu, X., Mochizuki, C., Sato, M., et al., 2011. The effect of composition of calcium phosphate composite scaffolds on the formation of tooth tissue from human dental pulp stem cells. Biomaterials. 32, 7053–7059.

Zuk, P.A., 2008. Tissue engineering craniofacial defects with adult stem cells? Are we ready yet? Pediatr Res. 63, 478–486.

Hydrogels in craniofacial tissue engineering

4

Ehsan Zahedi[1], *Sahar Ansari*[1], *Benjamin M. Wu*[1], *Sompop Bencharit*[2] *and Alireza Moshaverinia*[1]
[1]University of California, Los Angeles, CA, United States,
[2]Virginia Commonwealth University, Richmond, VA, United States

4.1 Introduction

The repair of damaged craniofacial tissues is often required due to tumors, trauma, and congenital malformations. This reconstruction process is challenging for clinicians because craniofacial region has a complex structure. The reconstructive procedures for craniofacial tissue regeneration are usually very complex as the craniofacial region is a complex construct itself, consisting of bone, cartilage, soft tissue, and neurovascular bundles. For instance, for the reconstruction of damaged craniofacial bones, an array of surgical procedures is available (Moshaverinia et al., 2012). Tissue engineering (TE) has evolved as a promising and interdisciplinary area of research aimed to develop new treatment modalities to repair and regenerate damaged or diseased tissues. Tissue regeneration contain three major components, namely scaffold, cells, and growth factors that are essential for tissue repair or regeneration. So, it is very important to seed cells (cell growth) onto a scaffold that exactly mimics the native microenvironment of the tissue (i.e., extracellular matrix, ECM). An increasing demand to regenerate tissues from patient-derived sources has led to the development of cell-based therapies using autologous stem cells, thereby decreasing immune rejection of scaffolds coupled with allogeneic stem cells or allografts. Adult stem cells are multipotent and are readily available in tissues such as fat and bone marrow (Malgieri et al., 2010). They possess the ability to repair and regenerate tissue through the production of therapeutic factors, particularly vasculogenic proteins (Leslie et al., 2017).

Scaffolds are mechanical structures that act as carriers for cells and growth factors. Preferably, a biomaterial is utilized in reconstructive surgery not only to replace the lost tissue but also to provide a conducive environment by acting as a scaffold for tissue regrowth. The major purpose of such a framework is to replicate the ECM, which leads to migration, cellular adhesion, proliferation, and differentiation of local progenitor stem cells (Bose et al., 2012).

Some essential biomechanical criteria of a scaffold includes biocompatibility, adequate mechanical strength, bioresorbability, and sufficient porosity. Biocompatibility of a scaffold is defined as its ability to support normal cellular activity, including molecular signaling systems, without any local and systemic toxic effects to the host tissue (Williams, 2008). Furthermore, two scaffolds must possess adequate mechanical

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00004-9
© 2017 Elsevier Ltd. All rights reserved.

stability to withstand the implantation procedure and the mechanical forces that are typically experienced at the scaffold-tissue interface, as well as resist collapse during a patient's normal activities. Dense bioceramic scaffold has a mechanical strength similar to that of cortical bone, while polymers are similar to cancellous bone. Ceramic-polymer scaffolds are typically weaker than cancellous bone but can confer advantages of increased biodegradability and flexibility, improving the brittleness of ceramic scaffolds (Wahl and Czernuszka, 2006; Zhang et al., 2014). In addition, the materials used as model ECM often need specific and reproducible elastic moduli that can be manipulated in a controllable manner (Schneider et al., 2006). Mechanical properties such as stiffness can be controlled (independently) by physical factors such as the cross-linking density, cross-linker type, and molecular weight (MW) distribution, as well as by chemical modification of the polymer (Peppas, 2004). Following implantation, a proper scaffold should degrade at a controlled rate, allowing room for ingrowth of new tissue. The degradation pattern of a scaffold should occur at an appropriate pace specific to the host tissue; for example, degradation occurring within 3−6 month would be acceptable in the craniofacial skeleton, where there is lower mechanical demand (Bose et al., 2012). Additionally, to facilitate diffusion of essential nutrients and oxygen for cell survival, interconnected channels are important feature of scaffold design. Pores should be at least 100 μm in diameter (Bose et al., 2012).

Numerous efforts were made to create biomaterials that can mimic ECM and finally hydrogels (e.g., polyethylene glycol, alginate) have emerged as one of the widely employed biomaterials for TE applications due to their potential to hold large amounts of water (like ECM) and their excellent resistance against the non-specific adsorption of proteins. In this chapter, we emphasis on the application of hydrogel biomaterial and stem cells for potential applications in craniofacial tissue regeneration.

4.2 Hydrogel biomaterials

Hydrogels formed by the cross-linking of hydrophilic polymers with a bridging agent, called a cross-linker. Depending on the type and concentration of the utilized cross-linker, they have a capability to absorb water but do not be dissolved. Cross-linking prevents dissolution while the hydrophilic backbones attract water molecules allowing water absorption (Annabi et al., 2014). Hydrogel biomaterials reach their stabilized swelling when a balance takes place between osmotic driving forces, which absorbs biological fluids into the hydrophilic hydrogel, and the cohesive forces provided by the polymer strands within the hydrogel. The latter prevents hydrogel expansion. The hydrogel cross-linking density determines these forces (Guenet, 1992).

Therefore they resemble natural soft tissue compared to any other type of biomaterial. This characteristic makes them an ideal option for biomedical applications like TE and drug delivery. The insoluble network provides potent immobilization and release of biomolecules. So hydrogels have the potential to be used as a matrix

Table 4.1 Advantages and disadvantages of hydrogel biomaterials for applications in tissue engineering.

	Advantages	Disadvantages
Hydrogels	• Biocompatible • Injectable in vivo as a liquid that gels at body temperature • Can transport nutrients to cells	• Usually poor mechanical properties • Hard to handle • Not easily can be sterilized

for TE and simulate ECM topography and holding growth factors to promote tissue regeneration (Geckil et al., 2010) (Table 4.1). They are proper choice for cell encapsulation due to high rate of biocompatibility and permeability to oxygen and other water-soluble metabolites/nutrients (Lee et al., 2008; Zhu, 2010). These injectable biomaterials can be delivered through minimally invasive means and they spontaneously mold to the shape of even the most complicated defects. This has important implications for reducing inflammatory side effects and subsequent scar formation stemming from invasive surgery and imprecise scaffold fit. Injectable biomaterials have been tested in the context of TE and may be appropriate for facilitating osteogenesis in craniofacial defects (Van Vlierberghe et al., 2011; Ni et al., 2014).

For the purpose of using hydrogels as scaffolds in TE, their mechanical characteristics are of high importance. It has been proved that ECM has a certain level of isometric tension between the cells in a tissue, which is variable according to tissue type. In addition, the response of cells to the tensions can be in a wide spectrum from form alterations to alterations in gene expression (Anseth et al., 1996). For this reason, hydrogel scaffolds may need to be designed with tissue specific mechanical characteristics. For instance, it is well-established that the differentiation of mesenchymal stem cells (MSCs) can be controlled by hydrogel stiffness (Lv et al., 2015). In this context the cross-linking density has a crucial role because it can make effects on cell encapsulation within hydrogel networks. Biomaterials utilized in scaffolds are divided into natural (e.g., dextran, chitosan), synthetic hydrogels (e.g., polylactic acid, polyglycolic acid) bioactive ceramics and glass (e.g., hydroxyapatite (HA), β-tricalcium phosphate), metals and composite scaffolds that are made up of 2 or more materials.

Biodegradable hydrogels have been considered as a popular scaffolds since they have controllable degradation, biocompatibility, and ease of processing (Yusop et al., 2012). These scaffolds are degraded by hydrolysis and gradually resorbed, allowing the supported tissue to gradually recover functionality. Biodegradable hydrogels can be divided into two categories: natural-including polysaccharides (e.g., chitosan) and proteins (e.g., collagen)—and synthetic, such as poly(lactic acid) (PLA). Synthetic biodegradable hydrogels can be produced under controlled conditions and therefore exhibit reproducible mechanical and physical properties (Ahmad et al., 2008).

Table 4.2 Classification of hydrogel biomaterials with examples for each category

Hydrogel Biomaterials	Natural hydrogels	Protein origin	Silk Collagen Fibrin Gelatin
		Polysaccharides origin	Alginate Dextran Agarose Chitosan Glycosaminoglycans
	Synthetic hydrogels	Polymer origin	Polylactic acid Polyglycolic acid Polypropylene fumarate
Hydrogels	**Advantages**		**Disadvantages**
Natural	High biocompatibility Intrinsic cellular interactions Biodegradable Cell controlled degradability Low toxicity byproducts		Mechanical strength Animal derived materials may pass on viruses
Synthetic	Precise control and mass produced Designable to meet specific requirements Low immunogenicity Minimize risk of biological pathogens or contaminants		Low biodegradability May include toxic substances

Natural and synthetic hydrogels (Table 4.2) are often used as frameworks for bone TE because of biodegradability, biocompatibility, porosity, and ease of handling (Chen et al., 2002; Ji et al., 2006). Naturally derived materials, such as collagen and fibrin proteins, or chitin-derived chitosan polysaccharide, are also an option for bone TE. Although such materials may grant greater cell adhesion and functional support properties than synthetic ones, but in most cases, they offer less control over mechanical properties, sometimes exhibit immunogenicity, and frequently exist in limited amounts. In fact, the major disadvantages of natural materials are poor mechanical properties as well as cell adhesion (Moshaverinia et al., 2015). Synthetic hydrogels, however, do not have such disadvantages and have been a more important source of biomaterials for osteoconductive purposes (Liu and Ma, 2004).

Hydrogel scaffolds could mimic mechanical characteristics of ECM. Cells may be suspended within or attached to the 3D network. By adding cell-binding motifs such as RGD (arginine-glycine-aspartic acid) adhesion peptide sequence, the rate of cellular attachment can be increased. It has been verified that RGD domains boost migration, proliferation, growth, and organization of cells for TE purposes (Shin et al., 2003).

4.2.1 Natural hydrogels

4.2.1.1 Dextran

Dextran is a nontoxic, hydrophilic homopolysaccharide, mainly composed of linear α-1,6-linked d-glucopyranose residues with a low percentage of α-1,2-, α-1,3-, and α-1,4-linked side chains. The biocompatibility of dextran has been well documented; thus dextran has been extensively explored in biomedical and pharmaceutical applications (Sun and Chu, 2006; Rodríguez-Velázquez et al., 2015; Mitra et al., 2001). Dextran has volume expansive properties and therefore its inclusion can improve blood flow (Dubniks et al., 2009). In addition, dextran has been largely used as coating material to protect and improve biocompatibility (Estevanato et al., 2012). Other polysaccharides, such as chitosan, alginate, and hyaluronic acid (HA) have various functional groups (e.g., amine and amide) but dextran only has hydroxyl groups, which do not support cell attachment. Some biopolymers, such as chitosan, possess functionality owing to their amine groups; chemical modification could convert some amine groups into other groups and thus change the molecule's initial functional characteristics.

4.2.1.2 Chitosan

Chitin and chitosan are naturally derived polymer and of special interest due to, as natural components of living structures, their biological and chemical similarities to natural tissues. They are biopolymers from renewable resources, obtainable from shells of shellfish, the wastes of the seafood industry. Their distinctive advantages include availability, biocompatibility, biodegradability, nontoxicity, antimicrobial properties, heavy metal ions chelation, gel forming properties, ease of chemical modification, and high affinity to proteins (Krajewska, 2005).

Chitosan-based scaffolds possess some special properties for use in TE. Chitosan can be formed as interconnected-porous structures by freezing and lyophilizing of chitosan solution or by processes such as an "internal bubbling process (IBP)" where $CaCO_3$ is added to chitosan solutions to generate chitosan−$CaCO_3$ gels in specific shapes by using suitable molds (Chow and Khor, 2000).

One of the properties of chitosan is that it confers considerable antibacterial activity against a broad spectrum of bacteria. It has been shown that chitosan can reduce the infection rate of experimentally induced osteomyelitis by *Staphylococcus aureus* in rabbits. The cationic nature of chitosan by amino group is related to anions on the bacterial cell wall. The interaction between positively charged chitosan and negatively charged microbial cell wall leads to the leakage of intracellular constituents (Aimin et al., 1999).

The field of wound healing has been another major emphasis in chitosan-based medical applications research. A number of researchers have examined the host tissue response to various chitosan-based implants. In general, these materials have been found to evoke a minimal foreign body reaction, with little or no fibrous encapsulation. The combination of chitosan with other materials appears to be a common theme in various reports. Blends with synthetic and natural polymers can

imbibe the wide range of physicochemical properties and processing techniques of synthetic polymers as well as the biocompatibility and biological interactions of natural polymers. Huang et al. (2005) blended chitosan with gelatin to improve the biological activity since (1) gelatin contains Arg-Gly-Asp (RGD)-like sequence that promotes cell adhesion and migration, and (2) forms a polyelectrolyte complex. Addition of gelatin-affected the stiffness of 2D and 3D scaffolds, facilitated the degradation rate and maintained the dimension in the presence of lysozyme (Huang et al., 2005).

4.2.1.3 Collagen

Collagen is a fibrous protein with helical structure which provides mechanical support to the connective tissue and responsible for cell distribution and capillary formation. There are three major collagen types: type I (skin-bone), type II (cartilage), and type III (walls of blood vessels). A frequent way for making porous collagen scaffold is freeze-drying after suspending collagen in the presence of salt crystals (O'Brien et al., 2005; Zhong et al., 2007).

4.2.1.4 Glycosaminoglycans

GAGAs are extensively found in the ECM. It has been proved that combining collagen and GAG can form nanofibrous scaffold. They are long unbranched chain of disaccharide units with carboxylic and/or sulfate ester groups. These groups could connect collagens and make networks. HA, is a biocompatible, nonimmunogenic anionic GAGA that is found in connective tissues, synovial fluid, and the ECM of cartilage (Burdick and Prestwich, 2011). HA is an important part of the ECM and has a crucial role in wound healing, cell signaling, angiogenesis, and matrix organizations (Allison and Grande-Allen, 2006). However, HA could be degraded by hyaluronidase in vivo. But by attaching synthetic materials, the durability, toughness, and strength could be raised (Fisher et al., 2015). There are few reports of encapsulating cells by HA because they do not have cell-binding sites (Lam et al., 2014).

4.2.1.5 Silk

Silks are fibrous proteins that are made as a fiber by silkworms, spiders, and other insects. Specifically, silks from *Bombyx mori* silkworms have been investigated for use as biomaterials for many different implementations. The outer filament proteins of silk (sericins) coat the inner fibroin brins. The sericins are removed by washing the silk filament in water because they may induce early inflammatory process. There are various biomaterials from silk such as gels, sponges, and nets that has been proposed for medical applications. Silk has inner fibroin brins that has proper biocompatibility and could support the growth of human cells (Motta et al., 2002a,b).

4.2.1.6 Agarose

Agarose is a polysaccharide polymer extracted from algae. It is very soluble in water due to a plenty of hydroxyl groups. They can be degraded by agarases and the concentration of the gel controls permeability and strength. Agarose is popular in tissue culture systems because it allows cells to grow inside the three-dimensional network (Rahfoth et al., 1998).

4.2.1.7 Gelatin

Gelatin is a biopolymer that can be derived from fish, insects, and the skin of land animals. This soluble protein is made through an irreversible process of partial hydrolysis of collagen (Zogbi and Pignatello, 2011). Physically (Helminger et al., 2014) and chemically (Lee et al., 2014) cross-linking methods can make gelatin hydrogels. Short degradation rates, poor mechanical properties, and lack of thermal stability are main disadvantages of gelatin hydrogels (Tsang et al., 2015) (Table 4.3).

4.2.1.8 Alginate

Alginates are polysaccharides isolated from brown algae such as laminaria hyperborea and lessonia found in coastal waters around the globe. They belong to a family of linear block polyanionic copolymers composed of (1-4)-linked-d-mannuronic acid (M units) and (1−4)-linked-l-guluronic acid (G units) residues (Zhang et al., 2009; Mitrano et al., 2010). It forms an ionic network in the presence of divalent or multivalent ions. Alginate microbeads are fabricated by dropping the cell-alginate solution into a calcium chloride solution.

Table 4.3 The advantages and disadvantages of two popular natural hydrogel biomaterials

Natural hydrogels	Advantages	Disadvantages
Alginate	• Biocompatible biodegradable (without lowering PH) • Gentile gelation • Hydrophilicity • Low cost • Easy handling (attractive feature for TE) • Highly hydrated tissue-like environment (encourage cell viability) • Prolonged shelf life	• Unstable mechanical • Properties • Lack of the specific cell-recognition signals • Limited absorption ability of serum proteins
Gelatin	• Bioresorbable • Biocompatible • Rapid degradation • Better solubility and less antigenicity • (compared to collagen)	• Poor mechanical properties • Short degradation rates • Lack of thermal stability

As a biomaterial, alginate has a number of advantageous features including biocompatibility and nonimmunogenicity and they are likely related to its hydrophilicity (Shapiro and Cohen, 1997).

Alginate and agarose provide little control over the gelation process, particularly in a clinical setting. Once cross-linking is induced, by the addition of an ionic solution or a temperature change, the process cannot be stopped or accelerated. Therefore the need for a new biomaterial or method for cell encapsulation that provides control over gel formation and shape maintenance led to the development of the photopolymerization system for TE applications (Elisseeff et al., 1999).

Generally, by increasing the concentration of polymer, the stiffness will be increased, especially in high-MW alginates (LeRoux et al., 1999). They can also be reinforced mechanically by forming polyelectrolyte complexes. The addition of cationic poly(ethyleneimine) (PEI) to alginate can ameliorate its mechanical properties (Kong and Mooney, 2003).

Gelling conditions and type of cross-linker can alter mechanical properties. Low temperatures reduce the diffusion rate of Ca^{2+} ions, which leads to slower cross-linking, a more ordered network structure and improves mechanical properties (Drury et al., 2004). Irrespective of the cross-linking method, alginate gels can keep their initial size and shape (Rowley and Mooney, 2002).

The degradation rate of alginate gels can be controlled by gamma-irradiation (Lee et al., 2003). Partial oxidation is another way because alginate can be hydrolyzed via reaction with sodium periodate (Bouhadir et al., 2001).

Since alginate gels do not promote cell attachment, they have to be covalently modified to provide significant attachment (Lansdown and Payne, 1994). One method to furnish such an adhesion is binding cellular adhesion molecules like laminin (Dhoot et al., 2004), fibronectin (Mosahebi et al., 2003), and collagen (Augst et al., 2006) to alginate but they can make nonspecific interactions. Some type of short amino acid sequences that are present in ECM molecules can provide such an adhesion instead. The fibronectin-derived adhesion peptide arginine-glycine-aspartic acid (RGD) is commonly used (Ruoslahti, 1996).

Presence of cell-binding peptides, such as RGD (arginine-glycine-aspartic acid tripeptide), in the structure of the alginate scaffold could be advantageous because these peptides mimic the cell–matrix interaction typical of the ECM (Moshaverinia et al., 2013). Studies have shown that alginate hydrogels have multiple growth factor delivery capacity, which make the scaffold of choice for complex tissue regeneration purposes (Borselli et al., 2011; Moshaverinia et al., 2015).

Encapsulation of various substances with minimal trauma which results of gentle gelling behavior is the major advantage of alginate (Klöck et al., 1997). A chemically modified alginate is currently used clinically to deliver proteins with the purpose of promoting regeneration of mineralized tissue (Bratthall et al., 2001) and as a vehicle for transplanted cells (Augst et al., 2006). Alginate hydrogel can serve as a scaffold for bone TE. However, it has several disadvantages such as poor mechanical properties, uncontrollable degradation, and lack of cellular interactions, which limits its biomedical applications (Masuda et al., 2003). Nevertheless, it is possible to control and characterize their biodegradation profile via oxidation process.

Also, different strengthening additives such as HA bioceramics have been added to make a composite material (Bouhadir and Mooney, 2002).

Alginate is soft in nature and mimic the characteristics of the ECM. The variation of compression modulus of alginate is between 1 and 1000 kPa with a shear modulus from 0.02 to 40 kPa that is much less than collagen and elastin (Drury et al., 2004). Source of the polymer, processing procedure, concentration, cross-linking ion, and environment could determine the mechanical properties of alginate (Drury and Mooney, 2003). Usually, G-rich alginate is more powerful than M-rich alginate (Kandalam et al., 2012). The most notable characteristic of alginate is the sol−gel conversion, which is controlled by the accessible free divalent ion. Increasing the concentration of calcium can enhance more cross-links bonds inside the network and finally makes the alginate more tough (Wan et al., 2008).

Alginate lacks cell-binding motifs. Therefore it requires chemical modification for efficient use in TE utilizations. By adding RGD domains the cell attachment and proliferation could be upgraded within the microarchitecture. According to a study, osteogenic markers genes ALP and RUNX-2 have detected more when RGD-coupled alginate hydrogel entrap MSCs (Wan et al., 2008).

Various hydrogels have been developed for several purposes. Their applications as cell transplantation vehicles, drug delivery systems, and scaffold are discussed in this section.

Alginate hydrogels could be widely useful for the sustained and localized delivery of traditional low-MW drugs and macromolecules for tissue regeneration. The traditional drug delivery systems are poor in targeting the tissue of interest, which can lead to side effects and low effectiveness (Mazué et al., 1993). Delivery of a small amount of drug through controllable fashion from an encapsulated polymer is favorable to offer high concentrations of drug over a sustained period of time with minimal side effects at other sites (Langer, 2000).

The release of proteins from hydrogels can significantly enhance their efficacy and targeting. Growth factors can foster or hinder cell differentiation (Li et al., 2006). Proliferation and/or tissue growth (Richmon et al., 2005), and vascularization (Schumacher et al., 1998). Protein drugs have to be injected in high doses because they degrade rapidly. So, localized delivery of drugs by polymers can guarantee sustained local exposure without any side effects (Laham et al., 1999).

Alginate gels are used as cell delivery carriers in TE to provide transplanted cells in a localized site and control over their fate (Atala and Koh, 2005). The addition of adhesion molecules to alginate gels can help to signal cells in the gel and affect their function. RGD−alginate gels significantly enhance bone formation in vivo by transplanted calvarial osteoblasts (Alsberg et al., 2001). Most tissue types consist of more than one cell type, whose interactions may be crucial to tissue formation and regeneration (Alsberg et al., 2002). The exact mechanisms that control this process are still poorly understood but may be linked to differential adhesion or response to chemotactic gradients.

One of the most recent advances in TE is using of microencapsulation systems for drug delivery devices. Hydrogels and specially alginate provide the free exchange of oxygen and nutrients between the entrapped cell and their environment whilst keeping the cell in it's place.

4.2.2 Synthetic hydrogels

Synthetic hydrogels are made on a large scale. They can make fine control over mechanical and physical properties and have a well-documented history of clinical application in craniofacial bone reconstruction, especially in children. Synthetic polymers like PLA, poly(glycolic acid) (PGA), and various iterations of combined poly(lactic-co-glycolic acid) (PLGA) have been used for a range of clinical applications, including critical-sized craniofacial defect repair (Yusop et al., 2012; Caballero et al., 2015).

4.2.2.1 Polylactic acid

PLA is an FDA-approved synthetic biomaterial that has several properties conducive to bone TE, including controllable biodegradation rate, biocompatibility, and good mechanical strength (Lee et al., 2001). However, it's poor osteoconductive properties have made it a poor candidate as a framework for craniofacial bone (Zhang et al., 2015).

4.2.2.2 Polyglycolic acid

PGA is another FDA-approved synthetic biomaterial with a variety of TE applications but its softness and inability to hold space have made it the last option for craniofacial defect repair. PGA and PLA alone are not proper frameworks, but their respective softness and low osteoinductivity have been partially addressed by combining them to form a PLGA composite scaffold (Mooney et al., 1996). PLGA has been shown to have a controllable degradation rate (through varying composition of its constituent homopolymers) in addition to supporting osteoblast attachment, growth, and differentiation both in vitro and in vivo (Liu and Ma, 2004; Mooney et al., 1996; Ishaug-Riley, 1997; Vacanti and Vacanti, 1994; Gentile et al., 2014). Nevertheless, PLGA's mechanical properties and osteoconductivity do not meet requirements for bone TE, and it is most often used as part of a composite material with ceramics, bioglass, or other more osteoconductive materials (Gentile et al., 2014; Pan and Ding, 2012).

4.2.2.3 Polypropylene fumarate

Polypropylene fumarate (PPF) is a synthetic, unsaturated, linear polyester polymer that is biodegradable, biocompatible, osteoconductive, injectable, and sufficiently strong for craniofacial bone TE (Henslee et al., 2012; Hedberg et al., 2005). A two-phase PPF cement incorporating cross-linked microparticles to increase strength and lower setting temperature has been developed. This PPF-based system has improved injectability, setting temperature, and setting time over clinically available polymethyl methacrylate bone cement and is believed to be suitable for application in craniofacial bone regeneration. PPF has also been used as a copolymer with polycaprolactone (PCL) as a scaffold for osteoblastic differentiation and maturation in vitro (Becker et al., 2015).

Polyamide (PA) is a synthetic polymeric collagen analog that provides excellent strength as well as biocompatibility. Those properties have made PA a promising partner for HA or other bioceramics in osteoconductive composite scaffolds. As a BMP-7-transduced MSC-laden composite with HA nanoparticles, PA has been successfully used to repair mandibular defects in rabbits (Li et al., 2010). PCL is a polyester polymer that has been used as TE scaffold material, especially for bone (Van Aalst et al., 2005). It is biocompatible, possesses excellent mechanical strength and compared with PLA, PGA, or PLGA, has a much longer degradation time, lasting up to 3 years (Engstrand et al., 2014). This feature could make PCL a proper scaffold material for cartilage TE in facial reconstructive surgery.

4.2.2.4 Polyethylene glycol

Polyethylene glycol (PEG) is a synthetic material with major characteristics of biocompatibility, water solubility, and low cost (Lutz and Hoth, 2006). It has numerous applications such as drug delivery and wound dressing (Zander et al., 2015; Ni et al., 2014) but could not support cell adhesion and proliferation. By adding cell-binding motifs like RGD peptides into the PEG hydrogel network, cell adhesion can be considered as a crucial feature of PEG-based hydrogel (Lee et al., 2015; Caiazzo et al., 2016).

4.2.2.5 Polyacrylamide

Polyacrylamide (PAM)-based hydrogel has many applications in the biomedical fields, drug delivery, and biosensor fluids in recent years (Tsou et al., 2016). Contact lense is the most important field that PAM has been used due to its bioinert and hydrophilic properties (Darnell et al., 2013; Fernández et al., 2005). It has low toxicity and poor cell adhesion. Therefore the incorporation with natural materials (e.g., alginate, collagen) and conjugation with peptides like RGD are the prerequisite steps before making any PAM-based hydrogels (Wang et al., 2014).

4.2.2.6 GelMA

Many of the available hydrogels have poor mechanical properties, cell binding, and viability. Collagen has been used to synthesize cell-laden microgels, but providing abiding micropatterns is still remained a big problem due to deficient mechanical strength. As discussed previously, incorporating the binding sequence Arg-Gly-Asp (RGD) has been proved that can ameliorate cell binding but if cells are unable to degrade the 3D network of hydrogel, the arrangement and movement of cells would be limited (Hedberg et al., 2005).

A lot of alternative biomaterials have been advanced for specific biomedical applications such as hydrogels made from both synthetic (e.g., poly(vinyl alcohol), poly(ethylene oxide), and poly(-acrylic acid)) and naturally arisen (e.g., alginate, agarose, and chitosan) polymers (Becker et al., 2015). One promising alternative hydrogel is GelMA, that is produced by incorporating methacrylate groups to the amine-containing side-groups of gelatin, leading to a photocrosslinkable material

that is stable at 37°C and provides long-term cell viability (Estevanato et al., 2012). It contains gelatin that can furnish proper cell adhesion sites and biodegradation through proteolysis (Becker et al., 2015).

Gelatin is inexpensive, denatured collagen that can be obtained from various sources. It can keep cell binding motifs, such as RGD and MMP-sensitive degradation sites (Vacanti and Vacanti, 1994; Gentile et al., 2014). In addition, the presence of carboxylic acid, thiol and hydroxyl in GelMA permits covalent binding of cytokines or growth factors with GelMA for better function. These binding sites are distributed on all polymer chains. Therefore adhesion, proliferation, and migration of cells on the surface of micropatterns can easily be done within the 3D network. The mechanical stability and potential of controlled shaping of microgels in a system containing Gela increased compared to collagen. By controlling the degree of methacrylation and gel concentration a wide range of mechanical properties for various implementations can be utilized.

Generally speaking, GelMA owns proper characteristics to consider as TE scaffolds because it is biocompatible, biodegradable, noncytotoxic, and nonimmunogenic it has been well established that various cells can bind and grow on the surface of GelMA substrates and can be encapsulated within the GelMA hydrogel matrixes with excellent viability (Peppas, 2004; Gentile et al., 2014).

4.3 Summary: current status and future prospects

Application of hydrogel biomaterials (natural and synthetic) in TE presents an immense opportunity for the entire medical field. These biomaterials are the attractive candidates for stem cell therapies and drug and growth factor delivery applications. The applications of hydrogel-delivered MSCs for craniofacial tissue regeneration are wide, ranging from regenerating bone defects due to oral disease to tissue reconstruction when the patient prefers not to have bone graft. The advantages of application of hydrogel microencapsulation system are simplicity, injectability, and biodegradability, yielding a three-dimensional, cell delivery scaffold for craniofacial TE. However, these applications do not come without the inherent challenges associated with new clinical procedures.

References

Ahmad, N., Lyles, J., Panchal, J., 2008. Outcomes and complications based on experience with resorbable plates in pediatric craniosynostosis patients. J. Craniofac. Surg. 19, 855–860.

Aimin, C., Chunlin, H., Juliang, B., Tinyin, Z., Zhichao, D., 1999. Antibiotic loaded chitosan bar: an in vitro, in vivo study of a possible treatment for osteomyelitis. Clin. Orthopaed. Related Res. 366, 239–247.

Allison, D.D., Grande-Allen, K.J., 2006. Review. Hyaluronan: a powerful tissue engineering tool. Tissue Eng. 12, 2131–2140.

Alsberg, E., Anderson, K.W., Albeiruti, A., Rowley, J.A., Mooney, D.J., 2002. Engineering growing tissues. Proc. Natl. Acad. Sci. 99, 12025–12030.

Alsberg, E., Anderson, K., Albeiruti, A., Franceschi, R., Mooney, D., 2001. Cell-interactive alginate hydrogels for bone tissue engineering. J. Dent. Res. 80, 2025–2029.

Annabi, N., Tamayol, A., Uquillas, J.A., Akbari, M., Bertassoni, L.E., Cha, C., et al., 2014. 25th anniversary article: rational design and applications of hydrogels in regenerative medicine. Adv. Mater. 26, 85–124.

Anseth, K.S., Bowman, C.N., Brannon-Peppas, L., 1996. Mechanical properties of hydrogels and their experimental determination. Biomaterials. 17, 1647–1657.

Atala, A., Koh, C., 2005. Applications of tissue engineering in the genitourinary tract. Expert Rev. Med. Dev. 2, 119–126.

Augst, A.D., Kong, H.J., Mooney, D.J., 2006. Alginate hydrogels as biomaterials. Macromol. Biosci. 6, 623–633.

Becker, J., Lu, L., Runge, M.B., Zeng, H., Yaszemski, M.J., Dadsetan, M., 2015. Nanocomposite bone scaffolds based on biodegradable polymers and hydroxyapatite. J. Biomed. Mater. Res. A. 103, 2549–2557.

Borselli, C., Cezar, C.A., Shvartsman, D., Vandenburgh, H.H., Mooney, D.J., 2011. The role of multifunctional delivery scaffold in the ability of cultured myoblasts to promote muscle regeneration. Biomaterials. 32, 8905–8914.

Bose, S., Roy, M., Bandyopadhyay, A., 2012. Recent advances in bone tissue engineering scaffolds. Trends Biotechnol. 30, 546–554.

Bouhadir, K., Mooney, D., 2002. Synthesis of hydrogels: alginate hydrogels. In: Atala, A., Lanza, R.P. (Eds.), in Methods Tissue Eng. 1. Academic Press, pp. 653–662.

Bouhadir, K.H., Lee, K.Y., Alsberg, E., Damm, K.L., Anderson, K.W., Mooney, D.J., 2001. Degradation of partially oxidized alginate and its potential application for tissue engineering. Biotechnol. Prog. 17, 945–950.

Bratthall, G., Lindberg, P., Havemose-Poulsen, A., Holmstrup, P., Bay, L., Söderholm, G., et al., 2001. Comparison of ready-to-use EMDOGAIN®-gel and EMDOGAIN® in patients with chronic adult periodontitis. J. Clin. Periodontol. 28, 923–929.

Burdick, J.A., Prestwich, G.D., 2011. Hyaluronic acid hydrogels for biomedical applications. Adv. Mater. 23, H41–H56.

Caballero, M., Morse, J.C., Halevi, A.E., Emodi, O., Pharaon, M.R., Wood, J.S., et al., 2015. Juvenile swine surgical alveolar cleft model to test novel autologous stem cell therapies. Tissue Eng. C Methods. 21, 898–908.

Caiazzo, M., Okawa, Y., Ranga, A., Piersigilli, A., Tabata, Y., Lutolf, M.P., 2016. Defined three-dimensional microenvironments boost induction of pluripotency. Nat. Mater. 15, 344–352.

Chen, G., Ushida, T., Tateishi, T., 2002. Scaffold design for tissue engineering. Macromol. Biosci. 2, 67–77.

Chow, K.S., Khor, E., 2000. Novel fabrication of open-pore chitin matrixes. Biomacromolecules. 1, 61–67.

Darnell, M.C., Sun, J.-Y., Mehta, M., Johnson, C., Arany, P.R., Suo, Z., et al., 2013. Performance and biocompatibility of extremely tough alginate/polyacrylamide hydrogels. Biomaterials. 34, 8042–8048.

Dhoot, N.O., Tobias, C.A., Fischer, I., Wheatley, M.A., 2004. Peptide-modified alginate surfaces as a growth permissive substrate for neurite outgrowth. J. Biomed. Mater. Res. A. 71, 191–200.

Drury, J.L., Mooney, D.J., 2003. Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials. 24, 4337–4351.

Drury, J.L., Dennis, R.G., Mooney, D.J., 2004. The tensile properties of alginate hydrogels. Biomaterials. 25, 3187−3199.

Dubniks, M., Persson, J., Grände, P.-O., 2009. Comparison of the plasma volume-expanding effects of 6% dextran 70, 5% albumin, and 6% HES 130/0.4 after hemorrhage in the guinea pig. J. Trauma Acute Care Surg. 67, 1200−1204.

Elisseeff, J., Anseth, K., Sims, D., Mcintosh, W., Randolph, M., Langer, R., 1999. Transdermal photopolymerization for minimally invasive implantation. Proc. Natl. Acad. Sci. 96, 3104−3107.

Engstrand, T., Kihlström, L., Neovius, E., Skogh, A.-C.D., Lundgren, T.K., Jacobsson, H., et al., 2014. Development of a bioactive implant for repair and potential healing of cranial defects: technical note. J. Neurosurg. 120, 273−277.

Estevanato, L.L., Lacava, L.M., Carvalho, L.C., Azevedo, R.B., Silva, O., Pelegrini, F., et al., 2012. Long-term biodistribution and biocompatibility investigation of dextran-coated magnetite nanoparticle using mice as the animal model. J. Biomed. Nanotechnol. 8, 301−308.

Fernández, E., López, D., López-Cabarcos, E., Mijangos, C., 2005. Viscoelastic and swelling properties of glucose oxidase loaded polyacrylamide hydrogels and the evaluation of their properties as glucose sensors. Polymer. 46, 2211−2217.

Fisher, S.A., Anandakumaran, P.N., Owen, S.C., Shoichet, M.S., 2015. Tuning the microenvironment: click-crosslinked hyaluronic acid-based hydrogels provide a platform for studying breast cancer cell invasion. Adv. Funct. Mater. 25, 7163−7172.

Geckil, H., Xu, F., Zhang, X., Moon, S., Demirci, U., 2010. Engineering hydrogels as extracellular matrix mimics. Nanomedicine. 5, 469−484.

Gentile, P., Chiono, V., Carmagnola, I., Hatton, P.V., 2014. An overview of poly (lactic-co-glycolic) acid (PLGA)-based biomaterials for bone tissue engineering. Int. J. Mol. Sci. 15, 3640−3659.

Guenet, J.-M., 1992. Thermoreversible Gelation of Polymers and Biopolymers. Academic Press, London.

Hedberg, E.L., Kroese-Deutman, H.C., Shih, C.K., Crowther, R.S., Carney, D.H., Mikos, A. G., et al., 2005. In vivo degradation of porous poly (propylene fumarate)/poly (DL-lactic-co-glycolic acid) composite scaffolds. Biomaterials. 26, 4616−4623.

Helminger, M., Wu, B., Kollmann, T., Benke, D., Schwahn, D., Pipich, V., et al., 2014. Synthesis and characterization of gelatin-based magnetic hydrogels. Adv. Funct. Mater. 24, 3187−3196.

Henslee, A.M., Gwak, D.H., Mikos, A.G., Kasper, F.K., 2012. Development of a biodegradable bone cement for craniofacial applications. J. Biomed. Mater. Res. A. 100, 2252−2259.

Huang, Y., Onyeri, S., Siewe, M., Moshfeghian, A., Madihally, S.V., 2005. In vitro characterization of chitosan−gelatin scaffolds for tissue engineering. Biomaterials. 26, 7616−7627.

Ishaug-Riley, S.L., 1997. Bone Formation by Three-Dimensional Osteoblast Culture in Biodegradable Poly(Alpha-Hydroxy Ester) Scaffolds. Rice University, Houston, TX.

Ji, Y., Ghosh, K., Shu, X.Z., Li, B., Sokolov, J.C., Prestwich, G.D., et al., 2006. Electrospun three-dimensional hyaluronic acid nanofibrous scaffolds. Biomaterials. 27, 3782−3792.

Kandalam, U., Cabel, A.I., Omidian, H., Stelnicki, E.J., 2012. Viability of human umbilical cord-derived mesenchymal stem cells in G-rich and M-rich alginates. J. Bioactive Compatible Polym. 27, 174−182.

Klöck, G., Pfeffermann, A., Ryser, C., Gröhn, P., Kuttler, B., Hahn, H.-J., et al., 1997. Biocompatibility of mannuronic acid-rich alginates. Biomaterials. 18, 707−713.

Kong, H.J., Mooney, D.J., 2003. The effects of poly (ethyleneimine)(PEI) molecular weight on reinforcement of alginate hydrogels. Cell Transplant. 12, 779−785.

Krajewska, B., 2005. Membrane-based processes performed with use of chitin/chitosan materials. Sep. Purif. Technol. 41, 305−312.

Laham, R.J., Sellke, F.W., Edelman, E.R., Pearlman, J.D., Ware, J.A., Brown, D.L., et al., 1999. Local perivascular delivery of basic fibroblast growth factor in patients undergoing coronary bypass surgery. Circulation. 100, 1865−1871.

Lam, J., Lowry, W.E., Carmichael, S.T., Segura, T., 2014. Delivery of iPS-NPCs to the stroke cavity within a hyaluronic acid matrix promotes the differentiation of transplanted cells. Adv. Funct. Mater. 24, 7053−7062.

Langer, R., 2000. Biomaterials in drug delivery and tissue engineering: one laboratory's experience. Acc. Chem. Res. 33, 94−101.

Lansdown, A., Payne, M., 1994. An evaluation of the local reaction and biodegradation of calcium sodium alginate (Kaltostat) following subcutaneous implantation in the rat. J. R. College Surg. Edinburgh. 39, 284−288.

Lee, D.W., Choi, W.S., Byun, M.W., Park, H.J., Yu, Y.-M., Lee, C.M., 2003. Effect of γ-irradiation on degradation of alginate. J. Agric. Food Chem. 51, 4819−4823.

Lee, J., Cuddihy, M.J., Kotov, N.A., 2008. Three-dimensional cell culture matrices: state of the art. Tissue Eng. B Rev. 14, 61−86.

Lee, S.H., Lee, Y., Chun, Y.W., Crowder, S.W., Young, P.P., Park, K.D., et al., 2014. In situ crosslinkable gelatin hydrogels for vasculogenic induction and delivery of mesenchymal stem cells. Adv. Funct. Mater. 24, 6771−6781.

Lee, S.J., Park, Y.J., Park, S.N., Lee, Y.M., Seol, Y.J., Ku, Y., et al., 2001. Molded porous poly (L-lactide) membranes for guided bone regeneration with enhanced effects by controlled growth factor release. J. Biomed. Mater. Res. A. 55, 295−303.

Lee, T.T., García, J.R., Paez, J.I., Singh, A., Phelps, E.A., Weis, S., et al., 2015. Light-triggered in vivo activation of adhesive peptides regulates cell adhesion, inflammation and vascularization of biomaterials. Nat. Mater. 14, 352−360.

Leroux, M.A., Guilak, F., Setton, L.A., 1999. Compressive and shear properties of alginate gel: effects of sodium ions and alginate concentration. J. Biomed. Mater. Res. 47, 46−53.

Leslie, S.K., Kinney, R.C., Schwartz, Z., Boyan, B.D., 2017. Microencapsulation of stem cells for therapy. Cell microencapsulation: methods and protocols. Methods Mol. Biol. 1479, 251−259.

Li, C., Vepari, C., Jin, H.-J., Kim, H.J., Kaplan, D.L., 2006. Electrospun silk-BMP-2 scaffolds for bone tissue engineering. Biomaterials. 27, 3115−3124.

Li, J., Li, Y., Ma, S., Gao, Y., Zuo, Y., Hu, J., 2010. Enhancement of bone formation by BMP-7 transduced MSCs on biomimetic nano-hydroxyapatite/polyamide composite scaffolds in repair of mandibular defects. J. Biomed. Mater. Res. A. 95, 973−981.

Liu, X., Ma, P.X., 2004. Polymeric scaffolds for bone tissue engineering. Ann. Biomed. Eng. 32, 477−486.

Lutz, J.-F., Hoth, A., 2006. Preparation of ideal PEG analogues with a tunable thermosensitivity by controlled radical copolymerization of 2-(2-methoxyethoxy) ethyl methacrylate and oligo (ethylene glycol) methacrylate. Macromolecules. 39, 893−896.

Lv, H., Li, L., Sun, M., Zhang, Y., Chen, L., Rong, Y., et al., 2015. Mechanism of regulation of stem cell differentiation by matrix stiffness. Stem Cell Res. Ther. 6, 103.

Malgieri, A., Kantzari, E., Patrizi, M.P., Gambardella, S., 2010. Bone marrow and umbilical cord blood human mesenchymal stem cells: state of the art. Int. J. Clin. Exp. Med. 3, 248−269.

Masuda, K., Sah, R.L., Hejna, M.J., Thonar, E.J., 2003. A novel two-step method for the formation of tissue-engineered cartilage by mature bovine chondrocytes: the alginate-recovered-chondrocyte (arc) method. J. Orthopaed. Res. 21, 139−148.

Mazué, G., Newman, A.J., Scampini, G., Torre, P.D., Hard, G.C., Iatropoulos, M.J., et al., 1993. The histopathology of kidney changes in rats and monkeys following intravenous administration of massive doses of FCE 26184, human basic fibroblast growth factor. Toxicol. Pathol. 21, 490−501.

Mitra, S., Gaur, U., Ghosh, P., Maitra, A., 2001. Tumour targeted delivery of encapsulated dextran−doxorubicin conjugate using chitosan nanoparticles as carrier. J. Controlled Release. 74, 317−323.

Mitrano, T.I., Grob, M.S., Carrión, F., Nova-Lamperti, E., Luz, P.A., Fierro, F.S., et al., 2010. Culture and characterization of mesenchymal stem cells from human gingival tissue. J. Periodontol. 81, 917−925.

Mooney, D., Mazzoni, C., Breuer, C., Mcnamara, K., Hern, D., Vacanti, J., et al., 1996. Stabilized polyglycolic acid fibre-based tubes for tissue engineering. Biomaterials. 17, 115−124.

Mosahebi, A., Wiberg, M., Terenghi, G., 2003. Addition of fibronectin to alginate matrix improves peripheral nerve regeneration in tissue-engineered conduits. Tissue Eng. 9, 209−218.

Moshaverinia, A., Chen, C., Akiyama, K., Ansari, S., Xu, X., Chee, W.W., et al., 2012. Alginate hydrogel as a promising scaffold for dental-derived stem cells: an in vitro study. J. Mater. Sci. Mater. Med. 23, 3041−3051.

Moshaverinia, A., Chen, C., Xu, X., Akiyama, K., Ansari, S., Zadeh, H.H., et al., 2013. Bone regeneration potential of stem cells derived from periodontal ligament or gingival tissue sources encapsulated in RGD-modified alginate scaffold. Tissue Eng. A. 20, 611−621.

Moshaverinia, A., Chen, C., Xu, X., Ansari, S., Zadeh, H.H., Schricker, S.R., et al., 2015. Regulation of the stem cell−host immune system interplay using hydrogel coencapsulation system with an anti-inflammatory drug. Adv. Funct. Mater. 25, 2296−2307.

Motta, A., Fambri, L., Migliaresi, C., 2002a. Regenerated silk fibroin films: thermal and dynamic mechanical analysis. Macromol. Chem. Phys. 203, 1658−1665.

Motta, A., Migliaresi, C., Lloyd, A.W., Denyer, S.P., Santin, M., 2002b. Serum protein absorption on silk fibroin fibers and films: surface opsonization and binding strength. J. Bioactive Compatible Polym. 17, 23−35.

Ni, P., Ding, Q., Fan, M., Liao, J., Qian, Z., Luo, J., et al., 2014. Injectable thermosensitive PEG−PCL−PEG hydrogel/acellular bone matrix composite for bone regeneration in cranial defects. Biomaterials. 35, 236−248.

O'brien, F.J., Harley, B., Yannas, I.V., Gibson, L.J., 2005. The effect of pore size on cell adhesion in collagen-GAG scaffolds. Biomaterials. 26, 433−441.

Pan, Z., Ding, J., 2012. Poly (lactide-co-glycolide) porous scaffolds for tissue engineering and regenerative medicine. Interface Focus. 2, 366−377.

Peppas, N., 2004. Hydrogels. Biomaterials Science: An Introduction to Materials in Medicine. 2, 100−107.

Rahfoth, B., Weisser, J., Sternkopf, F., Aigner, T., Von Der Mark, K., Bräuer, R., 1998. Transplantation of allograft chondrocytes embedded in agarose gel into cartilage defects of rabbits. Osteoarthritis Cartilage. 6, 50−65.

Richmon, J.D., Sage, A.B., Shelton, E., Schumacher, B.L., Sah, R.L., Watson, D., 2005. Effect of growth factors on cell proliferation, matrix deposition, and morphology of human nasal septal chondrocytes cultured in monolayer. Laryngoscope. 115, 1553−1560.

Rodríguez-Velázquez, E., Alatorre-Meda, M., Mano, J., 2015. Polysaccharide-based nanobiomaterials as controlled release systems for tissue engineering applications. Curr. Pharmaceut. Des. 21, 4837−4850.

Rowley, J.A., Mooney, D.J., 2002. Alginate type and RGD density control myoblast phenotype. J. Biomed. Mater. Res. 60, 217−223.

Ruoslahti, E., 1996. RGD and other recognition sequences for integrins. Annu. Rev. Cell Dev. Biol. 12, 697−715.

Schneider, A., Francius, G., Obeid, R., Schwinté, P., Hemmerlé, J., Frisch, B., et al., 2006. Polyelectrolyte multilayers with a tunable Young's modulus: influence of film stiffness on cell adhesion. Langmuir. 22, 1193−1200.

Schumacher, B., Pecher, P., Von Specht, B., Stegmann, T., 1998. Induction of neoangiogenesis in ischemic myocardium by human growth factors. Circulation. 97, 645−650.

Shapiro, L., Cohen, S., 1997. Novel alginate sponges for cell culture and transplantation. Biomaterials. 18, 583−590.

Shin, H., Jo, S., Mikos, A.G., 2003. Biomimetic materials for tissue engineering. Biomaterials. 24, 4353−4364.

Sun, G., Chu, C.-C., 2006. Synthesis, characterization of biodegradable dextran−allyl isocyanate−ethylamine/polyethylene glycol−diacrylate hydrogels and their in vitro release of albumin. Carbohydr. Polym. 65, 273−287.

Tsang, K., Annabi, N., Ercole, F., Zhou, K., Karst, D.J., Li, F., et al., 2015. Facile one-step micropatterning using photodegradable gelatin hydrogels for improved cardiomyocyte organization and alignment. Adv. Funct. Mater. 25, 977−986.

Tsou, Y.-H., Khoneisser, J., Huang, P.-C., Xu, X., 2016. Hydrogel as a bioactive material to regulate stem cell fate. Bioactive Mater. 1, 39−55.

Vacanti, C., Vacanti, J., 1994. Bone and cartilage reconstruction with tissue engineering approaches. Otolaryngol. Clin. North Am. 27, 263.

Van Aalst, J.A., Eppley, B.L., Hathaway, R.R., Sadove, A.M., 2005. Surgical technique for primary alveolar bone grafting. J. Craniofac. Surg. 16, 706−711.

Van Vlierberghe, S., Dubruel, P., Schacht, E., 2011. Biopolymer-based hydrogels as scaffolds for tissue engineering applications: a review. Biomacromolecules. 12, 1387−1408.

Wahl, D., Czernuszka, J., 2006. Collagen-hydroxyapatite composites for hard tissue repair. Eur. Cell Mater. 11, 43−56.

Wan, L.Q., Jiang, J., Arnold, D.E., Guo, X.E., Lu, H.H., Mow, V.C., 2008. Calcium concentration effects on the mechanical and biochemical properties of chondrocyte-alginate constructs. Cell. Mol. Bioeng. 1, 93−102.

Wang, Y., Zhao, Q., Zhang, H., Yang, S., Jia, X., 2014. A novel poly (amido amine)-dendrimer-based hydrogel as a mimic for the extracellular matrix. Adv. Mater. 26, 4163−4167.

Williams, D.F., 2008. On the mechanisms of biocompatibility. Biomaterials. 29, 2941−2953.

Yusop, A., Bakir, A., Shaharom, N., Abdul Kadir, M., Hermawan, H., 2012. Porous biodegradable metals for hard tissue scaffolds: a review. Int. J. Biomater.2012.

Zander, Z.K., Hua, G., Wiener, C.G., Vogt, B.D., Becker, M.L., 2015. Control of mesh size and modulus by kinetically dependent cross-linking in hydrogels. Adv. Mater. 27, 6283−6288.

Zhang, Q., Shi, S., Liu, Y., Uyanne, J., Shi, Y., Shi, S., et al., 2009. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J. Immunol. 183, 7787−7798.

Zhang, X., Chang, W., Lee, P., Wang, Y., Yang, M., Li, J., et al., 2014. Polymer-ceramic spiral structured scaffolds for bone tissue engineering: effect of hydroxyapatite composition on human fetal osteoblasts. PloS One. 9, e85871.

Zhang, Y., Wang, J., Wang, J., Niu, X., Liu, J., Gao, L., et al., 2015. Preparation of porous PLA/DBM composite biomaterials and experimental research of repair rabbit radius segmental bone defect. Cell Tissue Bank. 16, 615−622.

Zhong, S.P., Teo, W.E., Zhu, X., Beuerman, R., Ramakrishna, S., Yung, L.Y.L., 2007. Development of a novel collagen−GAG nanofibrous scaffold via electrospinning. Mater. Sci. Eng. C. 27, 262−266.

Zhu, J., 2010. Bioactive modification of poly (ethylene glycol) hydrogels for tissue engineering. Biomaterials. 31, 4639−4656.

Zogbi, L., Pignatello, P., 2011. Biomaterials Applications for Nanomedicine. InTech Rijeka, Croatia.

Dental biocomposites

5

Touraj Nejatian[1], *Zohaib Khurshid*[2], *Muhammad S. Zafar*[3,4], *Shariq Najeeb*[5], *Sana Zohaib*[6], *Masoud Mazafari*[7], *Louise Hopkinson*[8] *and Farshid Sefat*[8]
[1]Newcastle University, Newcastle upon Tyne, United Kingdom, [2]King Faisal University, Al-Ahsa, Saudi Arabia, [3]Taibah University, Madinah Al Munawwarah, Saudi Arabia, [4]Riphah International University, Islamabad, Pakistan, [5]Al-Farabi Colleges, Riyadh, Saudi Arabia, [6]King Faisal University, Al-Hofuf, Saudi Arabia, [7]Materials and Energy Research Center (MERC), Tehran, Iran, [8]University of Bradford, Bradford, United Kingdom

5.1 Introduction

Biocomposites for oral and dental tissue engineering have developed mainly by the use of multiple material blends by taking advantage of their compatible nature (Mohanty et al., 2005). They are biocompatible, with superior mechanical properties, and biodegradability. The most common examples of natural biocomposites are bone, teeth, skin, cartilage, tendon, and ligaments as well as resin-based dental composites for tooth filling in dentistry. By definition *composites* are blends of materials made from two different structures where the best properties of each are retained, and the inferior properties are lost (Milton, 2002; Tsai, 1992; John and Thomas, 2008). For example, weak and ductile polymers can be turned to strong and ductile material if mixed with ceramic particles that deflect or stop crack propagation. These types of blending result in composites or if its use in human body called as *biocomposites* (Mohanty et al., 2000) a term which was used for the first time in 1991 (Bernard and Picha, 1991). Before designing any composite structure, three interdependent factors must be considered (1) a selection of the suitable matrix and dispersed materials, (2) a choice of appropriate fabrication and processing methods, (3) both internal and external design of the device itself. Currently, many biocomposites used as human repair, reconstruction, and replacement. In Fig. 5.1 a schematic diagram showing potential uses of biocomposites.

5.2 Classification of biocomposites

Biomaterials are broadly classified in six different systems, namely metallic systems, ceramics systems, polymeric systems, carbon materials systems, composite systems, and engineered biological materials (Ratner et al., 2004). These systems specifically describe the nature and properties. Biocomposite structures are made of biomaterial compounds with a filler element dispersed into the matrix material, or a

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00005-0
© 2017 Elsevier Ltd. All rights reserved.

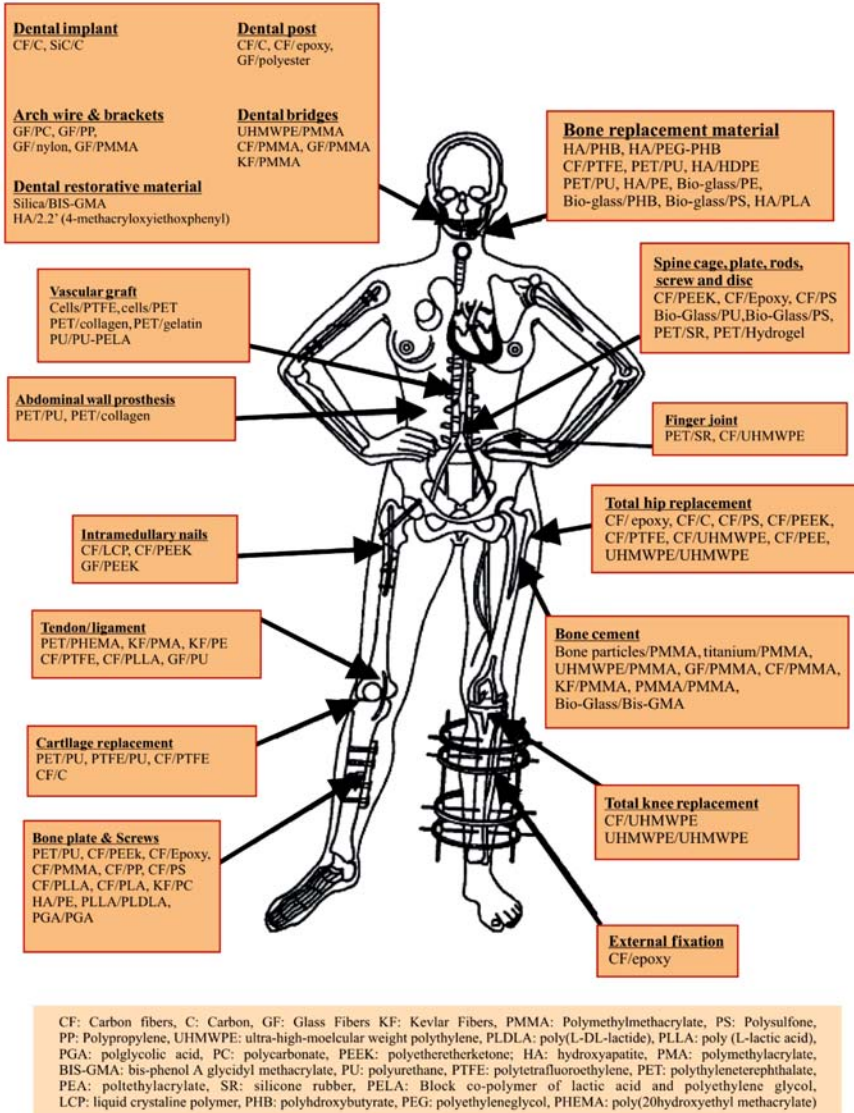


Figure 5.1 Various application of biocomposites in human body.
Adapted with permission from Ramakrishna, S., Mayer, J., Wintermantel, E., Leong, K.W., 2001. Biomedical applications of polymer-composite materials: a review. Compos. Sci. Technol. 61, 1189−1224.

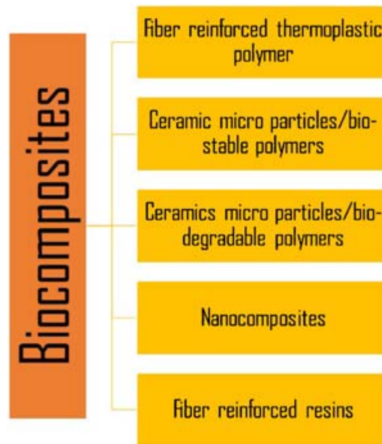


Figure 5.2 Classification of biocomposites (Mohanty et al., 2005).

construct with alternating sections of different materials (Ratner, 1993). Very advanced and recent classification of biocomposites are illustrated in Fig. 5.2.

5.3 Natural biocomposites

Composite materials, which exist naturally in animals and plants must be studied and understood in order to engineer synthetic biocomposites for various applications (Sommerfeldt and Rubin, 2001). Wood structure contains a polymer, cellulose (fibers) in combination with lignin is a common example of natural composite. These components when separated are weak materials, but in combination develop a strong composite. Both the combination and structural arrangement of materials develop impactful strength in resulting composite (Novaes et al., 2010; RB et al., 2012). Cotton is an example of a materials consisting of cellulose fibers alone, which is weaker compared to wood with more organized structure (Cao et al., 2016).

Bone, a dynamic component of human body, is also an example of natural biocomposites (Sefat, 2015; Nejatian, 2015). Its key constituents are hydroxylapatite (HAp) (hard and brittle mineral) and collagen (soft and flexible protein) with some other components, i.e., specialized cells, noncollagenous proteins, and mucopolysaccharides. Presence of these materials in specific ratios and their arrangement pattern gives suitable structure and properties to bone for performing its role in animal body. Collagen is also present in other natural biocomposites such as cartilage and finger nails but exhibits completely different properties. This diversity in appearance and characteristics is because of its combination with other materials and their arrangement patterns in each natural composite material.

In bioengineering, basic aim is to mimic natural composite materials with entire properties and structural details hoping to minimize inadvertent tissue reaction. Therefore it is immensely important to study natural materials in extensive detail.

Maximum descriptions can be obtained by studying them at variety of scales, i.e., at nano-, micro-, and macrolevel.

Tooth structure contains three different natural biocomposites, i.e., enamel, dentine, and cementum. HAp and collagen are main constituents of all these biocomposites alongside other components such as specialized cells, other proteins, and minerals. Enamel has brittle and hard structure in comparison to dentine which is soft. Enamel forms the outer surface of the tooth covering the inner dentine bulk. Enamel appears translucent and is unable to regenerate once damaged, but it can be restored with other synthetic biocomposites (dental resin, nanocomposite, or ceramic). While dentine has a hue which is reflected through enamel and is responsible for providing shade to tooth. Although limited, it has ability to regenerate itself if damaged and it is also subjected to changes due to ageing, physiological, and pathological stimuli. It must be noticed that, both biocomposites exhibit different properties, texture, and appearance despite their key constituents being similar. This is because the percentages and arrangements of these components is different in each. The arrangements vary at nano-, micro-, and macrolevels, which has a great impact on characteristics of each biocomposites (Khurshid et al., 2015). HAp in enamel is arranged in specific keyhole pattern forming interlocked enamel prisms.

Dentin and cementum are vital, hydrated, and natural composites with a matrix made of collagen type I filled by apatite which is calcium phosphate minerals (Dorozhkin, 2007). Dentin forms the main part of the tooth volume which is covered by enamel in oral cavity. Cementum covers dentine on tooth root surface, which acts as an intermediate layer to connect the root to the periodontal ligament. Periodontal ligament links cementum to the dental socket and helps suspend and hold the tooth in its position. As a result of higher percentage of organic constituents, dentin is less brittle than enamel, which makes it a suitable substructure to support enamel against functional forces. Enamel with higher hardness protects dentin against abrasive wear (Chun et al., 2014).

5.4 Synthetic biocomposites

Advanced production techniques and material science along with deeper understanding of biological reactions help developing synthetic biocomposites that play an important role in tissue engineering. Composite scaffolds are generally made up of two or more various scaffold materials such as synthetic polymer and inorganic materials. The aim of this combination of selected biomaterials is to enable tissue-engineered product to take advantage of each individual material and minimizes their disadvantages. Synthetic polymers such as bisphenol A-glycidyl methacrylate (Bis-GMA) or urethane dimethacrylate (UDMA) are mixed with inorganic filler particles or fibers to achieve required characteristics. This polymer-based composites are progressively becoming popular in restoring lost dentin and enamel. Polymethylmethacrylate (PMMA) is probably the most popular polymer, which has

been successfully used in dentistry and medicine for many decades either in plain or composite form (Furtos et al., 2016). Biocomposites with ceramics or glasses are also extensively used as scaffold for dental tissue regeneration. The filler particles are added to overcomes the inherent weakness of polymers such as lack of stiffness. Ease of fabrication is the advantage of polymers in comparison with other stiff and brittle materials such as ceramic scaffolds, which have poor processing ability to form highly porous structures. Polymers usually make the environment more acidic during the process of degradation. Resorption products of Ca/P scaffolds are capable of neutralizing this acidic environment, which result in less inflammation associated with the degradation of polymers. Over the past two decades, scientists have made a great progress toward tissue engineering of bone and dental tissue by fabricating highly porous polymer/ceramic composite scaffolds with osteoconductivity and required mechanical properties. Some of the synthetic material, which have been used, are biodegradable polymers: (1) polyesters such as polylactides, (2) polycyanoacrylates: isobutyl cyanoacrylate polymer, (3) polypeptides: poly(L-glutamic acid), poly(L-lysine), (4) polyanhydrides, polyphoshazenes, poly(ortho ester)s (Reis and Cohn, 2002).

Researchers demonstrated a great improvement in cell adhesion and cell viability within polymer-bioglass-composites as well as adequate biocompatibility and high bioactivity of the material for hard tissue repair. Dentin which is known to be one of the hardest natural materials needs proper repair with suitable biomaterials ideally biocomposites when damaged. Scientists recently showed great cell proliferation and differentiation when pure poly-lactic glycolic acid (PLGA) scaffold combined with either HA, TCP, and calcium deficient hydroxyapatite. Results suggested that Ca/P composite scaffolds effectively supported regeneration of tooth tissue and was the most suitable for dentin pulp regeneration among those PLGA/TCP scaffolds (Park et al., 1986). Zirconia hydroxyapatite (ZrO(2)/HA) composite is another synthetic porous material in tissue engineering of large bone defects with adequate biocompatibility and mechanical properties as well as excellent bone reconstruction and regeneration ability (Park and Bronzin, 2003).

5.5 Unique properties and adaptability of biocomposites

Biocomposites have become increasingly popular in dentistry due to their various desirable properties such as mechanical properties, biocompatibility, bioactivity, antibacterial activity, caries-inhibitory and regenerative activities, adhesion to the tooth structure, easy to use, and high aesthetic value. Biocomposites include such a large number of materials that the authors can discuss only some of them as examples here.

Interactions between different phases (e.g., continuous and discreet) of biocomposites determine their mechanical, physical, or biological behavior in living environment. Size, type, and weight fraction of inorganic filler particles have

a significant effect on bending strength, toughness, and surface hardness of PMMA (Nejatian et al., 2006). This effect is seen in PMMA-based bone cements where bone particles boost fatigue life and stiffness of the cement (Park et al., 1986). Similarly, fiber composite bone plates and femoral stems not only induce healing better, but also exhibit higher resilience than metal counterparts (Jockisch et al., 1992).

Biocompatibility is one of the key characteristics of biocomposites, however, defining this term is not straight forward. The definition of biocompatibility has been evolved throughout the years as biomaterials are being used for various purposes in different locations in human body. In addition, the interactions between materials and biological environments can cause wide range of local and systemic responses, which might be judged as curative, neutral, or toxic in a particular condition. Therefore there is still a great deal of uncertainty around the definition of biocompatibility. According to David Williams' latest definition,

> *Biocompatibility refers to the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimising the clinically relevant performance of that therapy*
>
> *(Williams, 2008).*

Biomaterials such as HAp, calcium phosphates (β-TCP and TTCP) wollastonite glass–ceramics (Saadaldin and Rizkalla, 2014), and bioactive glasses can induce bioactivity and bone bonding capability in neutral ceramics or titanium alloys (Ducheyne and Qiu, 1999; Tanzer et al., 2004). Bioactivity is the ability of materials to induce a specific biological response. Osteoconductivity, nontoxicity, noninflammatory, and nonimmunogenicity are the other properties of HAp which made it a popular constituent of restorative and regenerative materials (LeGeros, 1991). Bioglass stimulates osteoblast proliferation and osteogenesis by gene expressions and releasing calcium, phosphorous, and silicon ions.

Based on degradability of either matrix or filler particles, biocomposites are classified as biodegradable, partially biodegradable and nondegradable. Tissue engineering scaffolds and resorbable sutures are examples of biodegradable biocomposites. PMMA-based bone cements with biodegradable HAp filler particles are partially biodegradable biocomposites, which provide a matrix for bone opposition. HAp has osteoconductive and osteoinductive properties, which means that not only they serve as a scaffold for currently existing osteoblasts but they will also elicit formation of new osteoblasts (Barbieri et al., 2010; Kumar et al., 2013). Widely used resin (polymer)-based restorative and preventive composites in dentistry are examples of nonbiodegradable biocomposites. These are made of polymeric matrix such as UDMA, Bis-GMA, and PMMA, mixed with nonbiodegradable filler particles. The filler particles reduce polymerization shrinkage, enhance wear resistance, improve strength, and reduce water sorption of the composites. As a result, resin composites maintain shape, size, and appearance along with their mechanical and

physical properties throughout their service life (Lewandrowski et al., 2002). Although these polymers are considered to be cytotoxic or allergenic in unreacted forms and can cause inadvertent reactions specially among dental staff (Scott et al., 2004; Moharamzadeh et al., 2007), they are neutral and safe once polymerized. Biocomposites with polymeric matrix are corrosion resistant with higher aesthetic value in comparison with metal alloys. They also exhibit better fatigue strength and fracture toughness compared with ceramics (Furtos et al., 2013); however, they are not as radiopaque as metal alloys or ceramics (Furtos et al., 2012).

A group of bioactive dental composites have been developed to reduce caries activity either by suppressing harmful activity of oral bacteria or increasing acid resistance of the tooth structure. Bioactive glass containing 45S5 BAG fillers are introduced as pit and fissure sealants because of caries-inhibition activities and acceptable mechanical and physical properties (Yang et al., 2013). Glass ionomers, resin-modified glass ionomer, and compomers are other examples of restorative materials with composite structure, which can store and release fluoride in oral environment (Wiegand et al., 2007). The fluoride ion can replace hydroxide in the HAp crystal, forming more acid resistance fluoroapatite, facilitate remineralization of enamel, and inhibit metabolism of cariogenic bacteria such as *Streptococcus mutans* (Buzalaf et al., 2011). Bone cements may contain antibacterial agents either in the form of antibiotics such as gentamicin, tobramycin, vancomycin, and cephazolin (Bistolfi et al., 2011) or in the form of filler particles such as silver nanoparticles or calcium hydroxide particles (see paragraph below). Silver nanoparticles are shown to have antimicrobial activity against some of harmful bacterial strains and fungi such as *Candida albicans* (Mocanu et al., 2014).

Healing and regeneration of soft and hard tissues have been always the main focus of biomaterial sciences. Probably the most popular example of the material with such capability is calcium hydroxide, which is incorporated as main ingredient of some of routinely used pulp capping and root canal sealers to provoke dentinogenesis. These are used to either seal off and protect exposed vital pulpal tissue and provide chance for root maturation or closure (apexogenesis and apexification). The hydroxy ions released from this cement induces alkaline pH, which causes liquefactive necrosis in the superficial portion of the pulp, whereas the deeper area of the pulp retains neutral pH and stimulates hard tissue formation. In addition, alkaline environment supresses bacterial activity. Mineral trioxide aggregate is another example of these materials, which was introduced by Torabinejad et al. (1993) as a material for pulp capping, root canal filling, perforation repair, apexification, apical barriers, and revascularization (Nagy et al., 2014). In addition, composites of bioactive materials, such as bioglass or bioceramics, are used as coating to improve osteointegration of titanium and titanium-based implants (Ning and Zhou, 2002; Chu et al., 2006). It should be noted that not only the composition but also the structural and surface characteristics of biomaterials may also affect the tissue response. For example, only porous materials with pore size larger than 150 μm, when used in implants, allow tissue ingrowth (Li et al., 1994; Simmons et al., 1999).

Adhesion to tooth structure is another desirable properties of the restorative materials. Glass ionomer and resin-modified glass ionomer are examples of the

materials with such capability. Their bonding is through microretention and chemical bond to Ca ions in tooth structure (Almuhaiza, 2016). On the contrary, conventional resin composites lack this property; therefore they need an adhesive agent for retention. Newly developed self-adhesive resin composites showed promising in vivo results; however, sufficient clinical evidence is scarce (Makishi et al., 2015). Compomers have weak bonding to the tooth structure, only enough to retain small restorations in low-stress bearing areas. Generally, tooth bonding ability eliminated the need for destructive retentive features in cavity preparation and opened the doors to less invasive restorations.

Ceramics and polymer-based composites are becoming the most popular restorative materials mainly due their progressively improving aesthetic value, durability, and mechanical properties. They are easy to be used with different shade and translucency to mimic the natural tooth color or mask the discolored teeth. Polymer composites can set directly through either chemical or light-activated polymerization. Although polymerization shrinkage may pose stress on restoration and tooth bonding surface leading to microleakage and recurrent caries, in most of the cases it can be reliably controlled by a correct case selection and application technique.

5.6 Applications of biocomposites materials in dentistry

5.6.1 Oral and dental tissues engineering

Tissue engineering is the combined use of cells, biomaterials, engineering techniques, and various chemical and biological factors to repair, improve, or replace living tissue (Hutmacher, 2000). In the oral cavity, various pathological and traumatic processes may harm the soft and hard tissues. For instance, dental caries may lead to demineralization and cavitation of tooth structure, periodontal disease may cause alveolar bone resorption and infections and/or trauma may cause ulceration of the mucosa (Newman, 2012). Over the last few decades, many biomaterials, in combination with cells and growth factors, have been used to promote regeneration of diseased or damaged oral tissues. Guided tissue regeneration membranes, initially made of polytetrafluoroethylene, were used to act as a barrier membrane between the gingival epithelium and underlying periodontal bone to allow a space for the regeneration of the latter (Caffesse et al., 1990). However, because such materials were nonresorbable, secondary surgical procedures were required to remove the membrane, increasing the chances of infection and patient discomfort. Resorbable membranes made of synthetic polymers such as poly-L-lactic acid, PLGA, or natural materials such as collagen were introduced to overcome the problem (Sheikh et al., 2015). However, these materials have a number of limitations. Weak mechanical properties, poor handling characteristics and chances of allergies have limited their use (Bottino and Thomas, 2015; Bottino et al., 2012). Introduction of nanosized crystals such as HAp to polymers in order to produce

biocomposites not only increase the bioactivity of guided tissue regeneration (GTR) materials, they may also increase their mechanical properties (Hong et al., 2005). Composites of nanosized bioactive bioceramics and electrospun nanofibers have also been studied (Zafar et al., 2016). However, significantly more research is required to envisage the current status of these bioactive composites for tissue regeneration. More recent research has focused on using stem cells carried by various scaffolds made of biodegradable biocomposites in order to "kick-start" the regenerative process. Stem cells seeded on dental pulp may have the potential to regenerate damaged pulp, dentine, and enamel (Cordeiro et al., 2008). Indeed, natural polymers such as collagen have the potential to deliver recombinant growth factors to stimulate regeneration of dental tissues (Prescott et al., 2008). This combined use of growth factors, cells, and biocomposites has given rise to an entirely new paradigm of possibilities. It will only be possible to merely repairing oral tissues with synthetic materials but actual regeneration of living tissue is a possibility in the near future.

5.7 Restorative applications

Using dental materials to restore teeth dates back thousands of years. Ancient Egyptians and Mayans used metals and wood to place "donor teeth" into edentulous areas. Restoring cavities with gold foil was mainstay of restorative dentistry in the 19th and 20th century. Although, resin composites are popularly referred as composites among dental community most of dental materials have composite structure. Perhaps, one of the best examples of this is dental amalgam, an alloy of silver, mercury, and some other metal elements used to restore teeth. However, despites its excellent mechanical properties, dental amalgam cannot be used in anterior teeth as it is not tooth colored. Advent of aesthetic resin composites has brought a revolution in dentistry (Rizvi et al., 2016). Resin composites consist of a polymer matrix (such as Bis-GMA, UDMA, triethylene glycol dimethacrylate (TEGDM)) which binds together filler particles (such as silica) via coupling agent (Ferracane, 2011). As another example, glass ionomer cements, consisting of bioactive glass and acids, are tooth-adhering materials which are used in primary tooth restorations (Najeeb et al., 2016a). Partial dentures are routinely constructed of acrylic composites with fillers to impart particular properties. More recently, incorporation of bioactive ceramics into polymers such as polyetheretherketone has been a focus of interest to make of such materials as dental implants (Najeeb et al., 2016b, 2015).

5.8 Bone cements

Bone serves important functions in human body, e.g., support and protect vital organs. They produce red and white blood cells and store minerals. This important role reflects the importance of bone in human (Homo sapiens). The number of patients with bone defects due to trauma, metabolic diseases, and tumor are huge,

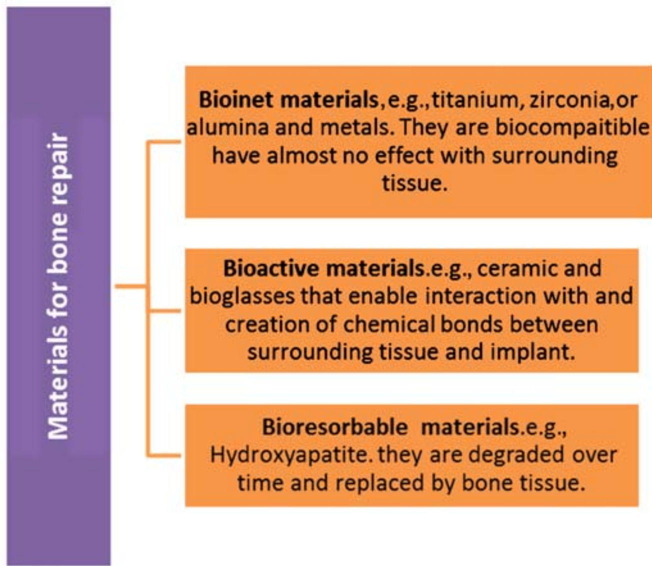


Figure 5.3 Different approaches for bone repair (Dorozhkin, 2010a, 2013, 2011).

so need of bone replacement are enormous. Therefore the development of bone repair materials has attracted much attention (Snoddy and Jayasuriya, 2016). Different approaches available nowadays for the management of bone repair depend on clinical situation. Few available materials used in bone repair are discussed in Fig. 5.3 (Bauer and Muschler, 2000; McAuliffe, 2003; Vaibhav et al., 2007). With the help of biomaterials implants, plates, bone grafts, and bone cements were made taking into account bone architecture and dynamics (Sheikh. Z et al., 2014).

Bone grafting is a surgical procedure that replaces missing bone in order to repair bone fractures that are extremely complex, pose a significant health risk to the patient, or fail to heal properly. Bone replacement grafts serve as a structural framework for clot formation, maturation, and remodeling that ultimately leads to bone formation in bone defects. Based on the origin, bone replacement grafts can generally be divided into natural and synthetic grafts. The natural sources of these grafts could be human or animal tissues (Table 5.1) (Dorozhkin, 2010b; Nauth et al., 2015; Moore et al., 2001).

5.8.1 Autogenous bone grafts

Autogenous grafts (commonly referred as autografts) are taken from one site (donor site) to insert at another site (recipient) within the same individual. The autografts remain gold standard to compare all other grafting biomaterials. Being from the host itself, there is no risk of antigenicity and graft compliance is near ideal. Few merits and demerits of autogenous bone grafts are in Table 5.2. Autologous grafts are usually obtained from chin, tuberosity, or iliac crest.

Table 5.1 **Bone replacement graft tissues and materials**

Bone replacement grafts
1. *Human bone sources*
a. Autogenous grafts (autografts)
i. Extraoral (e.g., iliac crest)
ii. Intraoral (e.g., tuberosity or chin)
b. Allogeneic grafts (allografts)
i. Fresh-frozen bone allografts
ii. Freeze-dried bone allografts (FDBA)
iii. Demineralized freeze-dried bone allografts (DFDBA)
2. *Bone substitutes (living nonhuman sources)*
a. Xenogeneic grafts (xenografts)
i. Bovine (derived) HA (porous, nonresorbable)
ii. Coralline calcium carbonate
3. *Synthetic bone substitutes (alloplasts)*
a. Polymeric materials (PGA, PLA, PGLA, etc.)
b. Bioactive glasses
c. Bioceramics
i. HA
– Dense, nonporous, nonresorbable HA
– Resorbable HA derived at low temperature)
ii. Other calcium phosphates (tricalcium phosphate, brushite, monetite)

PGA, poly-glycolic acid; *PLA*, poly-lactic acid; *PGLA*, poly-glycolic-lactic acid; *HA*, hydroxyapatite.

Table 5.2 **Key advantages and disadvantages of using autografts materials**

Advantages of autografts	Disadvantages of autografts
Highly biocompatible	Additional surgery to harvest bone and extensive hospitalization
High osteogenic potential	Less time and cost effective
Osteoconductive	Postoperative pain, increased blood loss
Osteoinductive	Prone to fracture
Comparable mechanical strength providing structural support	Dead bone is at potential risk for infection to set in
Easier incorporation into host site (none or minimal immune response)	High variability and difficult to control the quality of grafted bone
Availability in cortical and cancellous forms	Limited amount of graft tissue can be harvested

Table 5.3 Types of allogeneic bone grafts

Types	Description
Fresh or fresh-frozen bone	Highest osteoconductive and osteoinductive potential among all the available allografts. The risk of disease transmission, antigenicity, and extensive cross-matching and treatment required has rendered the use of frozen iliac allografts unacceptable in orthopedics
Freeze-dried bone allograft (FDBA)	FDBA possess inferior osteoinductive and mechanical properties as compared to fresh or frozen allografts
Demineralized freeze-dried bone allograft (DFDBA)	DFDBA has osteogenic potential

5.9 Allogeneic bone grafts

Bone grafts taken from a genetically nonidentical member of same species are called allografts. Allograft bone can be taken from cadavers that have donated their bone so that it can be used for living people who are in need of it; it is typically sourced from a bone bank (Dorozhkin, 2010b; Nauth et al., 2015; Moore et al., 2001; Zizzari et al., 2016). There has been a great interest in allografts due to their theoretical unlimited abundance and also because they match closely with the recipient in constitutional elements and architecture. Though allografts undergo various treatments prior to use, risk of disease transmission is still a possibility. It has been estimated that the risk of HIV transmission is 1 in 1.6 million with use of allografts (Buck et al., 1990). There have been some reports of cross-infection and incidences of disease transmission. Despite these risks, the recognition of the distinct advantages associated with allografts has resulted in an increase in bone grafting procedures with them. Allografts are mostly prepared as fresh, frozen, freeze-dried, mineralized, and demineralized, and each of these are available as cortical chips, cortical granules, cortical wedges, or cancellous powdered grafts (Table 5.3) (Mroz et al., 2008).

5.10 Xenografts

These are graft materials that are shared in between species. Popularly used xenografts for bone replacement therapy and regenerative applications are bovine bone and natural coral (Robson et al., 1999; Vacanti and Bonassar, 1999). Both sources provide bone grafts that are biocompatible and structurally similar to human bone; however, their processing techniques are quite different. Xenografts are osteoconductive in nature and are readily available for clinical use.

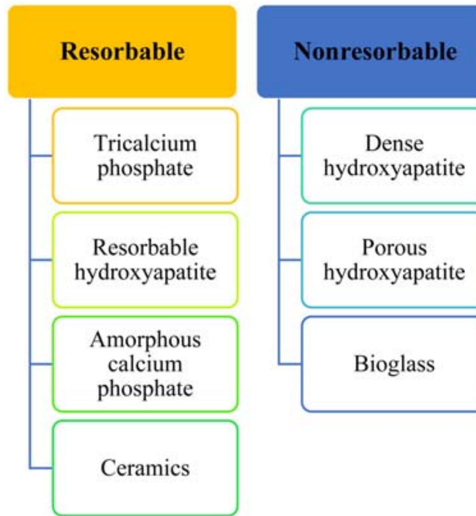


Figure 5.4 Types of alloplastic grafts.

5.11 Alloplastic grafts

Alloplastic grafts are usually made up from HAp, a mineral that is also the main component of bone. These are viable alternatives to autografts. Some other sources are bioactive glass, ceramics, and polymer. They can be resorbable and nonresorbable (Fig. 5.4).

Alloplastic graft materials have greatest usefulness as autogenous graft adjuncts to provide sufficient total volume of graft material. In the near future, these synthetic grafts may also serve as carriers for substances, such as antibiotics and growth factors. Therefore these graft materials are an ideal tool in the regenerative surgeon's armamentarium. Summary of all bone grafts in Table 5.4 (Vacanti and Bonassar, 1999).

5.12 Shortcomings of existing biocomposites and recent developments

Although polymer-based composites are becoming increasingly popular they are still far from an ideal restorative material. These materials have less elastic modulus than enamel and have relatively significant polymerization shrinkage. Low elastic modulus can direct functional stress towards tooth structure and cause fracture. The polymerization shrinkage may result in stress concentration on tooth-restoration interface, which may lead to microleakage and bacterial invasion. High creep and

Table 5.4 Summary of all bone grafts with description, examples and properties

Type	Description	Example	Property
Autografts	Used alone		Osteoconductive, osteogenic, and osteoinductive
Allograft based	Allograft bone used alone or in combination with other materials	Allegro, orthoblast, grafton	Osteoinductive and osteoconductive
Ceramic based	Includes calcium phosfate, calcium sulfate, and bioactive glass used alone or in combination	Osteograf, osteoset, NovaBone	Limited osteoinductive when mixed with bone marrow and Osteoconductive
Polymer based	Includes degradable and nondegradable polymers used alone and in combination with other materials	Cortoss, OPLA, Immix	Bioresorbable in degradable polymer and osteoconductive

wear rate are the other problems associated with polymer-based composites (Papadogianis et al., 1985; Cramer et al., 2011).

There is a growing concern about health and safety of nanoparticles and nanofibers used in medical devices and dental biomaterials specially polymer-based nanocomposites. Manufacturers, researchers, dental practitioners, and patients can be exposed to nanoparticles released to the environment either during experiments, production process, operation by dental practitioners (e.g., polishing or removal) or functional wear. Inhaled nanoparticles can hardly be cleared from human being respiratory system due to their small size. The particles can accumulate in lungs, enter systemic circulation, and accumulate in different organs passing through cell membranes. Nanoparticles can also gain access to blood circulation through skin and mucosa. The hazard caused by particles depends on their surface physico-chemical characteristics. With increasing application of different types of nanoparticles in dental biomaterials the need for assessing the heat hazards caused by short and long-term exposure to these materials should be investigated meticulously (2009).

Different strategies have been implemented to overcome the shortcomings of polymer-based restorative composites. Reduced light intensity at the early stage of polymerization and intermittent radiation, soft-start, and pulse-curing techniques have been used to increase the conversion level of monomer and reduce the stress developed during polymerization (Cramer et al., 2011). It is also shown that replacing TEGDMA with monomethacrylates such as morpholine carbonyl methacrylate

can increase the percentage of conversion and speed of polymerization and decrease the volumetric shrinkage of Bis-GMA composites (Lu et al., 2005). In a different approach, hybrid polymers have been developed using the combination of monomers with different setting reactions such as methacrylate via free-radical polymerization and vinyl ether via cationic mechanism. The resulting interpenetrating polymer network demonstrates less polymerization shrinkage stress (Lin, 2005). In another strategy, bicyclic monomers that engage in either radical or cationic double-ring-opening polymerization have been explored for their potential to reduce polymerization shrinkage in polymer-based composites (Chappelow et al., 2008). In addition, ring opening polymerization systems have been developed in order to reduce the polymerization shrinkage. Silorane material (Filtek LS) by dental composite filling materials (3M/ESPE) is a commercially available example of this type of composites. Silorane is a light-activated cationically ring-opening photopolymerizable composite used with a dedicated adhesive. Silorane and free-radical methacrylate materials can provide comparable properties either initially or after water storage (Ilie et al., 2009). As mention earlier in this chapter, fluoride as a caries preventive element was incorporated into restorative materials including compomers, giomers, resin-modified glass ionomers, and glass ionomer. These materials can release fluoride in ascending order with glass ionomer at the highest.

Concerns over the cytotoxicity of the amine co-initiators, ethyl-4-dimethylaminobenzoate, commonly used with camphorquinone as photoinitiator drive the investigation of alternative systems such as *N*,*N*-dimethylaminobenzyl alcohol, 4-(*N*,*N*-dimethylamino) phenethyl alcohol, and *N*,*N*-3,5-tetramethylaniline. Alternative monomers such as polyhedral oligomeric silsesquioxane methacrylates have been investigated as an alternative for the currently used matrix monomers, Bis-GMA and UDMA, in order to improve properties such as conversion, water sorption, volume shrinkage, and shrinkage stress. Despite some promise results, improvements in overall properties relative to Bis-GMA/TEGDMA are generally insignificant (Cramer et al., 2011).

Currently, efforts are focused to invent self-adhesive resin composites by incorporation of acidic functional groups like carboxylic acid and phosphoric acid into the monomer structure. However, further development on their mechanical and physical properties is required before replacing current tooth-adhesive-composite systems (López-Suevos and Dickens, 2008).

Thiol-ene photo polymerization mechanism explored as an alternative to conventional methacrylate polymerization exhibits significant reductions in polymerization shrinkage stress (Cramer et al., 2010). Thiol-ene photo polymerization of allyl sulfide and multifunctional monomers, allows the forming polymer chains to relax throughout the polymerization rather than before gelation stage only. This reduced its shrinkage stress significantly (Park et al., 2012).

High solubility and poor adhesion to the dentine makes calcium hydroxide prone to disintegration if exposed to oral environment due to microleakage in the restoration. This will leave a gap under the restoration, which is a route for bacterial invasion (Cox et al., 1996). Isolation of this materials from oral environment using tooth-bonded water insoluble materials such as polymer composites is recommended.

5.13 Conclusions

Biocomposites are playing a crucial role in the field of dental biomaterials and their importance in healthcare sector applications. Over the last decade significant improvement in the properties of the materials has enabled us to offer less invasive restorations. Efforts have been made to improve biocompatibility, strength, aesthetic, tooth adhesion, antibacterial activity, and wear resistance of biocomposites. Polymerization shrinkage and microleakage and following bacterial invasion are the main causes of restoration failures. Significant amount of studies has been carried out to address these issues by modifying polymer matrix, filler particle technologies, and improving adhesive systems. However, ideal properties yet to be achieved.

References

Almuhaiza, M., 2016. Glass-ionomer cements in restorative dentistry: a critical appraisal. J. Contemp. Dent. Pract. 17, 331−336.

Barbieri, D., Renard, A.J., De Bruijn, J.D., Yuan, H., 2010. Heterotopic bone formation by nano-apatite containing poly(D,L-lactide) composites. Eur. Cell. Mater. 19, 252−261.

Bauer, T.W., Muschler, G.F., 2000. Bone graft materials. An overview of the basic science. Clin. Orthop. Relat. Res. 371, 10−27.

Bernard, S., Picha, G., 1991. The use of coralline hydroxyapatite in a “biocomposite” free flap. Plast. Reconstr. Surg. 87 (1), 96−105.

Bistolfi, A., Massazza, G., Verne, E., Masse, A., Deledda, D., Ferraris, S., et al., 2011. Antibiotic-loaded cement in orthopedic surgery: a review. ISRN Orthop. 2011, 290851.

Bottino, M.C., Thomas, V., 2015. Membranes for periodontal regeneration—a materials perspective. Front. Oral Biol. 17, 90−100.

Bottino, M.C., Thomas, V., Schmidt, G., Vohra, Y.K., Chu, T.-M.G., Kowolik, M.J., et al., 2012. Recent advances in the development of GTR/GBR membranes for periodontal regeneration—A materials perspective. Dent. Mater. 28, 703−721.

Buck, B.E., Resnick, L., Shah, S.M., Malinin, T.I., 1990. Human immunodeficiency virus cultured from bone. Implications for transplantation. Clin. Orthop. Relat. Res.249−253.

Buzalaf, M.A., Pessan, J.P., Honorio, H.M., Ten Cate, J.M., 2011. Mechanisms of action of fluoride for caries control. Monogr. Oral Sci. 22, 97−114.

Caffesse, R.G., Smith, B.A., Duff, B., Morrison, E.C., Merrill, D., Becker, W., 1990. Class II furcations treated by guided tissue regeneration in humans: case reports. J. Periodontol. 61, 510−514.

Cao, J., Sun, X., Lu, C., Zhou, Z., Zhang, X., Yuan, G., 2016. Water-soluble cellulose acetate from waste cotton fabrics and the aqueous processing of all-cellulose composites. Carbohydr. Polym. 149, 60−67.

Chappelow, C.C., Pinzino, C.S., Chen, S.-S., Kotha, S.P., Glaros, A.G., Eick, J.D., 2008. Tetraoxaspiroalkanes for polymerization stress reduction of Silorane resins. J. Appl. Polym. Sci. 108, 3738−3747.

Chu, C., Xue, X., Zhu, J., Yin, Z., 2006. Fabrication and characterization of titanium-matrix composite with 20 vol% hydroxyapatite for use as heavy load-bearing hard tissue replacement. J. Mater. Sci. Mater. Med. 17, 245−251.

Chun, K.J., Choi, H.H., Lee, J.Y., 2014. Comparison of mechanical property and role between enamel and dentin in the human teeth. J. Dent. Biomech. 5, 1758736014520809.

Cordeiro, M.M., Dong, Z., Kaneko, T., Zhang, Z., Miyazawa, M., Shi, S., et al., 2008. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J. Endodont. 34, 962−969.

Cox, C., Sübay, R., Ostro, E., Suzuki, S., Suzuki, S., 1996. Tunnel defects in dentin bridges: their formation following direct pulp capping. Oper. Dent. 21, 4−11.

Cramer, N.B., Couch, C.L., Schreck, K.M., Carioscia, J.A., Boulden, J.E., Stansbury, J.W., et al., 2010. Investigation of thiol-ene and thiol-ene−methacrylate based resins as dental restorative materials. Dent. Mater. 26, 21−28.

Cramer, N.B., Stansbury, J.W., Bowman, C.N., 2011. Recent advances and developments in composite dental restorative materials. J. Dent. Res. 90, 402−416.

Dorozhkin, S.V., 2007. A hierarchical structure for apatite crystals. J. Mater. Sci. Mater. Med. 18, 363−366.

Dorozhkin, S.V., 2010a. Amorphous calcium (ortho)phosphates. Acta Biomater. 6, 4457−4475.

Dorozhkin, S.V., 2010b. Calcium orthophosphates as bioceramics: state of the art. J. Funct. Biomater. 1, 22−107.

Dorozhkin, S.V., 2011. Biocomposites and hybrid biomaterials based on calcium orthophosphates. Biomatter. 1, 3−56.

Dorozhkin, S.V., 2013. Calcium orthophosphates in dentistry. J. Mater. Sci. Mater. Med. 24, 1335−1363.

Ducheyne, P., Qiu, Q., 1999. Bioactive ceramics: the effect of surface reactivity on bone formation and bone cell function. Biomaterials. 20, 2287−2303.

Ferracane, J.L., 2011. Resin composite-state of the art. Dent. Mater. 27, 29−38.

Furtos, G., Silaghi-Dumitrescu, L., Lewandowska, K., Sionkowska, A., Pascuta, P., 2016. Biocomposites for orthopedic and dental application. Key Eng. Mater.672.

Furtos, G., Silaghi-Dumitrescu, L., Moldovan, M., Baldea, B., Trusca, R., Prejmerean, C., 2012. Influence of filler/reinforcing agent and post-curing on the flexural properties of woven and unidirectional glass fiber-reinforced composites. J. Mater. Sci. 47, 3305−3314.

Furtos, G., Tomoaia-Cotisel, M., Prejmerean, C., 2013. Resin composites reinforced by glass fibers with potential biomedical applications. Structure and mechanical properties. Particul. Sci. Technol. 31, 332−339.

Hong, Z., Zhang, P., He, C., Qiu, X., Liu, A., Chen, L., et al., 2005. Nano-composite of poly (l-lactide) and surface grafted hydroxyapatite: mechanical properties and biocompatibility. Biomaterials. 26, 6296−6304.

Hutmacher, D.W., 2000. Scaffolds in tissue engineering bone and cartilage. Biomaterials. 21, 2529−2543.

Ilie, N., Hickel, R., Watts, D.C., 2009. Spatial and cure-time distribution of dynamic-mechanical properties of a dimethacrylate nano-composite. Dent. Mater. 25, 411−418.

Jockisch, K.A., Brown, S.A., Bauer, T.W., Merritt, K., 1992. Biological response to chopped-carbon-fiber-reinforced peek. J. Biomed. Mater. Res. 26, 133−146.

John, M.J., Thomas, S., 2008. Biofibres and biocomposites. Carbohydr. Polym. 71, 343−364.

Khurshid, Z., Zafar, M., Qasim, S., Shahab, S., Naseem, M., Abureqaiba, A., 2015. Advances in nanotechnology for restorative dentistry. Materials. 8, 717.

Kumar, P., Vinitha, B., Fathima, G., 2013. Bone grafts in dentistry. J. Pharm. Bioallied Sci. 5, S125−S127.

LeGeros, R.Z., 1991. Calcium Phosphates in Oral Biology and Medicine. Karger, Basel, Switzerland.

Lewandrowski, K.U., Bondre, S.P., Shea, M., Untch, C.M., Hayes, W.C., Hile, D.D., et al., 2002. Composite resorbable polymer/hydroxylapatite composite screws for fixation of osteochondral osteotomies. Biomed. Mater. Eng. 12, 423−438.

Li, P., Ohtsuki, C., Kokubo, T., Nakanishi, K., Soga, N., De Groot, K., 1994. The role of hydrated silica, titania, and alumina in inducing apatite on implants. J. Biomed. Mater. Res. 28, 7−15.

Lin Y, S.J., 2005. The impact of water on photopolymerization kinetics of methacrylate/vinyl ether hybrid systems. Polym. Adv. Technol. 16, 195−199.

López-Suevos, F., Dickens, S.H., 2008. Degree of cure and fracture properties of experimental acid-resin modified composites under wet and dry conditions. Dent. Mater. 24, 778−785.

Lu, H., Stansbury, J.W., Nie, J., Berchtold, K.A., Bowman, C.N., 2005. Development of highly reactive mono-(meth)acrylates as reactive diluents for dimethacrylate-based dental resin systems. Biomaterials. 26, 1329−1336.

Makishi, P., Pacheco, R.R., Sadr, A., Shimada, Y., Sumi, Y., Tagami, J., et al., 2015. Assessment of self-adhesive resin composites: nondestructive imaging of resin−dentin interfacial adaptation and shear bond strength. Microsc. Microanal. 21, 1523−1529.

McAuliffe, J.A., 2003. Bone graft substitutes. J. Hand Ther. 16, 180−187.

Milton, G.W., 2002. Theory Compos by Graeme W Milton. Cambridge Univ Press, Cambridge.

Mocanu, A., Furtos, G., Rapuntean, S., Horovitz, O., Flore, C., Garbo, C., et al., 2014. Synthesis; characterization and antimicrobial effects of composites based on multi-substituted hydroxyapatite and silver nanoparticles. Appl. Surf. Sci. 298, 225−235.

Mohanty, A., Misra, M. & Hinrichsen, G. 2000 Biofibres, biodegradable polymers and biocomposites: an overview., 276−277. [accessed 2016 August 22].

Mohanty, A.K., Misra, M., Drzal, L.T., 2005. Natural Fibers, Biopolymers, and Biocomposites. CRC Press.

Moharamzadeh, K., van Noort, R., Brook, I.M., Scutt, A.M., 2007. Cytotoxicity of resin monomers on human gingival fibroblasts and HaCaT keratinocytes. Dent. Mater. 23, 40−44.

Moore, W.R., Graves, S.E., Bain, G.I., 2001. Synthetic bone graft substitutes. ANZ J. Surg. 71, 354−361.

Mroz, T.E., Joyce, M.J., Steinmetz, M.P., Lieberman, I.H., Wang, J.C., 2008. Musculoskeletal allograft risks and recalls in the United States. J. Am. Acad. Orthopaed. Surg. 16, 559−565.

Nagy, M.M., Tawfik, H.E., Hashem, A.A., Abu-Seida, A.M., 2014. Regenerative potential of immature permanent teeth with necrotic pulps after different regenerative protocols. J. Endod. 40, 192−198.

Najeeb, S., Khurshid, Z., Matinlinna, J.P., Siddiqui, F., Nassani, M.Z., Baroudi, K., 2015. Nanomodified peek dental implants: bioactive composites and surface modification—a review. Int. J. Dent. 2015, 7.

Najeeb, S., Khurshid, Z., Zafar, M., Khan, A., Zohaib, S., Martí, J., et al., 2016a. Modifications in glass ionomer cements: nano-sized fillers and bioactive nanoceramics. Int. J. Mol. Sci. 17, 1134.

Najeeb, S., Zafar, M.S., Khurshid, Z., Siddiqui, F., 2016b. Applications of polyetheretherketone (PEEK) in oral implantology and prosthodontics. J. Prosthodont. Res. 60, 12−19.

Nauth, A., Lane, J., Watson, J.T., Giannoudis, P., 2015. Bone graft substitution and augmentation. J. Orthopaed. Trauma. 29, S34−S38.
Nejatian, T., Johnson, A., van Noort, R., 2006. Reinforcement of denture base resin. Adv. Sci. Technol. 49, 124−129.
Nejatian, T., Sefat, F., Johnson, T., 2015. Impact of packing and processing technique on mechanical properties of acrylic denture base materials. Materials, ISSN 1996-1944.
Newman, M.G., 2012. Carranza's Clinical Periodontology. Saunders, Philadelphia, PA;, London.
Ning, C.Q., Zhou, Y., 2002. In vitro bioactivity of a biocomposite fabricated from HA and Ti powders by powder metallurgy method. Biomaterials. 23, 2909−2915.
Novaes, E., Kirst, M., Chiang, V., Winter-Sederoff, H., Sederoff, R., 2010. Lignin and biomass: a negative correlation for wood formation and lignin content in trees. Plant Physiol. 54, 555−561.
Papadogianis, Y., Boyer, D.B., Lakes, R.S., 1985. Creep of posterior dental composites. J. Biomed. Mater. Res. 19, 85−95.
Park, H.C., Liu, Y.K., Lakes, R.S., 1986. The material properties of bone-particle impregnated PMMA. J. Biomech. Eng. 108, 141−148.
Park, H.Y., Kloxin, C.J., Abuelyaman, A.S., Oxman, J.D., Bowman, C.N., 2012. Stress relaxation via addition-fragmentation chain transfer in high T(g), high conversion methacrylate-based systems. Macromolecules. 45, 5640−5646.
Park, J.B., Bronzin, J.D., 2003. Biomaterials: Principles and Applications. CRC Press, Boca Raton, FL.
Prescott, R.S., Alsanea, R., Fayad, M.I., Johnson, B.R., Wenckus, C.S., Hao, J., et al., 2008. In vivo generation of dental pulp-like tissue by using dental pulp stem cells, a collagen scaffold, and dentin matrix protein 1 after subcutaneous transplantation in mice. J. Endodont. 34, 421−426.
Ratner, B., 1993. New ideas in biomaterials science—a path to engineered biomaterials. J. Biomed. Mater. Res. 27, 837−850.
Ratner, B., Hoffman, A., Schoen, F., Je, L., 2004. Biomaterials Science: An Introduction to Materials in Medicine. Academic Press, Boston.
RB, S., Capanema, E., Balakshin, M., Chang, H., Jameel, H., 2012. Lignin structural variation in hardwood species. J. Agric. Food Chem. 60, 4923−4930.
Reis, R.L., Cohn, D., 2002. Polymer Based Systems on Tissue Engineering, Replacement and Regeneration. *Kluwer Academic Publishers*, *Dordrecht*, p. 86.
Rizvi, A., Zafar, M.S., Al-Wasifi, Y., Fareed, W., Khurshid, Z., 2016. Role of enamel deminerlization and remineralization on microtensile bond strength of resin composite. Eur. J. Dentist. 10, 376−380.
Robson, S.C., Schulte Am Esch 2nd, J., Bach, F.H., 1999. Factors in xenograft rejection. Ann. N. Y. Acad. Sci. 875, 261−276.
Saadaldin, S.A., Rizkalla, A.S., 2014. Synthesis and characterization of wollastonite glass−ceramics for dental implant applications. Dent. Mater. 30, 364−371.
Scott, A., Egner, W., Gawkrodger, D.J., Hatton, P.V., Sherriff, M., van Noort, R., et al., 2004. The national survey of adverse reactions to dental materials in the UK: a preliminary study by the UK Adverse Reactions Reporting Project. Br. Dent. J. 196, 471−477.
Sefat, F., Khaghani, S.A., Nejatian, T., Genedy, M., Abdeldayem, A., Salehi Moghadam, Z., et al., 2015. Transforming growth factor beta (TGF-β) isomers influence cell detachment of MG-63 bone cells. Tissue Cells. 47, 567−574.
Sheikh, Z., Najeeb, S., Khurshid, Z., Verma, V., Rashid, H., Glogauer, M., 2015. Biodegradable materials for bone repair and tissue engineering applications. Materials. 8, 5273.

Sheikh, Z., Abdallah, M., Nader, H., 2014. In: Matinlinna, J.P. (Ed.), Handbook of Oral Biomaterials. Pan Stanford Publishing Pte. Ltd, Singapore.
Simmons, C.A., Valiquette, N., Pilliar, R.M., 1999. Osseointegration of sintered porous-surfaced and plasma spray-coated implants: an animal model study of early postimplantation healing response and mechanical stability. J. Biomed. Mater. Res. 47, 127−138.
Snoddy, B., Jayasuriya, A.C., 2016. The use of nanomaterials to treat bone infections. Mater. Sci. Eng. C. 67, 822−833.
Sommerfeldt, D.W., Rubin, C.T., 2001. Biology of bone and how it orchestrates the form and function of the skeleton. Eur. Spine J.S86−S95.
Tanzer, M., Gollish, J., Leighton, R., Orrell, K., Giacchino, A., Welsh, P., et al., 2004. The effect of adjuvant calcium phosphate coating on a porous-coated femoral stem. Clin. Orthopaed. Relat. Res. 424, 153−160.
Torabinejad, M., Watson, T.F., Pitt Ford, T.R., 1993. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J. Endodont. 19, 591−595.
Tsai, S.W., 1992. Theory of Composites Design. Think Composites, Dayton, OH.
Vacanti, C.A., Bonassar, L.J., 1999. An overview of tissue engineered bone. Clin. Orthop. Relat. Res. 367, S375−S381.
Vaibhav, B., Nilesh, P., Vikram, S., Anshul, C., 2007. Bone morphogenic protein and its application in trauma cases: a current concept update. Injury. 38, 1227−1235.
Wiegand, A., Buchalla, W., Attin, T., 2007. Review on fluoride-releasing restorative materials-;Fluoride release and uptake characteristics, antibacterial activity and influence on caries formation. Dent. Mater. 23, 343−362.
Williams, D.F., 2008. On the mechanisms of biocompatibility. Biomaterials. 29, 2941−2953.
Yang, S.-Y., Piao, Y.-Z., Kim, S.-M., Lee, Y.-K., Kim, K.-N., Kim, K.-M., 2013. Acid neutralizing, mechanical and physical properties of pit and fissure sealants containing melt-derived 45S5 bioactive glass. Dent. Mater. 29, 1228−1235.
Zafar, M., Najeeb, S., Khurshid, Z., Vazirzadeh, M., Zohaib, S., Najeeb, B., et al., 2016. Potential of electrospun nanofibers for biomedical and dental applications. Materials. 9, 73.
Zizzari, V.L., Zara, S., Tetè, G., Vinci, R., Gherlone, E., Cataldi, A., 2016. Biologic and clinical aspects of integration of different bone substitutes in oral surgery: a literature review. Oral. Surg. Oral Med. Oral Pathol. Oral Radiol. 122, 392−402.

Further reading

Current Intelligence Bulletin 60, 2009. Interim Guidance for Medical Screening and Hazard Surveillance for Workers Potentially Exposed to Engineered Nanoparticles.
Ramakrishna, S., Mayer, J., Wintermantel, E., Leong, K.W., 2001. Biomedical applications of polymer-composite materials: a review. Compos. Sci. Technol. 61, 1189−1224.

Surface modification of dental implants

6

Jonathan Wirth, Mohammadreza Tahriri, Kimia Khoshroo, Morteza Rasoulianboroujeni, Andrew R. Dentino and Lobat Tayebi
Marquette University School of Dentistry, Milwaukee, WI, United States

6.1 Introduction

Dental implants are biocompatible posts surgically placed through the mucosa in the edentulous ridges of a patient's mouth. They may be utilized as the fixtures for the abutments of various prostheses, such as fixed crowns, bridges, and removable implant-supported overdentures. Titanium is the material of choice in terms of biocompatibility and its capacity to resist corrosion and low toxicity (Albrektsson et al., 1981; Fatehi et al., 2008; Khosroshahi et al., 2008; Fatehi et al., 2014). Most importantly, it has the properties which make it ideal for osseointegration which is defined as a direct functional and structural connection between living bone and the surface of a load carrying implant. Osseointegration is a fundamental determinant of an implant's success in terms of healing and longevity.

Methods of testing implant/tissue interface include histomorphometry, electron microscopy, and laser scanning profilometry (Chai et al., 2011, 2012). It has been confirmed that surface topography has valid effect on the implant success; thus, evaluation of the surface with these methods is considered useful in prediction of success. According to Wennerberg and Albrektsson (2000), laser scanning profilometry is the most valid profilometric device on measuring material topography. Histomorphometry is the analysis of the tissue/implant interface in vivo and offers a more direct understanding of the adaptation of bone architecture to the modification being tested. Ultimately, the success of any design method should be evaluated at a clinical level (Al-Nawas et al., 2008).

Removal torque tests are often implemented to test the stability of the implant at a given phase. Force of removal torque has a direct relationship with bone-to-implant contact, rendering it an acceptable method of testing osseointegration (Cho and Park, 2003).

Albrektsson et al. (1981) identified implant design and surface finish two fundamental determinants in the manipulation of osseointegration. This review will describe the various methods to improve those parameters via surface modification techniques.

Various surface modification procedures can improve osseointegration overtime as well as promote shorter healing processes (Jemat et al., 2015).

Implant surface modification aims to modify surface topography as well as surface energy to promote cell proliferation and growth in the local environment,

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00006-2
© 2017 Elsevier Ltd. All rights reserved.

thus accelerating osseointegration (Jemat et al., 2015). The extent with which an implant will osseointegrate is not attributed to any one surface characteristic; rather, a multitude: titanium surface modification research focuses on improving the results of methods in isolation and how they enhance each other in tandem (Jemat et al., 2015). Many methods are being tested to create improvement on the design of titanium surface, with an emphasis on manipulating the biochemical (Lamolle et al., 2009) and physical topography (Yeo, 2014) of the implant's interface. In terms of the material of the implant, titanium is already a corrosive resistant, high strength metal with good formability and machinability (Albrektsson et al., 1981). Surface modification mostly improves clot wettability, cell-implant adhesion, proliferation, and osseointegration, all of which contribute to the end of shorter healing time (Jemat et al., 2015).

Surface wettability describes the contact angle of a hydrophilic substance as it preads out over to the surface of the interface in question (titanium). It is directly proportional to a substance's hydrophilicity and is a key factor in osseointegration in that cell migration and adhesion to an implant's surface is driven by adsorption of osteogenic proteins at the surface of the implant. These proteins have a higher affinity with hydrophilic surfaces rather than hydrophobic surfaces (Gray, 2004).

While many different surface modification methods exist, they can be subdivided into two broad categories: additive methods and subtractive methods (Jemat et al., 2015). Additive methods are those which describe impregnation or coating of the material (Jemat et al., 2015). Impregnation is the process by which a material or chemical agent is fully integrated into the titanium core while coating techniques are superficial additions to the implants surface. Conversely, subtractive techniques are procedures which remove or deform material to increase roughness of the surface (Jemat et al., 2015). A variety of subtractive techniques are employed to yield a wide range of surface topography, utilizing different blasting materials, grits, and even anodization methods (Garg et al., 2012). Etchants and plasma sprays are further examples of subtractive methods (Cho and Park, 2003).

6.2 Surface treatment methods

6.2.1 Subtractive methods

Subtractive methods are utilized to manipulate the surface morphology and roughness of the titanium implant's surface. Roughness of the implant's surface alone can drastically improve an implant's success. Prospective research on surface modification methods lays emphasis on subtractive methods, especially in terms of spray plasma coating and acid etching (Jemat et al., 2015). This section will review a variety of subtractive methods.

Increasing this roughness alone via blasting processes can significantly improve bone fixation, with optimal roughness at about 1.5 μm (Garg et al., 2012). Optimization of this roughness in terms of configuration of surface topography is currently under investigation and is the aim of the majority of subtractive methods.

6.2.1.1 Grit blasting

Machines surfaces are defined as turned, milled, or polished surfaces (Wennerberg and Albrektsson, 2000). The turning of an implant yields a surface with an average roughness of 0.96 μm and an average peak spacing of 8.6 μm (Garg et al., 2012). This method in many of the studies involved in the writing of this review is the comparative control in testing significant impact of surface modification methods.

Grit blasting is a method by which pressurized particles are used to blast the surface of a turned titanium implant, usually using silica or ceramic material. This could be sand, hydroxyapatite, alumina, or TiO_2 (Ban et al., 2006). Conventionally, this process is usually followed by acid etching in order to remove residual blast particles while attributing further surface roughness (Ban et al., 2006). This process can also aid in the decontamination of the surface of the implant (Coelho et al., 2009).

It has been demonstrated in a study by Al-Radha et al. (2012) that surface-free energy is the most important factor in determining bacterial adhesion upon an implant surface by culturing saliva pellicles upon blasted titanium discs in vitro.

6.2.1.2 Acid etching

Acid etching describes the use of acids on metals to both sterilize and modify roughness (Jemat et al., 2015). Hydrofluoric (HF), nitric (HNO_3), and sulfuric (H_2SO_4) acid etchants are usually employed, as well as solutions of these acids (Jemat et al., 2015). This method provides the substrate with homogeneous roughening of its surface (Guo et al., 2012). There is a direct relationship between acid concentration and yielded roughness.

6.2.1.3 Dual acid etching

Continuing acid etching, dual acid etching (DAE) describes the treatment of titanium surface with different solutions of acids. Dual etching has proven to be an effective method to achieve rapid osseointegration with greatly improved resistance to torque after healing when compared to machined surface implants (Chou and Chang, 2001).

6.2.1.4 Blasting and acid etching (SLA)

Gobbato et al. (2012) performed a 5-year split mouth experiment to test sandblasting and acid etching (SLA) implant's performance in early loading (6 weeks) compared to a titanium plasma sprayed control. The results demonstrated that SLA implants yielded very high success rates with four times greater resistance to removal torque, superior histomorphometry, and stronger bone responses when relative to turned surfaces. Xue et al. (2005) also demonstrated in a study on dogs that SLA treatment showed improved early bone growth and osseointegration.

6.2.1.5 Anodized surface implants

Anodized surface implants undergo electrolyte and current treatment by being placed as the anodes of galvanic cells with phosphoric acid acting as the electrolyte (Wennerberg and Albrektsson, 2010). This anodic oxidation of the surface promotes growth of a native titanium oxide layer with a porous topography, developing a moderately rough surface (Gaggl et al., 2000). This method also impregnates the surface layer with 5% phosphorus in the form of phosphates, with amorphous TiO_2 and crystalline grains present on the amorphous matrix layer.

An animal study on pigs concluded that the anodized surface had a biocompatibility similar to hydroxyapatite-coated surface implants, both of which have higher biocompatibility than turned surface titanium implants. Another clinical study shows evidence that clinical outcomes of anodized versus turned surfaces are more successful when placed on patients with poor bone quality compared to machined titanium implants (Glauser et al., 2003).

When compared to SLA, anodic oxidation methods demonstrated weaker osseointegration. SLA surface roughness can be controlled to achieve optimal roughness. Studies demonstrate evidence that removal torque test resistance and histomorphometric measurements are similar in both SLA and anodized surface implants (Yeo et al., 2008; Al-Nawas et al., 2008).

6.2.1.6 Laser etching and microarc oxidation

Laser etching is a relatively new treatment that may have the potential to provide an oxidized layer while controlling surface roughness. A direct relationship between voltage to all roughness, pore size, and thickness of resulting oxide layer in the laser etching procedures demonstrates prospect in this field. Electromicroscopy reveals a layer of Ti, C, O, Ca, and phosphonium develop at the surface layer after laser treatment. One study resulted in a removal torque that was 20% more resistant than machined and blasted implants (Pető et al., 2002). While contaminants of carbon and oxygen derived from CO_2 in the atmosphere were present in analysis of the postmodified surface (Deppe et al., 2005), it is actually the least contaminating surface treatment in comparison to more well-researched and commonly utilized surface modification techniques such as acid etching, sand blasting, and plasma spraying.

The process involves an ultrasonic pretreatment followed by etching using an Nd:YAG laser at 50 kW, with a frequency of 7.5 kHz and 16.4 current. Postetching, the implant is processed in an electrolyte solution containing 3.5% glycerophosphate disodium salt pentahydrate and 1.2% calcium acetate monohydrate for 15 s at a voltage of 350 V and frequency of 800 Hz. This will induce microarc oxidation of the implant surface that demonstrates a more optimal surface roughness than conventional anodized surface oxidation. Roughness, pore size, and thickness of this oxide layer can be modulated by its direct relationship with voltage.

6.2.2 Additive methods

In this subsection, additive surface modification methods will be discussed. These are those which involve impregnation or coating of the titanium implant.

6.2.2.1 Ceramic coating methods

There are a variety of ceramic coating methods utilized as described below.

1. ***Plasma spraying.*** Plasma spraying offers the advantage of a thin and even coat (50−70 μm). It has surmounted the greatest interest in combination with acid etching techniques and is arguably the most preferable surface coating method to date. This method utilized a stream of HA powder blown at a very high temperature, ionizing the powder and projecting it onto the titanium implant's surface. This method uses a carrier gas that ionizes the forming plasma and superheats the particles of HA: This partially melted substrate is propelled at the surface of the implant, fabricating around 50-μm thick coatings (Garg et al., 2012). The retention of this coat relies on mechanical interlocking; thus, plasma spraying is often prefaced by a grit blasting or etching modification. The subtractive method of choice is DAE in most conventional products due to the reduced contamination yield from acid treatment (Santiago et al., 2005). The adhesion between the metal and the coating is still considered weak; thus, consolidation with optimal roughness for osseointegration as well as mechanical interlocking of plasma sprayed coats should be investigated.
2. ***Vacuum deposition***. In vacuum deposition, the implant is placed in a vacuum chamber and is bombarded with the coating substrate, resulting in sputtered or ablated atoms being coated on the surface. Sputtering can be accomplished by a variety of methods, including ion beam, radiofrequency, and pulsed laser deposition (Garg et al., 2012).
3. ***Sol−gel and dip-coating.*** In this method, the coating is heated at 800−900°C to melt the carrier glass to obtain its bonding to the metallic substrate. The implant is placed into this solution and is withdrawn at a prescribed rate. It is then sintered to form a more dense coating (Garg et al., 2012).
4. ***Electrolytic process***. In this process, the titanium implant is placed in a solution arranged in a galvanic cell. The solution contains coating nanoparticles in a mixture of phosphoric acid and calcium nitrate with a Ca:P molar ratio of 1.67 in the presence of all liquid crystalline phase including surfactants, water, and water-insoluble organic solvents. This phase acts as a template, hindering deposition and limiting the particle size of 5 nm, yielding a relatively evenly distributed coating. The surfactants are burned away at 550°C for 5 min in an atmosphere of nitrogen, resulting in a thin layer of HA. The presence of TiO has been reported to improve osteogenesis at the site of the implant.

6.2.2.2 Fluoride-modified surface

Surface hydrophilicity is increased when treated with fluoride containing acids. This produces osseointegration with shorter healing periods as well as firmer bone anchorage when compared to unmodified implants (Roccuzzo and Wilson Jr., 2009). This is understood to be due to formation of fluoridated HA and fluorapatite in the tissue. There was a significant increase resulting from this modification in all seeding rate, alkaline phosphatase activity and incorporation of new collagen in the newly formed boney matrix. A study in rabbits demonstrated an increase in gene expression levels of osteogenic markers and higher bone mineral densities with fluoride surface modification. In one clinical trial, in conjunction with sand blasting, fluoride treatment allowed for the successful functional loading of the implant within 3 weeks in a maxillary molar (Roccuzzo and Wilson Jr., 2009). Another 5-year study demonstrated high survival rates with early loading (Mertens and Steveling, 2011).

6.2.2.3 Biochemical and organic compound coating

There are two approaches towards biochemical methods of surface modification discussed in this section: coats which promote cell-adhesion and coats that promote osteotropic effects.

There has been a focus on studying compounds such as proteins and peptide that may promote bone formation. These are referred to as bone morphogenetic proteins or compounds. Recombinant human bone morphogenetic protein 2 (rhBMP-2) showed a significant enhancement of bone affinity and healing capacity when coated on an oxidized titanium surface versus an uncoated, oxidized titanium implant (Yeo, 2014). Peptide coatings also show promising cell-adhesion from osteoblasts while not eliciting an adaptive immune response. While Kim et al. demonstrated enhancement due to rhBMP-2, there have not been many more studies that have reproduced this result (Yeo, 2014). To date, there are no clinical trials utilizing this surface implant modification. Applications in cell-adhesion coating utilize molecules like fibronectin, vitronectin, type I collagen, osteogenin, and bone sialoprotein (Yeo, 2014).

6.3 Prospective surface modification methods

6.3.1 Discrete crystalline deposition (DCD)

Calcium phosphate particles of 20–100 nm are deposited on a dual acid etched surface by a sol–gel technique. There are reports of higher adhesive force to the implant surface by the calcium phosphate particles, which make up 50% of the surface after coating. It is speculated that this process reduces incidence of periimplantitis due to a lower bacterial adhesion when compared to SLA-modified implants (Smeets et al., 2016).

Superior mechanical results were reported in one study by Mendes et al. (2009) where DCD surface-modified implants and controls were inserted in the femur of rats. Disruptive forces were induced on the bone-implant interface after 9 days in vivo, showing higher bone-implant interface in DCD implants when compared to the control (Mendes et al., 2009). A 1-year clinical trial followed 139 DCD implants placed in 42 patients, resulting in reports of a 99.4% survival rate with mean marginal bone resorption of 1.01 mm. These studies had no comparative controls and further observation of these studies are needed (Mendes et al., 2009).

6.3.2 Laser ablation

This method addresses interest in integration of surrounding soft tissue into the implant surface. Nanoscale surface manufacturing techniques are implemented on the implant collar to promote gingival attachment. The collar is processed with a laser to generate a pattern of micro- and nanoscale channels, which have been

proposed to act as a biological seal, facilitating connective tissue attachment and bone, while inhibiting epithelial downgrowth (Pecora et al., 2009).

One animal study by Nevins et al. reported connective tissue formation around laser-ablated abutments, which were organized in a perpendicular arrangement, yielding a dense cervical seal that prevented apical migration of the junctional epithelium (Nevins et al., 2012).

A controlled clinical trial evaluated 20 laser-ablated implants placed in 15 patients with control implants manufactured with conventional machined collars inserted neighboring them. Results report a mean probing depth of 2.3 mm for laser-lock implants as opposed to 3.6 mm for control implants with crestal bone loss of 0.59 mm when compared to 1.94 mm for controls. This suggests significant development of connective tissue around the laser-ablated implant collar. These benefits are in line with a high 2-year survival rate consistent with that of conventional implants (96.1%) (Farronato et al., 2014).

6.3.3 Photofunctionalization

In photofunctionalization, dental implant surfaces undergo a UV treatment which alters titanium dioxide on the surface. It is thought to enhance bioactivity and osseointegration, promoting interactions of cells and proteins at a molecular level: a property defined as osteoconductivity in a study by Gao et al. (2013).

This group used a dog model to investigate wettability induced by UV-A irradiation. The study reports 2 weeks of enhanced healing due to UV radiation but no significant healing rates after 4 weeks.

Currently, clinical data are very limited; however, one case followed up seven implants placed in four patients with compromised bone and reported significant increases in implant stability quotient of the photofunctionalized implants.

6.3.4 Extracellular matrix protein coating

It is thought that coating the implant with extracellular matrix proteins may act as a guide for osteoprogenitor cell migration towards the implant surface via the action of integrins on the cell surface and Arg-Gly-Asp (RGD) motifs on fibronectin. Reports show an increase in bone volume and mineralization for collagen type II-coated implants compared to uncoated controls in one study in dogs (Murray et al., 2014). Little clinical evidence exists which has found no significant difference compared to controls (Smeets et al., 2016).

6.3.5 Peptide coating

The intent is to provide structural peptides in the site to facilitate cell-adhesion in osseointegration or provide an antibacterial coating. Arg-Gly-Asp RGD is an important peptide in the sequence of extracellular matrix proteins released by fibroblasts to induce osteogenesis. RGD is the binding site for integrin receptors in the adoption and chemotaxis of osteogenic cells. One study in dogs demonstrated a

significantly higher degree of bone-implant contact with RGD coating compared to machined titanium implants (Garg et al., 2012).

Long-term implant failure is attributed to periimplantitis. Innovations in bactericidal coatings are being examined in peptide-coating methods. Specifically, GL13K is a defense-derived protein found in saliva and has compatibility with titanium in vitro. However, this modification method requires further research before becoming a clinical reality (Garg et al., 2012).

6.3.6 Antisclerostin immunoglobulin coating

The progression of osteogenesis begins with woven bone formation which is transformed into trabecular bone. A series of messenger molecules modulates osteoblast and osteoclast interactions: sclerostin, a messenger molecule secreted by osteocytes to inhibit osteogenesis by blocking osteoblastic bone formation, provides a negative feedback to control the progression of osteogenesis, acting as a bone morphogenetic antagonist. Administration of antibodies that block sclerostin may improve bone anchorage of titanium implants in patients with osteoporosis or poor bone quality. No clinical studies are yet available in this field (Smeets et al., 2016).

6.3.7 Organic compounds coatings

HA coatings provide reliable local drug delivery systems for various organic compounds.

Statins are suggested to liberate bone morphogenetic proteins, thus promoting osteogenesis.

Bisphosphonates are antiresorptive drugs, inhibiting osteoclast activity (Drake et al., 2008). This implementation in coating is indicated in patients with metastatic bone disease or osteoporosis.

6.3.8 Nanotechnology coatings

There has been a significant number of studies in nanotechnology, which would provide a method by which dental implants could be designed with a surface roughness modification controlled at a nanoscale level, as well as incorporating growth factors and coatings optimally for the purpose of promoting protein adsorption and cell-adhesion (Tomsia et al., 2011).

Very few studies have reported modifications in roughness; however, deposition of nanoparticles like biomimetic calcium phosphate, alumina, titania, and zirconia is being investigated. It has been reported that nanocoating of smooth-surface implants by BMP-2 and collagen promotes periimplant bone formation better than uncoated smooth-surface titanium implants (Wennerberg and Albrektsson, 2010).

The advantages and disadvantages of different surface modification methods to improve the surface characteristics and biological performance of titanium and its alloys are summarized in Table 6.1.

Table 6.1 **Merits and demerits of surface modification methods of titanium and its alloys (John et al., 2016)**

	Acid etching and sand blasting	Surface coating	Alkali heat treatment	Plasma treatment	Ion implantation
Merits	• Eliminates contaminants from the surface • Selective removal of pollutants • Relatively long lasting	• Intermediate layer between the bone and implant surface	• Powerful bone-implant bonding • Creation of greatly bioactive layer • Encourages the apatite formation ability • Superior biocompatibility • Enhanced life span	• Increased osteoblast cell-adhesion • High durability • Economical	• High life span • Enhanced antibacterial activity • Improved wear resistance
Limitations	• Surface destructions • Changing mechanical characteristics • Mainly incorporated with other methods for surface treatment	• Cracking oxide layer • Less longevity	• Feasibility of ectopic bone development • High working temperature	• No remarkable chemical alternations	• Costly • Existence of high pollutants • Nonpermanent • Very deep profiles are muddled

6.4 Conclusion

Surface modifications can address a multitude of challenges in implant dentistry, especially in terms of compensating for poor bone quality and reducing healing time. Many preclinical studies demonstrate significant differences in results of various surface implant modifications; however, further clinical trials must be performed to confirm the clinical relevance of these findings.

Trends in surface implant modification research suggest that there is a deviation from improving on osseointegrative potential towards decreasing incidence of periimplantitis and improving success rates in poor bone quality. There also seems to be a trend in macro-design changes regarding collar modification for integrating soft tissue, reducing pocket depths and thus incidence of periimplant diseases.

Achieving optimal roughness is possible via many routes, among which the most conventional being the combined action of grit blasting and DAE, which increases surface area and wettability. Laser etchants may lead to further improvement and are more efficient at forming an optimal surface with less contaminants. It is likely that nanotechnology will one day be able to bring the optimization of this process to its pinnacle but an established methodology for this approach is still not available yet. One of the drawbacks of utilizing surface roughening in titanium is that all methods decrease surface purity as the machined, unmodified implant surfaces yield the highest purity titanium implant. Surface-modified implants provide superior implant stability when compared to a purer surfaced, machined implant. The significant decrease in contaminants in laser etching is promising in this regard. Otherwise, methods aimed to decrease decontamination while utilizing surface roughness should be further investigated.

The goals of future implant studies endeavor to design a surface which offers polyvalence, enhanced clinical behavior and improvement on current modifications which facilitate osseointegrative factors such as osteogenesis and cellular growth, as well as soft-tissue attachment and inhibition of microbial colonization on the implant surface to reduce the incidence of periimplant diseases.

References

Albrektsson, T., Brånemark, P.-I., Hansson, H.-A., Lindström, J., 1981. Osseointegrated titanium implants: requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. Acta Orthop. Scand. 52, 155–170.

Al-Nawas, B., Groetz, K., Goetz, H., Duschner, H., Wagner, W., 2008. Comparative histomorphometry and resonance frequency analysis of implants with moderately rough surfaces in a loaded animal model. Clin. Oral. Implants Res. 19, 1–8.

Al-Radha, A.S.D., Dymock, D., Younes, C., O'sullivan, D., 2012. Surface properties of titanium and zirconia dental implant materials and their effect on bacterial adhesion. J. Dent. 40, 146–153.

Ban, S., Iwaya, Y., Kono, H., Sato, H., 2006. Surface modification of titanium by etching in concentrated sulfuric acid. Dent. Mater. 22, 1115–1120.

Chai, W., Moharamzadeh, K., Brook, I., Van Noort, R., 2011. A review of histomorphometric analysis techniques for assessing implant-soft tissue interface. Biotech. Histochem. 86, 242−254.

Chai, W.L., Brook, I.M., Emanuelsson, L., Palmquist, A., Van Noort, R., Moharamzadeh, K., 2012. Ultrastructural analysis of implant−soft tissue interface on a three dimensional tissue-engineered oral mucosal model. J. Biomed. Mater. Res. A. 100, 269−277.

Cho, S.-A., Park, K.-T., 2003. The removal torque of titanium screw inserted in rabbit tibia treated by dual acid etching. Biomaterials. 24, 3611−3617.

Chou, B.-Y., Chang, E., 2001. Interface investigation of plasma-sprayed hydroxyapatite coating on titanium alloy with ZrO_2 intermediate layer as bond coat. Scr. Mater. 45, 487−493.

Coelho, P.G., Cardaropoli, G., Suzuki, M., Lemons, J.E., 2009. Histomorphometric evaluation of a nanothickness bioceramic deposition on endosseous implants: a study in dogs. Clin. Implant. Dent. Relat. Res. 11, 292−302.

Deppe, H., Warmuth, S., Heinrich, A., Körner, T., 2005. Laser-assisted three-dimensional surface modifications of titanium implants: preliminary data. Lasers Med. Sci. 19, 229−233.

Drake, M.T., Clarke, B.L., Khosla, S., 2008. Bisphosphonates: mechanism of action and role in clinical practice. Mayo Clinic Proceedings. Elsevier, Rochester, pp. 1032−1045.

Farronato, D., Mangano, F., Briguglio, F., Iorio-Siciliano, V., Riccitiello, F., Guarnieri, R., 2014. Influence of Laser-Lok surface on immediate functional loading of implants in single-tooth replacement: a 2-year prospective clinical study. Int. J. Periodontics Restorative Dent. 34, 79−89.

Fatehi, K., Moztarzadeh, F., Solati-Hashjin, M., Tahriri, M., Rezvannia, M., Ravarian, R., 2008. In vitro biomimetic deposition of apatite on alkaline and heat treated Ti_6A1_4V alloy surface. Bull. Mater. Sci. 31, 101.

Fatehi, K., Moztarzadeh, F., Tahriri, M., Khoshroo, K., Heidari, S., Sadeghi, A., 2014. Biomimetic synthesis, characterization, and adhesion properties of bone-like apatite on heat and alkaline-treated titanium alloy. Synth. React. Inorg., Metal-Org. Nano-Metal Chem. 44, 1535−1540.

Gaggl, A., Schultes, G., Müller, W., Kärcher, H., 2000. Scanning electron microscopical analysis of laser-treated titanium implant surfaces—a comparative study. Biomaterials. 21, 1067−1073.

Gao, Y., Liu, Y., Zhou, L., Guo, Z., Rong, M., Liu, X., et al., 2013. The effects of different wavelength UV photofunctionalization on micro-arc oxidized titanium. PLoS ONE. 8, e68086.

Garg, H., Bedi, G., Garg, A., 2012. Implant surface modifications: a review. J. Clin. Diagn. Res. 6, 319−324.

Glauser, R., Lundgren, A., Gottlow, J., Sennerby, L., Portmann, M., Ruhstaller, P., et al., 2003. Immediate occlusal loading of Brånemark TiUnite™ implants placed predominantly in soft bone: 1-year results of a prospective clinical study. Clin. Implant. Dent. Relat. Res. 5, 47−56.

Gobbato, L., Arguello, E., Martin, I.S., Hawley, C.E., Griffin, T.J., 2012. Early bone healing around 2 different experimental, HA grit-blasted, and dual acid-etched titanium implant surfaces. A pilot study in rabbits. Implant. Dent. 21, 454−460.

Gray, J.J., 2004. The interaction of proteins with solid surfaces. Curr. Opin. Struct. Biol. 14, 110−115.

Guo, C.Y., Matinlinna, J.P., Tang, A.T.H., 2012. Effects of surface charges on dental implants: past, present, and future. Int. J. Biomater. 2012, 5.

Jemat, A., Ghazali, M.J., Razali, M., Otsuka, Y., 2015. Surface modifications and their effects on titanium dental implants. BioMed Res. Int. 2015, 11.

John, A.A., Jaganathan, S.K., Supriyanto, E., Manikandan, A., 2016. Surface modification of titanium and its alloys for the enhancement of osseointegration in orthopaedics. Curr. Sci. 111, 1003.

Khosroshahi, M., Mahmoodi, M., Tavakoli, J., Tahriri, M., 2008. Effect of Nd:Yttrium-aluminum-garnet laser radiation on Ti_6Al_4V alloy properties for biomedical applications. J. Laser. Appl. 20, 209–217.

Lamolle, S.F., Monjo, M., Lyngstadaas, S.P., Ellingsen, J.E., Haugen, H.J., 2009. Titanium implant surface modification by cathodic reduction in hydrofluoric acid: surface characterization and in vivo performance. J. Biomed. Mater. Res. A. 88, 581–588.

Mendes, V.C., Moineddin, R., Davies, J.E., 2009. Discrete calcium phosphate nanocrystalline deposition enhances osteoconduction on titanium-based implant surfaces. J. Biomed. Mater. Res. A. 90, 577–585.

Mertens, C., Steveling, H.G., 2011. Early and immediate loading of titanium implants with fluoride-modified surfaces: results of 5-year prospective study. Clin. Oral. Implants Res. 22, 1354–1360.

Murray, I.R., West, C.C., Hardy, W.R., James, A.W., Park, T.S., Nguyen, A., et al., 2014. Natural history of mesenchymal stem cells, from vessel walls to culture vessels. Cell. Mol. Life Sci. 71, 1353–1374.

Nevins, M., Camelo, M., Nevins, M.L., Schupbach, P., Kim, D.M., 2012. Connective tissue attachment to laser-microgrooved abutments: a human histologic case report. Int. J. Periodontics Restorative Dent. 385–392.

Pecora, G.E., Ceccarelli, R., Bonelli, M., Alexander, H., Ricci, J.L., 2009. Clinical evaluation of laser microtexturing for soft tissue and bone attachment to dental implants. Implant. Dent. 18, 57–66.

Pető, G., Karacs, A., Pászti, Z., Guczi, L., Divinyi, T., Joób, A., 2002. Surface treatment of screw shaped titanium dental implants by high intensity laser pulses. Appl. Surf. Sci. 186, 7–13.

Roccuzzo, M., Wilson Jr., T.G., 2009. A prospective study of 3 weeks' loading of chemically modified titanium implants in the maxillary molar region: 1-year results. Int. J. Oral Maxillofac. Implants. 24, 65–72.

Santiago, A.S., Santos, E.A., Sader, M.S., Santiago, M.F., Soares, G.D.A., 2005. Response of osteoblastic cells to titanium submitted to three different surface treatments. Braz. Oral. Res. 19, 203–208.

Smeets, R., Stadlinger, B., Schwarz, F., Beck-Broichsitter, B., Jung, O., Precht, C., et al., 2016. Impact of dental implant surface modifications on osseointegration. BioMed Res. Int. 2016, 16.

Tomsia, A.P., Launey, M.E., Lee, J.S., Mankani, M.H., Wegst, U.G., Saiz, E., 2011. Nanotechnology approaches for better dental implants. Int. J. Oral. Maxillofac. Implants. 26, 25.

Wennerberg, A., Albrektsson, T., 2000. Suggested guidelines for the topographic evaluation of implant surfaces. Int. J. Oral Maxillofac. Implants. 15, 231–244.

Wennerberg, A., Albrektsson, T., 2010. On implant surfaces: a review of current knowledge and opinions. Int. J. Oral Maxillofac. Implants. 25, 63–74.

Xue, W., Liu, X., Zheng, X., Ding, C., 2005. In vivo evaluation of plasma-sprayed titanium coating after alkali modification. Biomaterials. 26, 3029–3037.

Yeo, I.-S., 2014. Reality of dental implant surface modification: a short literature. Open Biomed. Eng. J. 8, 114–119.

Yeo, I.S., Han, J.S., Yang, J.H., 2008. Biomechanical and histomorphometric study of dental implants with different surface characteristics. J. Biomed. Mater. Res., B: Appl. Biomater. 87, 303–311.

Characterization of biomaterials

7

Meisam Omidi[1,2], *Atena Fatehinya*[2], *Masomeh Farahani*[2], *Zahra Akbari*[2], *Saleheh Shahmoradi*[2], *Fatemeh Yazdian*[3], *Mohammadreza Tahriri*[4], *Keyvan Moharamzadeh*[5], *Lobat Tayebi*[4] and *Daryoosh Vashaee*[6]
[1]Shahid Beheshti University, Tehran, Iran, [2]Shahid Beheshti University of Medical Sciences, Tehran, Iran, [3]University of Tehran, Tehran, Iran, [4]Marquette University School of Dentistry, Milwaukee, WI, United States, [5]University of Sheffield, Sheffield, United Kingdom, [6]North Carolina State University, Raleigh, NC, United States

7.1 Introduction

A biomaterial is a natural or synthetic material that can be used for different applications such as tissue engineering, bioelectrodes and biosensors, drug delivery, gene therapy, diagnosis of disease, and the improvement of the healthcare. The main characteristic of all biomaterials is their ability to be in the living systems, although the effects of both the biomaterial on the host tissues, and the living system on the biomaterial lead to immunological interactions (local and systemic) and thereby may cause device failure, patient's distress, or even fatality; therefore the biomaterials must be biologically sustainable and biocompatible. Biocompatibility is the behavior of a material in a living tissue that does not cause an adverse effect such as producing a toxic, a physiological reactivity, or an immunological response. Biomaterials can be metals, ceramics, polymers, hydrogels, or composite materials that used vastly in literature for oral and dental tissue engineering or relevant applications (Razavi et al., 2015; Yazdimamaghani et al., 2014; Razavi et al., 2014b; Yazdimamaghani et al., 2013; Razavi et al., 2014a; Heidari et al., 2015). The first challenge in employing the right material for specific application is investigating the chemical, physical, and biological characteristics of it, which is the focus of this chapter.

7.2 Chemical characterization techniques

The determination and analysis of the chemical characteristics and molecular structures of biomaterials often is required for different applications. This section presents an overview of the important chemical characterization strategies and spectroscopic techniques, including infrared (IR), Raman, X-ray photoelectron spectroscopy (XPS), ultraviolet–visible (UV/Vis), nuclear magnetic resonance (NMR), mass spectrometry (MS), and their relative applications.

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00007-4
© 2017 Elsevier Ltd. All rights reserved.

7.2.1 Infrared spectroscopy

IR spectroscopy is a commonly used optical technique for identification of compounds and their chemical structure analysis. Diverse functional groups within a molecule absorb IR radiation at various frequencies; therefore IR spectroscopy infers the presence or absence of certain chemical functional groups in a molecule. This technique is nondestructive and can be applied to analyze gaseous, liquid, or solid materials. The energy of the IR photons is not adequate to cause transition of the valence electrons; however, the vibrational and rotational motions are excited in molecules by IR radiation. The spectra are achieved by passing (reflecting) a beam of IR radiation through (from) a material. The resulting spectrum consists of a plot of absorption, transmission, or refection intensity versus wavelength or frequency. The IR can be divided into three spectral subregions; the near extends from 800 to 2500 nm (NIR), the mid from 2500 to 15000 nm (MIR), and the far-IR from approximately 15,000 to 100,000 nm (FIR).

The most common type of IR instrument is the Fourier transform infrared (FTIR) spectrometer owing to its high signal-to-noise ratio, easy to use, and relatively low cost. An FTIR enables measuring all wavelengths at once; therefore the whole spectral information is gathered at the same time (Yu et al., 2009; Kassi et al., 2010). Fig. 7.1 demonstrates the principle operation of an FTIR. As shown, the FTIR is like a Michelson interferometer with the only difference that one of the mirrors is movable and is controlled by a motor. Therefore it is possible to change the time delay between the reflected beams off the two mirrors continuously using

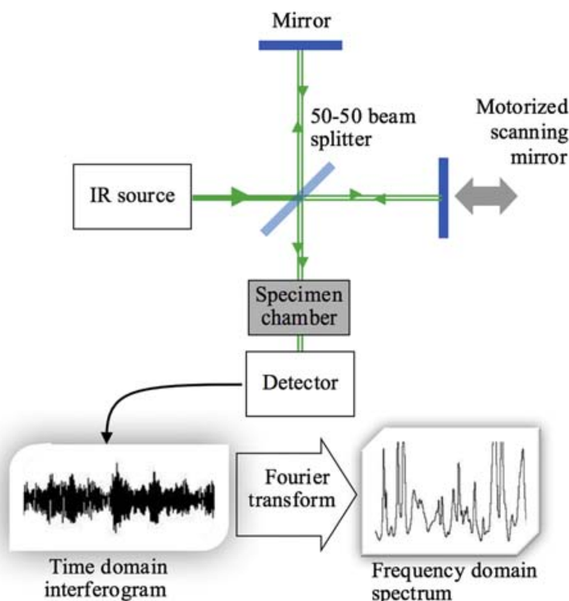


Figure 7.1 The basic configuration of the FTIR spectrometer.

the movable mirror. The specimen is located in front of the detector where the interferogram is recorded. The interferogram data are not useful as it is; however its Fourier transform reveals information about the spectral response of the specimen. The specimen chamber may be in a configuration to send out the transmitted or reflected beam.

FTIR spectroscopy is a valuable tool that is widely used by researchers. As a biomedical example, FTIR has been used for the investigation of the conversion degree in dental composites and the polymerization process (Ahuja and Scypinski, 2010; Araujo et al., 2015; Moraes et al., 2008; Ilie and Durner, 2014). Also, a specific technique named attenuated total reflection FTIR (ATR-FTIR) spectroscopy is frequently employed to characterize biomaterials. The main advantage of the ATR-FTIR is the feasibility of direct assessment without using any pretreatment or destructing the specimen during the preparation procedures (Durner et al., 2012).

7.2.2 Raman spectroscopy

Raman scattering, another nondestructive and noninvasive spectroscopic method and complementary to IR absorption spectroscopy, is employed to assess molecular vibrations and has considerable potential as an analytical technique in the characterization of biomaterials in any state (solid, liquid, or gas). Selection rules that govern the principles of Raman scattering and IR absorption spectroscopy are completely different. Thus there exist certain vibrational mode excitations that are allowed in IR spectroscopy but are forbidden in Raman spectroscopy. The Raman spectra are generated due to the interaction of the photons and the specimen molecules and are collected using optical filters form the scattered light. The Raman spectra contain information about the chemical species, molecular structures, and the conformation of the materials and provide a "fingerprint" for their identification. Generally, in a Raman spectrum, the Raman intensity is plotted versus the Raman shift. The Raman shift is defined as the difference of frequencies between the Raman scattered and the incident light beam. The C−S, S−S, C−C, N=N, and C=C groups are the examples which provide strong Raman bands (Brittain, 2011; Petry et al., 2003; Bazin et al., 2009).

7.2.3 X-ray photoelectron spectroscopy

XPS is a very potent technique, suitable for characterizing the elemental and chemical composition of the very top surface (1−10 nm) of any solid surface. The phenomenon is based on the photoelectric effect that refers to the surface bombardment with X-ray photons. The electron ejection occurs using a monochromatic beam of soft X-rays in an ultrahigh vacuum environment. The electrons are then emitted from the shell of the atoms and their kinetic energy and number are simultaneously measured by detectors. The difference of the photon energy and the sum of the electron kinetic energy and the work function determines the electron binding energy. The work function depends

on both the spectrometer and the material, so it is often treated as an adjustable instrumental correction factor. The resulting XPS spectrum consists of a plot of the detected number of photoelectrons as a function of the binding energy. The binding energy of the electrons is a characteristic of the elements, but it is also affected by the formal oxidation state, the local bonding environment such as the identity of the nearest-neighbor atoms, bonding hybridization to the nearest-neighbor atoms, etc. Therefore XPS is sensitive to the chemical nature of the materials and provides somewhat different results for different chemical binding states.

The use of XPS is limited in the analysis of biological materials as they have relatively short lifetimes in high vacuum and under radiation damage caused by X-ray photons although the ambient-pressure XPS is a current area of development. There are, nevertheless, vast applications of XPS in biomaterials characterizations. Many different studies have underlined potential of XPS to examine and analyze the types of biomolecules, some of which include proteins and peptides, lipids, enzymes, and DNA. XPS enables investigating the extent of the functionality and specific adsorption or binding of these biomolecules onto various surfaces; hence, it helps to develop biosensors, bioarrays, and biofouling controls for medical or dental applications. XPS has been also applied to the analysis of hard tooth tissue in restorative dentistry, including the effects of different treatments and chemical agents for the health of the tooth, and understanding of the mechanisms underpinning the interactions between the biomaterial and the hard tissue (Artyushkova and Atanassov, 2013; McArthur et al., 2014).

7.2.4 Ultraviolet–visible spectroscopy

UV–Vis spectroscopy, one of the earliest characterization techniques, can be utilized to measure the absorbance of UV to visible light by a material as a function of the wavelength. The UV region extends from approximately 190–350 nm and the visible region from 350 to 800 nm. The absorption happens duo to the electronic transitions from the ground state to the excited state and its magnitude depends on the Beer–Lambert law:

$$A = abc$$

In which A is the absorbance, a is the wavelength-dependent absorption coefficient, b is the path length through the solution in an analytical cell, and c is the molar concentration of the absorbing analyte.

This nondestructive spectroscopic method offers both qualitative and quantitative information about a liquid sample. According to the Beer–Lambert law the absorbance of a particular substance in a solution is directly proportional to its concentration; hence, the absorption spectroscopy can be used for a quantitative analysis. Also the absorptivity of a molecule is a wavelength-dependent parameter and its intensity depends on the chemical nature. Therefore the technique is an excellent analytical tool for the characterization and evaluation of many materials including

dental biomaterials and composites (Ikemura et al., 2008; Ikemura et al., 2010; Ikemura and Endo, 2010).

7.2.5 Nuclear magnetic resonance spectroscopy

NMR spectroscopy provides a nondestructive method for the research on chemical structures, conformations, and dynamics of biomolecules. In contrast to the previously discussed spectroscopy techniques, NMR probes the nuclei of atoms and not the electrons. A spin-nuclei mechanism occurs in NMR that is often based on the behavior of specific atoms (1H, 13C, 15N, 31P, 19F) exposed to an external magnetic field. The atomic nuclei with nonzero spins are NMR active in the magnetic field and generate two low and high energy spin states with the energy difference of ΔE.

The schematic diagram of the NMR technique is shown in Fig. 7.2. An external magnetic field, which is often very strong in the range of 1–20 T for modern NMR instruments, is used to induce the energy difference ΔE between the two spin states of the nuclei. The sample is simultaneously exposed to a radio frequency (RF) transmitter. Depending on the magnitude of the ΔE, different wavelength of the RF field is absorbed, which is detected by an RF receiving circuit. The ΔE is a function of the external magnetic field as well as the characteristics of the specific atoms (1H, 13C, 15N, 31P, 19F) exposed to the external magnetic field. The NMR spectrum is collected either by sweeping the magnetic field while the frequency of the RF radiation id fixed, or by varying the frequency of the RF radiation under fixed magnetic field.

An NMR spectrum is exhibited as plot of the signal intensity (from the RF receiver) against chemical shift. The chemical shift can be described as the ratio of the difference between the frequencies of a given sample and that of a reference

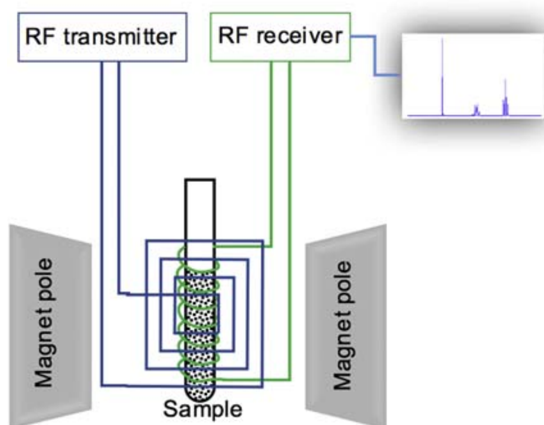


Figure 7.2 Schematic diagram of an NMR spectrometer.

compound, expressed in parts per million (ppm). The chemical shifts can reveal information about the covalent and noncovalent structure of the molecules.

NMR has also been applied in molecular level understanding of the mechanisms underpinning biomineralization, such as the onesused for bone repair and hard tissue regeneration of the teeth or in restorative dentistry (Goobes et al., 2007; Duer, 2015; Shetty and Kundabala, 2013).

7.2.6 Mass spectrometry

MS is a highly sensitive analytical technique which has been increasingly employed to characterize biomaterials based on the mass-to-charge ratio (m/z) of the gas-phase ions. The types of elements in the biomaterials and differences between the masses of the isotopic forms of a given atom can be determined by MS. A MS ionizes the material in the ionization source and employs mass-to-charge ratio (m/z) to separate the resulting ions in the mass analyzer. The ions are then detected, with or without fragmentation per their relative abundance.

The mass spectroscopy in conjunction with the chromatographic separation methods, including gas or liquid chromatography, has been often applied to characterize complex samples (Vékey et al., 2011).

Studies indicate that secondary ion mass spectrometry (SIMS) is a sensitive technique to characterize the biomaterials and biomineralized bone and dental tissues. Furthermore, the development of time-of-flight SIMS (TOF-SIMS) has also enhanced the mass spectroscopy sensitivity (Kanazawa et al., 2012; Ducheyne et al., 2015).

7.3 Physical characterizations techniques

7.3.1 Scanning electron microscope

Scanning electron microscope (SEM) is one of the common methods for imaging the microstructure and morphology of the materials. In SEM, an electron beam with low energy is radiated to the material and scans the surface of the sample. Several different interactions occur as the beam reaches and enters the material, which lead to the emission of photons and electrons from or near the sample surface (Sampath Kumar, 2013). In order to form an image the receiving signals produced from the electron–sample interactions are detected with different types of detectors depending on the mode of SEM being used. Different modes of SEM exist for characterization of materials (including biomaterials) such as the X-ray mapping, secondary electrons imaging, backscattered electrons imaging, electron channeling, and Auger electron microscopy (Sampath Kumar, 2013). A typical SEM consists of several components such as:

- The electron gun which is located on top of the column and emits electrons. The electrons are then accelerated to energy levels of typically 0.1–30 keV.

- Hairpin tungsten gun which makes a high diameter electron beam to form high-resolution images.
- Electromagnetic lenses and apertures which focus and shape the electron beam to form a small concentrated electron spot on the sample.
- A high-vacuum environment that allows electrons moving without being scattered or absorbed by the air.

7.3.2 Transmission electron microscope

Transmission electron microscopy (TEM) is often used to acquire direct information about the morphology, crystal structural, and composition of biomaterials to higher resolution than what is usually achievable with SEM. In this method, electrons are emitted from an electron gun located in a vacuum chamber and directed by electrostatic lenses onto the specimen (Sampath Kumar, 2013). Due to the interactions of the electrons with the sample, depending on the sample density, some electrons undergo scattering (or absorption), and some others pass through the sample. The sample must be thin enough so that the number of the transmitted electrons is sufficient for imaging. These electrons pass through the sample and hit the detector, such as a florescent screen, at the bottom of the microscope and an image. The image intensity follows the sample density. The denser the sample, the less electron pass through it, and the darker image is generated (Agrawal et al., 2013).

Achieving a high-resolution image in nanometer or less scale is the most important advantage of TEM. A small electron wavelength can lead to very high resolution in the order of less than a nanometer. The TEM has also some limitations like sample damage (due to the electron beam energy), low contrast in low atomic materials, and small depth of resolution that results in two-dimensional images (Sampath Kumar, 2013).

In comparison with SEM, TEM sample preparation, equipment operation, and data interpretation are more complex and require more time and skills (Agrawal et al., 2013).

7.3.3 Atomic force microscope

Atomic force microscopy (AFM) is a technique to image and analyze almost all kinds of surfaces (hard or soft, insulated or conductor, synthetic or natural). The AFM image reveals the three-dimensional features of the surface with spatial resolutions of nanometer or angstrom scale (Haugstad, 2012; Agrawal et al., 2013).

In AFM, a sharp tip is automatically dragged through the surface of the material and records its topographic image. The tip is attached to a flexible beam, and the force between the tip and the sample surface deflects the beam elastically. An optical system is used to record the beam deflection, which is proportional to the interatomic forces (Sampath Kumar, 2013).

Different modes of AFM have been developed based on the tip–surface interactions, as shown in Fig. 7.3:

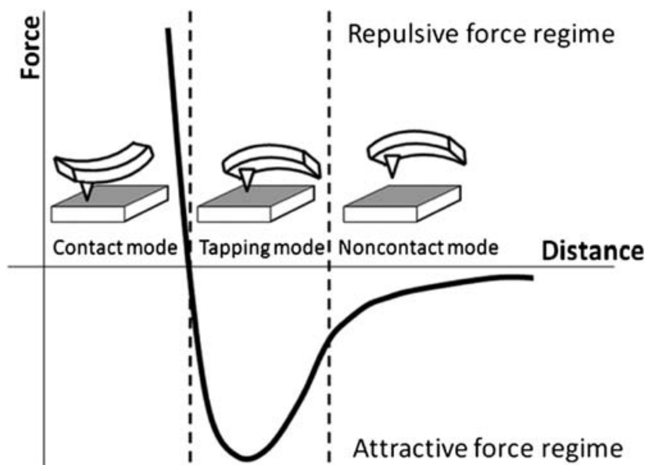


Figure 7.3 Schematic diagram of the different modes of AFM and the force versus distance for each mode (Sampath Kumar, 2013).

- Dynamic force or tapping mode, in which the tip oscillates and moves near the surface of the sample. The oscillations lead to periodic contact of the tip with the surface.
- Contact mode, in which the tip moves over the surface of the sample and experiences a strong repulsive force that bends the beam.
- Noncontact mode, in which the tip moves close to the surface (farther than the case of the dynamic mode and not touching the sample). The interaction forces in this mode are very low in the range of piconewtons. The noncontact mode does not damage the surface of the soft samples, which can happen in other modes due to the penetration of the probe into the sample (Sampath Kumar, 2013).

7.3.4 X-ray diffraction

X-ray diffraction (XRD) is a strong technique for the characterization of both the structure and composition of the materials. An X-ray diffractometer consists of an X-ray generator, a diffractometer which controls the direction of the X-ray beam and the position and orientation of the sample as well as the detector, a detector, and a computer to collect and analysis the data (Agrawal et al., 2013).

In this method, a monochromatic X-ray beam is radiated towards the material and the intensity of the diffracted beam is measured as a function of the angle of incident. The crystal interplanar space *d*, i.e., the distance between the atomic planes in the material, will be calculated as below:

$$2d = \sin\theta = \lambda$$

where λ is the wavelength of the X-ray used, and θ is the angle of incident. The interatomic plane spacing d and the diffraction angles reveal the material composition and structural features, such as shape and dimension of the unit cell, and the

width and shifts of the diffraction lines contain various information such as the crystallite sizes, residual stress, etc. (Sampath Kumar, 2013).

7.3.5 Contact angle measurement

The biocompatibility is one of the most important features of biomaterials that must be characterized carefully due the interactions with the tissues. Furthermore, the surface properties of biomaterials may also change due to exposure to the atmosphere or body environment. For example, this layers of oxide, carbon, or hydrocarbons may be formed on the surface of biomaterials even when they are exposed to normal atmosphere. Solid surfaces can be categorized in two groups of low- and high-energy surfaces. The first group has surface tension less than 100 dyn/cm, such as polymeric materials and organic compounds. In contrast, metallic and inorganic surfaces have surface tension of almost 200−500 dyn/cm and are placed in the second group (Sampath Kumar, 2013).

Quite often, special treatments are used to clean the surface of materials and change it into a hydrophilic surface. Contact angle measurement is one of the techniques to determine the hydrophilicity of the surfaces (Sampath Kumar, 2013). This measurement shows the physical properties of the surface or the coating such as adhesion, wettability, and cleanliness.

Wettability especially indicates the interaction between the liquid and the solid surface. Different techniques are used to measure this parameter, including direct optical methods and indirect force methods. Among these methods drop shape analysis and axisymmetric drop shape analysis are very common. The latter method is especially very accurate with a reproducibility of $\pm 2^\circ$ and is appropriate for contact angle measurement of ultra-small droplets (Yuan and Lee, 2013).

The angle between the surface and the tangent line at the contact point of the drop with the surface is called the contact angle as shown in Fig. 7.4. The contact angle is a quantitative value that determines the hydrophilicity of the surface. The angle decreases as the surface become more hydrophilic (Sampath Kumar, 2013). The contact angle can also indicate the amount of homogeneity or heterogeneity of a sample surface (Agrawal et al., 2013).

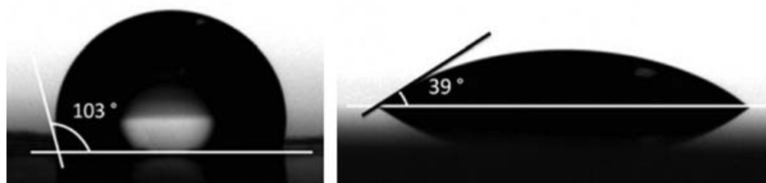


Figure 7.4 Contact angle image.

The spreading parameter, S, is a parameter that quantifies the wettability of a surface, and is defined as

$$S = \gamma_s - (\gamma_1 + \gamma_{s1})$$

when $S < 0$, liquid does not spread on the surface and the drop takes a spherical shape indicating partial or no wetting, whereas when $S > 0$, liquid spreads on the surface indicating a total wetting.

The contact angle can be measured in dynamic or static mode. The dynamic contact angle is produced during wetting, i.e., advancing angle or dewetting, i.e., receding angle. However, in the stats contact angle, the contact area between the surface and the liquid is not changed during the measurement. In the case of dynamic contact angle the advancing contact angle (θ_A) is simultaneously measured as the volume of the drop is increased on the surface while the receding contact angle (θ_R) is obtained when removing similar volume of liquid form the surface (dewetting). The advancing contact angle which occurs on wetting is usually larger than the receding angle which occurs on dewetting. The difference of the two angles, $\theta_A - \theta_R$, is the called hysteresis, which can be used to evaluate the roughness of the surface.

7.3.6 Mercury intrusion porosimetry

Porosity is described as the percentage of void space in a solid and is an important character of a biomaterial to permit cell migration and proliferation, cell nutrition, and vascularization for tissue regeneration. Additionally, a porous surface provides noteworthy mechanical stability by enhancing the mechanical interlocking between the inserted biomaterial in human body and the surrounding natural tissue. In recent years, porous biomaterials have been seriously researched and extensively used as scaffolds in tissue engineering/regeneration, as well as drug delivery system (Sampath Kumar, 2013). Although the minimum pore size needed to permit the in-growth of mineralized tissue is to the order of 50 μm, larger pore sizes can increase the depth of infiltration of mineralized tissues into the biomaterial; however, these larger pore sizes debilitate the mechanical characteristics. Smaller pore size creates larger surface area, causing higher adsorption of cell-inducing proteins. As the optimal pore size is reliant on the biomaterial employed and its application, it is very crucial to examine the porosity and pore size of biomaterials (Sampath Kumar, 2013; Karageorgiou and Kaplan, 2005; Park and Lakes, 2007).

Porous materials may have two vital pore types: open and closed pores. The closed pores are inside the bulk material and not reachable to outside fluids. The open pore is a channel that is connected to the surface of the material and reachable to fluids. The open pores are categorized into dead-end pores, which terminate inside the material, and interconnected/through pores, which make the passage of fluids feasible. Based on the pore diameter the International Union of Pure and Applied Chemistry (IUPAC) has categorized pores into three groups;

micropores ($D < 2$ nm), mesopores ($2 \text{ nm} < D < 50$ nm), and macropores ($D > 50$ nm). Interconnected macropores are a crucial factor for scaffolds, as they provide the potential to enable tissue in-growth (Park and Lakes, 2007).

Mercury intrusion porosimetry (MIP) is widely used for measuring pore size and porosity. The principle is based on the moving of nonwetting liquid through the pores needing pressure (P), which is inversely proportional to the diameter (D) of the pores (assuming the pore is cylindrical) and directly proportional to the surface tension of the liquid (γ) and the angle of contact (θ) with the solid surface as described by the Washburn equation (Park and Lakes, 2007):

$$P = -4\gamma \cos \theta / D$$

Mercury is nonwetting to most solid biomaterials and thus, is best choice for intrusion porosimetry, also because it will not spontaneously penetrate pores by capillary action. For mercury-solid system (θ is usually taken as 140° and γ of the mercury is about 0.48 N/m), the applied pressure is inversely proportional to the size of the pores (i.e., only slight pressure being needed to intrude mercury into large macropores), whereas much more prominent pressures are needed to force the required equilibrated mercury into small pores. It also demonstrates that under given external pressure P, mercury can oppose passage into pores smaller than D, but cannot oppose passage into pores larger than D. Therefore for any pressure, it can be described which pore sizes have been occupied with mercury and which sizes have not. Recording the dynamic intrusion of mercury into a porous structure under rigorously controlled pressures allows the generation of pore size/volume distributions within the porous biomaterial (Sampath Kumar, 2013).

Regular MIP analysis includes putting the biomaterial specimen into a container and emptying the container to eliminate contaminant gases and vapors. Subsequently, while still emptied, mercury is permitted to fill the container and then the pressure is enhanced towards encompassing, while recording the volume of mercury entering larger openings in the specimen. When ambient pressure has been reached, diameters approximately 12 mm have been filled with mercury. Afterward, the specimen container is placed in a pressure vessel and the volume of mercury that intrudes into the specimen due to enhancement in pressure is described by substituting pressure values into Washburn's equation. In order to have a volume sensitivity down to <1 μL a capillary tube is connected to the specimen cup, which serves both as the mercury reservoir during test and as an element of the mercury volume transducer, since just a small volume of mercury is needed to produce a long "string" in a little capillary. The combination of specimen cup and capillary stem is called a penetrometer. The principle source of mercury is eliminated subsequent to filling, and the pressure applied to the mercury in the capillary is transmitted from the far end of the capillary to the mercury encompassing the specimen in the specimen cup. The outer surface of the glass capillary stem is plated with metal to shape a coaxial capacitor alongside the mercury column in the stem. The length of the mercury column is the only variable in the value of the capacitance, and a little volume of mercury entering or leaving a small

capillary causes a huge change in length (and area) of the mercury column. Therefore capacitance calculations of the stem establish a very high determination sensitivity and resolution for the mercury volume passing into or out of the specimen cup for changes in the outside pressure. The graph of the applied pressures and the cumulative volumes of mercury intruded at each pressure is named the intrusion curve. Thus as pressure is decreased, mercury extrudes the pores and a graph of this process gives the extrusion curve. The intrusion and extrusion curves do not have the same path due to the shape of the pores and other physical phenomena. Notwithstanding, both the intrusion and extrusion curves provide data about the pore network in the analyzed biomaterial. Finally, it is worth mentioning that MIP analysis establishes a border pore size distribution more precisely and faster than other techniques (Sampath Kumar, 2013).

7.3.7 Gas adsorption measurements

The surface area, particle size, and porous structure of biomaterials can be commonly examined by the determination of the amount of adsorbed inert gas such as nitrogen, argon, krypton, or carbon dioxide on solid surfaces. The gas adsorption can be categorized as either physical or chemical sorption. The physical adsorption of a gas includes weak molecular forces, such as van der Waals forces, whereas formation of a chemical bond establishes the driving force in chemical sorption. The physically adsorbed gases can be effortlessly eliminated by decreasing the partial pressure, but the chemically sorbed gases are generally difficult to eliminate from the solid surface. The physical sorption is employed not only to calculate surface area, but also to probe the entire surface, including irregularities and pore interiors. The equilibrium relation between the amounts of adsorbed gas with the partial pressure of the gas at constant temperature is called gas adsorption isotherm. In order to calculate the surface area and for pore analysis, the Brunauer, Emmett, and Teller adsorption isotherm is typically utilized, which includes adsorption of a monolayer of nitrogen gas molecules onto the surface of the powder by cooling and then heating to vaporize (desorption) the monolayer to calculate the amount of nitrogen adsorbed. The surface area is calculated from adsorption measurements by assuming spherical shape and unimodal distribution of the nanoparticles, and the surface area can then be simply converted into particle size. It is important to point out that, before calculating, the specimen is heat-treated in a vacuum or flowing gas to eliminate any contaminants (Sampath Kumar, 2013; Park and Lakes, 2007).

7.4 Biological characterization technique

Biocompatibility of biomaterials can be assessed using the in vitro and in vivo tests. In vitro tests are performed outside of the body, often employing cell culture systems and the aim is to simulate the clinical situation. In vivo tests are performed

Table 7.1 Advantage and disadvantage of in vivo and in vitro test

In vitro tests	In vivo tests
Advantage	Advantage
Experimentally controllable	Simulate real body condition
Repeatable	Clinically relevant
Fast	
Inexpensive	
Simple	
Small amount of test material is required.	
Decreasing the use of animal	
Have a quality control	
Range of applications is vast	
Disadvantage	Disadvantage
Chronic effects cannot be tested	Expensive
	Time consuming
	Ethical and regulatory issues

Table 7.2 Recommended standards by ISO, USP, and ASTM

ISO	USP	ASTM
Cytotoxicity	Cytotoxicity	Cell culture cytotoxicity
Sensitization	Sensitization	Sensitization
Genotoxicity	Genotoxicity	Skin irritation
Implantation	Implantation	Systemic toxicity
Chronic toxicity	Chronic toxicity	Short-term implantation
Carcinogenicity	Carcinogenicity	Long-term implantation
	Irritation	Genotoxicity
	Systemic toxicity (acute)	Carcinogenicity
	Subchronic toxicity	

inside the body of the living organism and are more clinically relevant than the in vitro tests. In vitro tests are frequently carried out as screening tests prior to in vivo assessment as the in vitro tests are simpler and cheaper than the in vivo tests and there are ethical issues associated with the use of animals for research. The in vivo and in vitro tests both have advantages and limitations. Table 7.1 displays the advantages and disadvantages of in vivo and in vitro tests (Cogliano et al., 2012; Di Silvio, 2008; Hanks et al., 1996).

A summary of the biocompatibility tests as recommended by the American Society for Testing Materials (ASTM), the United States Pharmacopeia (USP), and the International Organization for Standardization (ISO) for implantable devices and biomaterials is provided in Table 7.2 (Koschwanez and Reichert, 2007).

7.4.1 In vitro characterization

In vitro test systems often include three main components: a biological system, cell/material contact, and a biological end-point (Moharamzadeh et al., 2009). Biological system can be either 2D or 3D cell cultures or a whole organ culture. Cell/material contact can be established in three different ways: direct contact, indirect contact such as Agar overlay technique, and contact through extracts and eluates. Various biological endpoints can be assessed to record the response of the biological system to the tested materials.

7.4.1.1 Cytotoxicity testing

Cytotoxicity assay is a test for analyzing the cytotoxic effects of the material and medical device on the living organism (Rosengren et al., 2005). It was the earliest and simplest in vitro technique that was designed for biocompatibility evaluation of materials. Examples of biological endpoints used in cytotoxicity testing include:

- Morphological assessment using histology or ultrastructural analysis of the cells using the SEM or TEM.
- Cell viability and proliferation assays such as the Alamar blue assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, neutral red uptake, propidium iodide assay, lactate dehydrogenase assay, bromodeoxyuridine incorporation assay, 3H-thymidine incorporation assay, and DNA or protein content measurement.
- Cell function assays such as the measurement of the release of inflammatory markers, glutathione determination, heat-shock protein assay and apoptosis assay (Zhang et al., 2009; Camps and About, 2003; Seifalian et al.).

7.4.1.2 Hemocompatibility testing

Hemocompatibility testing is a method for studying of adverse effects (e.g., thrombosis, hemolysis, activation of platelets, and complement pathway) and interaction between the blood and the material or the medical device (Ratner et al., 2012). One of the main issues about hemocompatibility is inadequate standards for anticoagulation. Therefore it can be difficult to classify the material as hemocompatible or non-hemocompatible (Braune et al., 2013). The important aspects in hemocompatibility testing include physical and chemical characteristics, stability of the materials, test conditions, controls, reference materials, and plausibility aspects.

7.4.1.3 Genotoxicity and carcinogenicity testing

Genotoxicity and carcinogenicity testing are for studying the genotoxic effect (e.g., gene mutation, change in DNA, and chromosomal alterations) and the carcinogen effects of biomaterial/implant device on the living system (Ratner et al., 2012; Landsiedel et al., 2009). Genotoxic effects can be direct (DNA target) or indirect (non-DNA target) (Graziano and Jacobson-Kram, 2015). Genotoxic effects of nanomaterials have been evaluated by micronucleus assay, comet assay, and Ames test (bacterial reversed mutation assay) (Landsiedel et al., 2009). Ames test is a method

for evaluating mutagenic effects of implant device, chemicals, and drug utilizing bacteria to detect carcinogens and mutagens. The advantage of Ames test is its simplicity, low cost and being a fast assay (Seifalian et al.). Comet assay is another test to detect early DNA damage and it is a fast, sensitive, and simple technique (Kim et al., 2013; Seifalian et al.).

7.4.1.4 Reverse transcription-polymerase chain reaction

Reverse transcription-polymerase chain reaction (RT-PCR) is a sensitive in vitro method and has a crucial role in medical science and biomaterial fields. RT-PCR is used for detecting and comparing the levels of mRNA and the surface proteins (Leong et al., 2007; Wang and Brown, 1999). PCR can be performed in real-time PCR and end-point PCR. Relative and absolute quantification (aqPCR) are two models of real-time RT-PCR. The main role of end-point PCR in biomaterial fields is the measurement of gene expression. The absolute quantification is preferred to end-point PCR for detecting levels of gene expression but end-point PCR is usually used because it has low cost and needs simple equipment (Mauney et al., 2005).

7.4.2 In vivo characterization

In vivo testing of biomaterials and medical devices include implantation testing, sensitization testing, irritation testing, and toxicity tests (Di Silvio, 2008).

7.4.2.1 Sensitization, irritation, and toxicity tests

The innate immune system and adaptive immune system have important roles in immune responses (Di Silvio, 2008). Sensitization is an increase in immune response and acute or delayed hypersensitivity to a biomaterial in living system (Overmier, 2002) where utilization of the material can result in irritant effects and localized inflammatory reaction on skin (Robinson et al., 1990b). Sensitization testing can be time consuming as it requires a film of material/chemical agent in saline solution to be placed on the skin of the test subject and the symptoms of adverse effect of biomaterial to be monitored over time (Dumitriu and Popa, 2013). Guinea Pig Maximization, Buehler, and Murine Local Lymph Node Assay are examples of sensitization testing (Robinson et al., 1990a; Kimber et al., 2001). Intracutaneous reactivity, materials-mediated pyrogen test, acute systemic toxicity, subacute systemic toxicity, subchronic systemic toxicity, and chronic toxicity tests are examples of other in vivo tests that are often performed on animals to assess the irritation and toxicity of the materials (Ratner et al., 2012).

7.4.2.2 Implantation testing

In implantation tests, materials are implanted into the connective tissue, muscle, or into the bone of an animal to assess the adverse and pathological effects of products and materials on the function and structure of the tissues (Ratner et al., 2012). The pathological effects can be analyzed at gross level or at microscopic level using

histological techniques to demonstrate tissue necrosis and apoptosis, cell proliferation, thrombus formation, collagen deposition, and endothelization (Seifalian et al.). Short-term and long-term testing can be used to assess the immediate and delayed tissue responses to the implanted materials.

7.4.2.3 Biodegradation test

Some degradable biomaterials can leach out degradation products (e.g., impurities, catalysts, and corrosion products) to the adjacent tissues and distant organs. Biodegradation of materials occur through hydrolytic mechanisms. In vivo biodegradation tests have an important role for studying of effects biodegradation of material and medical device in living tissue. Histological analysis can identify biological and tissue responses in the living system (Park and Bronzino, 2002; Seifalian et al.; Ratner et al., 2012).

Acknowledgment

The authors would like to thank the financial supports from Osteo Science Foundation, AFOSR (FA9550-12-1-0225), and NSF (EEC-1160483, ECCS-1351533, and CMMI-1363485).

References

Agrawal, C.M., Ong, J.L., Appleford, M.R., Mani, G., 2013. Introduction to Biomaterials: Basic Theory with Engineering Applications. Cambridge University Press, Cambridge.

Ahuja, S., Scypinski, S., 2010. Handbook of Modern Pharmaceutical Analysis. Academic Press, USA.

Araujo, L.O.F., Barreto, O., Mendonça, A.A.M., França, R., 2015. Assessment of the degree of conversion in light-curing orthodontic resins with various viscosities. Appl. Adhes. Sci. 3, 1.

Artyushkova, K., Atanassov, P., 2013. X-ray photoelectron spectroscopy for characterization of bionanocomposite functional materials for energy-harvesting technologies. ChemPhysChem. 14, 2071–2080.

Bazin, D., Chappard, C., Combes, C., Carpentier, X., Rouzière, S., André, G., et al., 2009. Diffraction techniques and vibrational spectroscopy opportunities to characterise bones. Osteoporos. Int. 20, 1065–1075.

Braune, S., Grunze, M., Straub, A., Jung, F., 2013. Are there sufficient standards for the in vitro hemocompatibility testing of biomaterials? Biointerphases. 8, 1–9.

Brittain, H.G., 2011. Characterization of Pharmaceutical Compounds in the Solid State. Academic Press, New York.

Camps, J., About, I., 2003. Cytotoxicity testing of endodontic sealers: a new method. J. Endodont. 29, 583–586.

Cogliano, V.J., Luebeck, E.G., Zapponi, G.A., 2012. Perspectives on Biologically Based Cancer Risk Assessment. Springer, USA.

Di Silvio, L., 2008. Cellular Response to Biomaterials. Elsevier Science, Amsterdam, The Netherlands.

Ducheyne, P., Healy, K., Hutmacher, D.E., Grainger, D.W., Kirkpatrick, C.J., 2015. Comprehensive Biomaterials. Newnes, Boston.

Duer, M.J., 2015. The contribution of solid-state NMR spectroscopy to understanding biomineralization: atomic and molecular structure of bone. J. Magn. Resonan. 253, 98−110.

Dumitriu, S., Popa, V., 2013. Polymeric Biomaterials: Structure and Function. CRC Press, Boca Raton.

Durner, J., Obermaier, J., Draenert, M., Ilie, N., 2012. Correlation of the degree of conversion with the amount of elutable substances in nano-hybrid dental composites. Dent. Mater. 28, 1146−1153.

Goobes, G., Stayton, P.S., Drobny, G.P., 2007. Solid state NMR studies of molecular recognition at protein-mineral interfaces. Prog. Nucl. Magn. Resonan. Spectrosc. 50, 71.

Graziano, M.J., Jacobson-Kram, D., 2015. Genotoxicity and Carcinogenicity Testing of Pharmaceuticals. Springer International Publishing, Cham, NY.

Hanks, C.T., Wataha, J.C., Sun, Z., 1996. In vitro models of biocompatibility: a review. Dent. Mater. 12, 186−193.

Haugstad, G., 2012. Overview of AFM. Atomic Force Microscopy. John Wiley & Sons, Inc, New Jersey.

Heidari, F., Bahrololoom, M.E., Vashaee, D., Tayebi, L., 2015. In situ preparation of iron oxide nanoparticles in natural hydroxyapatite/chitosan matrix for bone tissue engineering application. Ceram. Int. 41, 3094−3100.

Ikemura, K., Endo, T., 2010. A review of the development of radical photopolymerization initiators used for designing light-curing dental adhesives and resin composites. Dent. Mater. J. 29, 481−501.

Ikemura, K., Ichizawa, K., Jogetsu, Y., Endo, T., 2010. Synthesis of a novel camphorquinone derivative having acylphosphine oxide group, characterization by UV-VIS spectroscopy and evaluation of photopolymerization performance. Dent. Mater. J. 29, 122−131.

Ikemura, K., Ichizawa, K., Yoshida, M., Ito, S., Endo, T., 2008. UV-VIS spectra and photoinitiation behaviors of acylphosphine oxide and bisacylphosphine oxide derivatives in unfilled, light-cured dental resins. Dent. Mater. J. 27, 765−774.

Ilie, N., Durner, J., 2014. Polymerization kinetic calculations in dental composites: a method comparison analysis. Clin. Oral Invest. 18, 1587−1596.

Kanazawa, E., Townsend, G., Takayama, H., 2012. New Directions in Dental Anthropology: Paradigms, Methodologies and Outcomes. University of Adelaide Press, Adelaide.

Karageorgiou, V., Kaplan, D., 2005. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials. 26, 5474−5491.

Kassi, S., Didriche, K., Lauzin, C., Vaernewijckb, X.D.G.D.E., Rizopoulos, A., Herman, M., 2010. Demonstration of cavity enhanced FTIR spectroscopy using a femtosecond laser absorption source. Spectrochim. Acta A: Mol. Biomol. Spectrosc. 75, 142−145.

Kim, H.R., Park, Y.J., Shin, D.Y., Oh, S.M., Chung, K.H., 2013. Appropriate in vitro methods for genotoxicity testing of silver nanoparticles. Environ. Health Toxicol. 28, e2013003.

Kimber, I., Basketter, D., Berthold, K., Butler, M., Garrigue, J.-L., Lea, L., et al., 2001. Skin sensitization testing in potency and risk assessment. Toxicol. Sci. 59, 198−208.

Koschwanez, H.E., Reichert, W.M., 2007. In vitro, in vivo and post explantation testing of glucose-detecting biosensors: current methods and recommendations. Biomaterials. 28, 3687−3703.

Landsiedel, R., Kapp, M.D., Schulz, M., Wiench, K., Oesch, F., 2009. Genotoxicity investigations on nanomaterials: methods, preparation and characterization of test material, potential artifacts and limitations—many questions, some answers. Mut. Res./Rev. Mut. Res. 681, 241−258.

Leong, D.T., Gupta, A., Bai, H.F., Wan, G., Yoong, L.F., Too, H.-P., et al., 2007. Absolute quantification of gene expression in biomaterials research using real-time PCR. Biomaterials. 28, 203−210.

Mauney, J.R., Jaquiéry, C., Volloch, V., Heberer, M., Martin, I., Kaplan, D.L., 2005. In vitro and in vivo evaluation of differentially demineralized cancellous bone scaffolds combined with human bone marrow stromal cells for tissue engineering. Biomaterials. 26, 3173−3185.

McArthur, S.L., Mishra, G., Easton, C.D., 2014. Applications of XPS in biology and biointerface analysis. Surface Analysis and Techniques in Biology. Springer, New York.

Moharamzadeh, K., Brook, I.M., Van Noort, R., 2009. Biocompatibility of resin-based dental materials. Materials. 2, 514−548.

Moraes, L.G.P., Rocha, R.S.F., Menegazzo, L.M., Araújo, E.B.D., Yukimito, K., Moraes, J.C.S., 2008. Infrared spectroscopy: a tool for determination of the degree of conversion in dental composites. J. Appl. Oral Sci. 16, 145−149.

Overmier, J.B., 2002. Sensitization, conditioning, and learning: can they help us understand somatization and disability? Scand. J. Psychol. 43, 105−112.

Park, J., Lakes, R.S., 2007. Biomaterials: An Introduction. Springer Science & Business Media, New York.

Park, J.B., Bronzino, J.D., 2002. Biomaterials: Principles and Applications. CRC Press, Boca.

Petry, R., Schmitt, M., Popp, J., 2003. Raman spectroscopy—a prospective tool in the life sciences. ChemPhysChem. 4, 14−30.

Ratner, B.D., Hoffman, A.S., Schoen, F.J., Lemons, J.E., 2012. Biomaterials Science: An Introduction to Materials in Medicine. Elsevier Science, Waltham.

Razavi, M., Fathi, M., Savabi, O., Razavi, S.M., Heidari, F., Manshaei, M., et al., 2014a. In vivo study of nanostructured diopside (CaMgSi 2 O 6) coating on magnesium alloy as biodegradable orthopedic implants. Appl. Surf. Sci. 313, 60−66.

Razavi, M., Fathi, M., Savabi, O., Vashaee, D., Tayebi, L., 2015. In vivo assessments of bioabsorbable AZ91 magnesium implants coated with nanostructured fluoridated hydroxyapatite by MAO/EPD technique for biomedical applications. Mater. Sci. Eng. C. 48, 21−27.

Razavi, M., Fathi, M.H., Savabi, O., Vashaee, D., Tayebi, L., 2014b. Biodegradation, bioactivity and in vivo biocompatibility analysis of plasma electrolytic oxidized (PEO) biodegradable Mg implants. Phys. Sci. Int. J. 4, 708.

Robinson, M.K., Nusair, T.L., Fletcher, E.R., Ritz, H.L., 1990a. A review of the Buehler guinea pig skin sensitization test and its use in a risk assessment process for human skin sensitization. Toxicology. 61, 91−107.

Robinson, M.K., Nusair, T.L., Robert Fletcher, E., Ritz, H.L., 1990b. A review of the Buehler guinea pig skin sensitization test and its use in a risk assessment process for human skin sensitization. Toxicology. 61, 91−107.

Rosengren, A., Faxius, L., Tanaka, N., Watanabe, M., Bjursten, L.M., 2005. Comparison of implantation and cytotoxicity testing for initially toxic biomaterials. J. Biomed. Mater. Res. A. 75A, 115−122.

Sampath Kumar, T.S., 2013. Physical and chemical characterization of biomaterials A2. In: Bandyopadhyay, A., Bose, S. (Eds.), Characterization of Biomaterials. Academic Press, Oxford, Chapter 2.

Seifalian, A.M., Salmasi, S., Prinsloo, N., Kalaskar, D.M., 2014. Inorganic biomaterials characterization. In: Zhang, X.C. (Ed.), Inorganic Biomaterials: Structure, Properties and Applications. Smithers Rapra, Shrewsbury, pp. 45−87.

Shetty, N., Kundabala, M., 2013. Biominerals in restorative dentistry. J. Interdiscipli. Dent. 3, 64.

Vékey, K., Telekes, A., Vertes, A., 2011. Medical Applications of Mass Spectrometry. Newnes, Oxford.

Wang, T., Brown, M.J., 1999. mRNA quantification by real time TaqMan polymerase chain reaction: validation and comparison with RNase protection. Anal. Biochem. 269, 198−201.

Yazdimamaghani, M., Pourvala, T., Motamedi, E., Fathi, B., Vashaee, D., Tayebi, L., 2013. Synthesis and characterization of encapsulated nanosilica particles with an acrylic copolymer by in situ emulsion polymerization using thermoresponsive nonionic surfactant. Materials. 6, 3727−3741.

Yazdimamaghani, M., Vashaee, D., Assefa, S., Shabrangharehdasht, M., Rad, A.T., Eastman, M.A., et al., 2014. Green synthesis of a new gelatin-based antimicrobial scaffold for tissue engineering. Mater. Sci. Eng. C. 39, 235−244.

Yu, P., Jonker, A., Gruber, M., 2009. Molecular basis of protein structure in proanthocyanidin and anthocyanin-enhanced Lc-transgenic alfalfa in relation to nutritive value using synchrotron-radiation FTIR microspectroscopy: a novel approach. Spectrochim. Acta A: Mol. Biomol. Spectrosc. 73, 846−853.

Yuan, Y., Lee, T.R., 2013. Contact angle and wetting properties. In: Bracco, G., Holst, B. (Eds.), Surface Science Techniques. Springer, Berlin, Heidelberg.

Zhang, H., Shen, Y., Ruse, N.D., Haapasalo, M., 2009. Antibacterial activity of endodontic sealers by modified direct contact test against *Enterococcus faecalis*. J. Endodont. 35, 1051−1055.

This page intentionally left blank

Biocompatibility of dental biomaterials

8

Mohamed S. Ibrahim[1,2], *Noha A. El-Wassefy*[2] *and Dina S. Farahat*[2]
[1]Marquette University School of Dentistry, Milwaukee, WI, United States,
[2]Faculty of Dentistry, Mansoura University, Mansoura, Egypt

8.1 Introduction

The term "biocompatibility" is used to describe the proper biological requirements of a biomaterial for medical usage. Biocompatibility of materials can be defined as "the property of not causing any opposing tissue responses" (Anderson, 2011). Harmful tissue reactions can trigger biological mechanisms that can cause failure of the biomaterial or prosthesis and prove harm to the host. Another possible definition is "the materials ability to act with a proper host response in a definite purpose."

The safety assessment of a biomaterial or prosthesis is mostly considered to be the same as its biocompatibility assessment. Biocompatibility assessment is a gauge of the extent and degree of the unfavorable changes of the host homeostatic mechanisms. The primary constituent of a material's biocompatibility is that, whatever the anticipated purpose of a material is, it shall do no harm.

Biomaterials can only accomplish functions if they elicit a response from the contact tissues, this tissue response should be compatible with and/or positively encourage those function. For a long-term implantable device, its biocompatibility denotes the ability to achieve its proposed functions, without provoking any objectionable local or systemic effects. However, for tissue engineering scaffolds, the use of inert materials has limited benefit.

Therefore biocompatibility can be redefined as "The ability of a biomaterial to achieve its anticipated purpose in medical therapy, without producing any objectionable local or systemic effects in the host or therapy, but rather, creating the highest suitable positive tissue response and enhancing the clinical implementation of therapy."

8.2 Concepts of biocompatibility testing

In the last several decades, biological risks that may be associated with the use of dental materials have diverted attention towards the importance of ensuring the safety of a material, as well as its effectiveness. Many experts and standards organizations have proposed tests and regulations to assure the compatibility of different materials with biological tissues. Standards suggested by different organizations,

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00008-6
© 2017 Elsevier Ltd. All rights reserved.

such as the Food and Drug Administration (FDA) and International Organization for Standardization (ISO), provide better comparability of results and may be preferred in many situations. Because of the different biological side effects that may arise from the interaction of a dental material with biological tissues, biocompatibility of the material cannot be established using a single test. It has to be done following a planned approach that consists of several sophisticated biological tests. Different standards, such as ISO 7405 Standard, ANSI/ADA Document No. 41, and ISO 10993 Standard, have provided the guidelines for biological testing of dental and medical devices. Evaluation of biocompatibility usually occurs in a linear progression from in vitro studies, to animal studies, and finally to usage tests.

8.3 In vitro tests

In vitro tests are those done outside the body of a living organism where the material under investigation is placed in direct or indirect contact with cells or an isolated component of a living system. There are two types of cells that can be used: primary cells and continuous cell lines. Primary cells are harvested from a living organism then directly established for growth in an artificial environment forming a cell culture. They grow for only a limited time in culture, but may retain many of the cell features in vivo. Continuous cell lines are transformed primary cells that can divide an indefinite number of times. Because of their transformation, these cells may not retain all the in vivo characteristics, but they consistently exhibit many features that they do retain. In vitro tests are generally quicker, cheaper, and more reproducible than in vivo tests. They allow for a controlled cellular environment and the detailed determination of the cellular response to test materials. However, the relevance of in vitro tests to the clinical scenarios remains questionable (Sakaguchi and Powers, 2012; Wataha, 2012).

8.3.1 Cytotoxicity tests

The International Standard (ISO 10993-5) provided basic guidelines for testing cytotoxicity of medical devices. To measure the cytotoxicity of a material a sample of the material or an extract is incubated with cultured cells for a specific amount of time and then the effect of the material on cells is evaluated. Negative and positive controls are placed in separate cultures and tested simultaneously following the same procedures used with test materials. Testing can be done in a variety of ways including the direct or indirect contact tests. In direct contact tests, samples of the material are placed in cultures directly contacting cells with no barriers in between. This test mimics the physiologic circumstances, but damage to cells might occur if the sample was moved or highly soluble toxicants are leached from it (Ratner et al., 2004).

In various clinical situations the materials used are not placed in direct contact with cells, so tests that use barriers (indirect tests) have been developed to assess

toxicity in such situations. An agar overlay test is an indirect test that is frequently used to evaluate toxicity of different materials. This involves adding culture medium containing 2% agar to cultured cells, then allowing the agar to solidify. A sample of the material is placed on the agar surface which acts as a barrier between the material and the cells. A vital stain (a stain that only stains vital cells) is used for visualization of any effects that a toxicant, diffusing from the sample through the agar barrier, might have on the cells and thus measures cytotoxicity (ISO, 2009; Ratner et al., 2004; Sjögren et al., 2000). To further simulate the oral environment, *dentin barrier cytotoxicity* tests have been introduced which use dentin discs as barriers between the material and the cells. The aim is to mimic the clinical situation and measure the ability of molecules, leached from a material, to traverse the dentinal tubules towards the pulp and the decrease in their concentration due to diffusion and adsorption through the dentine layer (Galler et al., 2005; Schmalz and Schweikl, 1994). Indirect contact tests provide a better concentration gradient of leachable toxicants and protect cells from physical damage, however, they require the use of a sample with a flat surface and a thermal shock may occur when using agar (Ratner et al., 2004).

The cytotoxic effect of a material is determined qualitatively or quantitatively, the later being more recommended. Qualitative assessment is achieved by microscopic inspection of morphological changes that may have been caused by the material such as vacuolization, cellular detachment, and cell lysis. For this sort of assessment the material is incubated with a monolayer of cells for the specified time, then the material is removed, cells are fixed and stained for microscopic examination. Live cells appear properly stained and attached to the culture plate, nevertheless, dead cells detach from the plate and are lost during fixation. Accordingly, toxicity is measured by the lack of cells below and around the borders of the sample. Damaged cells, that display anomalous morphology caused by material cytotoxicity, are detected at the boundaries between live and dead cells during microscopic investigation. Cell damage may be presented as increased vacuolization due to uptake of fluids and toxins by lysozymes, rounding of cells resulting from loss of attachment or swelling due to osmotic pressure.

A quantitative assessment of cytotoxicity is accomplished by measuring a series of different parameters; for example, the amount of "surviving" cells, cell proliferation, cell metabolism, protein, or DNA synthesis, enzyme activity, or the synthesis of inflammatory mediators. One of the most commonly used quantitative tests is the *methyl thiazolyl tetrazolium assay (MTT)* which measures the viability of cells via metabolic activity. It is a colorimetric assay which relies on the basis that mitochondrial dehydrogenase within living cells can reduce the yellow, water-soluble MTT into purple crystals of formazan. The quantity of crystals formed is determined by spectrophotometry and is directly related to the number of viable cells and their activity (van Meerloo et al., 2011). Membrane integrity tests are also frequently used to measure cytotoxicity. These tests measure the ease by which a stain passes through the cell membrane based on the concept that a loss in membrane permeability is comparable to cell death. For example, trypan blue stain and fluorescent probes such as propidium iodide are excluded by viable cell membranes but

are able to traverse compromised cell membranes thereby selectively staining nonvital cells (Cho et al., 2008). The *neutral red uptake* assay utilizes "neutral red" dye which is a vital dye that is taken up and retained by lysosomes of only vital cells but cannot be retained by damaged cells. Thus nonvital cells appear colorless on microscopic examination. The amount of dye absorbed by healthy cells is determined spectrophotometrically and used to calculate the relative cell viability (Repetto et al., 2008).

8.3.2 Mutagenicity and genotoxicity testing

A "genotoxic" material or "genotoxin" is any material that can cause damage to the DNA or other constituents of the cell that control the integrity of the genome, such as the spindle apparatus or DNA repair systems. The destructive effects of a material on a cellular genetic level may occur directly or indirectly and are not essentially accompanied by mutations. On the other hand a mutagenic material is any material that produces permanent changes in the genetic components of a cell resulting in DNA mutations. Hence, genotoxicity is a broader expression than "mutagenicity" and it can be concluded that not all genotoxins are mutagens, however, all mutagens are genotoxins. Permanent changes in DNA were found to be important predisposing factors in the malignancy process, as a result, genotoxicity tests can be used for understanding the results of carcinogenicity studies keeping in mind that not all positive genotoxic results imply a carcinogenic risk of the material to the living organism (Gad, 2010).

The *bacterial reverse mutation test* (Ame's test) is frequently used to assess the mutagenicity of materials. It utilizes special salmonella typhimurium bacterial strains that comprise mutations in the histidine locus affecting their ability to form histidine, which is typically synthesized by normal salmonella strains and is crucial for their growth. The material is incubated with the mutant bacterial strains in a histidine free medium and its mutagenic ability is evidenced by the capacity of the material to produce a mutation that reverses the original mutation, restoring the bacteria's ability to synthesize the amino acid essential for their growth. Mutagenicity is directly proportional to the number of colonies of revertant bacteria which regained their ability to form histidine, which is essential for their survival and growth (Phillips and Arlt, 2009; Schmalz, 2009).

Chromosomal aberrations or anomalies caused by a genotoxic dental material can be detected using the in vitro *micronucleus test.* If a genotoxic material damages chromosomes during cell division, the entire chromosome or parts of it may fail to incorporate in the nuclei of two daughter cells and rather form a separate micronucleus. This test identifies micronuclei in the cytoplasm of interphase mammalian cells after incubation with the material under investigation for a specified time. Additionally, this test facilitates the microscopic identification of structural changes due to breakage of chromosomes (clastogenic changes) as well as numerical changes in chromosomes resulting from chromosome loss (aneugenic changes) (Fenech, 2000; Phillips and Arlt, 2009; Schmalz and Arenholt-Bindslev, 2009).

8.3.3 Hemocompatibilty testing

Hemocompatibility tests are performed to study the compatibility of the material under investigation, with blood and its components. Chemicals, leachables, or even shear stress from a device in contact with blood can interact with red blood cells leading to the release of hemoglobin in a process called "hemolysis." The *hemolysis assay* is recommended for testing all dental devices and materials except the ones that are projected to be placed in contact with unbroken skin or mucous membranes. To test the hemocompatibility of a material, it is incubated with whole blood diluted in saline for a specific time period, then the amount of released hemoglobin is measured as an indicator of hemocompatibility. The blood may be from a human or a rabbit or sometimes from other species. An extract of the material can be incubated with blood in replacement of the material's sample (ASTM, 2000; Henkelman et al., 2009; Seyfert et al., 2002).

8.4 Animal tests

Animal tests are biocompatibility tests in which the material under evaluation is placed in the body of an animal, often mammals including mice, rats, rabbits, and guinea pigs. Animal tests allow the material to interact with the various biological systems present within the animal body, allowing an intact biological system to react to the material. Thus these tests are considered to be more comprehensive and more relevant than in vitro tests. While in vitro tests provide crucial information about cellular and molecular interactions with the material, animal tests are indispensable despite their many disadvantages. Moreover, these tests are used to evaluate the biological response that cannot be modeled in vitro, including blood interaction, infection, wound healing, hypersensitivity response, carcinogenesis, and chronic inflammation without putting humans at risk. They are also less expensive than clinical trials and provide for more expedient results. However, these type of tests have many drawbacks including the difference between the animal response and the human response. Therefore the results cannot be directly extrapolated to clinical situations in humans. Additionally the interpretation of results may be difficult due to the overlap of incidents that may affect the test outcome. Finally, ethical and legal concerns are common problems associated with this type of testing (De Souza Costa et al., 2014; Wataha, 2012, 2001).

8.4.1 Skin sensitization tests

Skin sensitization and consequent allergic contact dermatitis are common health and occupational hazards that result from an immunological response to chemical allergens that may be present in a body contacting skin (Gerberick et al., 2004). A material is not categorized as a skin sensitizer unless there is proof of sensitization by the material in a considerable quantity of people or enough positive results are reported from animal testing (Basketter et al., 2012). The immunological process

occurs in two stages. In the first stage, exposure to the chemical allergen in susceptible individuals induces a specific immunological memory and the individual becomes sensitized to that specific allergen. The second stage occurs if the sensitized subject is reexposed to the same allergen, as an aggressive immune response will be elicited and a cell-mediated or antibody-mediated allergic response will be produced (Kimber et al., 2011).

Three animal assays are regularly used to evaluate the skin sensitizing potentials of chemicals. In *guinea pig maximization tests* the material is injected intradermally followed by a 10–14 days induction period during which a hypersensitivity reaction may be induced. Then adhesive patches containing the material are applied and the skin reaction to the second exposure to the material is scored. The results may vary from no reaction to intense erythema and swelling (Sakaguchi and Powers, 2012). *Buehler occluded patch tests* also measure the sensitization potentials of chemicals in guinea pigs; however, they involve the use of occluded patches loaded with the test material in the induction phase without the need of intradermal injection of the material (Jack, 1994). The *local lymph node assay* is carried out in mice based on the principle that allergens stimulate the increase of lymphocytes in lymph nodes that drain the location of allergen application. The number of proliferating lymphocytic cells is quantified using radioactive labeling or other nonradioactive methods which measure cellular proliferation, such as measuring the level of ATP providing quantitative data (Idehara et al., 2008). A chemical is considered a sensitizer if it causes a threefold or a higher increase in cell numbers in comparison to numbers measured in control mice. The assay provides animal welfare advantages over guinea pig tests as fewer animals are used and the pain and distress suffered by the animals during testing is decreased as well (Gerberick et al., 2007).

8.4.2 Mucous membrane irritation tests

Mucous membrane irritation tests are considered for materials that will be used in direct contact with natural channels or tissues. Various assays are used to determine the inflammatory effects that materials can have on mucous membranes at a variety of sites such as oral, rectal, or vaginal mucous membranes. To estimate whether a dental material might be irritant to the oral mucosa the Chinese hamster cheek pouch irritation test is conducted. In this model a disc of the material is inserted in the cheek pouch of the hamster while placing the control material in the opposite pouch. After 10–14 days the discs are removed, the pouches are macroscopically examined and inflammatory changes are scored. The animal is then sacrificed, and biopsy specimens are prepared for histological evaluation (Jong et al., 2012).

8.4.3 Implantation tests

In implantation tests, test materials are surgically inserted into bone, subcutaneously, or intramuscularly in a laboratory animal. Different criteria govern the selection of the animal species to be used during the experiment, such as the size of the samples to be implanted, the number of samples, and the expected duration of the

test with regards to the projected lifespan of the animal. Rodents or rabbits are often used in cases when short-term testing is expected, whereas animals with rather long life expectancy such as rabbits, dogs, sheep, or pigs are more suitable for long-term testing. After implanting the material in the tissues for the designated time, the surrounding tissue is macroscopically investigated with documentation of any tissue reactions observed such as swelling, hematoma, or connective tissue encapsulation. The local draining lymph nodes should also be examined. The animal is then euthanized and the surrounding tissues are excised and histopathologically investigated for any changes such as necrosis, degeneration, and tissue changes such as granuloma formation (Ratner et al., 2004; Schmalz, 2009).

8.5 Usage tests

Usage tests are considered the most relevant to the clinical situation amongst the biocompatibility tests. They are done either in human volunteers, referred to as clinical trials, or in animals. Animal usage tests differ from other animal tests in that the material is placed in exactly the same site similar to the final-planned use. Also the test mimics the clinical situation in all aspects including time, location, and the procedure by which the material is manipulated. Despite the high convenience provided by usage tests, they are associated with many shortcomings such as their high cost, difficulty in interpreting test outcomes, and finally the legal and ethical complications related to the tests (Sakaguchi and Powers, 2012; Wataha, 2001).

8.5.1 Dental pulp irritation tests

Dental pulp irritation tests are carried out to appraise the compatibility of a material within the dental pulp. To measure the effect of a material on the dental pulp, first, class V cavities are prepared in intact none carious teeth of nonhuman primates or human volunteer's teeth scheduled for extraction due to orthodontics treatment. The material and control are placed in the prepared cavities for the required time and by the end of the test, teeth are extracted and prepared for microscopic examination. Irritants can affect the pulp leading to inflammation which classified from "none" in which the pulp contains minimal inflammatory cells to "severe" in which chronic inflammation results in substantial necrosis of pulp tissues. In addition, minor irritating stimuli can stimulate the pulp to generate tertiary or reactive dentin combined with the occlusion of dentinal tubules and the formation of sclerotic dentin. Stronger irritants can have a more damaging effect leading to the death of the odontoblasts. Dental pulp stem cells respond to the pulp assault by differentiating into secondary odontoblasts capable of forming reparative dentin with a less organized configuration (Murray and Garcia-Godoy, 2007; Sakaguchi and Powers, 2012; Schmalz, 2009; Tziafas et al., 2000).

8.5.2 Mucosa and gingiva usage tests

The influence of materials on the gingival and mucosal tissues can be investigated by placing the materials in direct contact with the tissue by inserting the test material in cavity preparations with subgingival extensions or in contact with buccal or palatal mucosa for different time periods. The number of inflammatory cells is noted and used to classify the tissue response into slight, moderate, or severe. The disadvantage of this test is that there is a high chance there will be some sort of inflammation in the gingival tissues due to different factors such as bacterial plaque or defective restorations which might affect the final results (Klötzer, 1978).

8.5.3 Endodontic usage tests

Studies that implicate the use of animal models (e.g., primates, dogs) have been used to determine the compatibility of different materials with periapical tissues. In these tests the pulp is extirpated and the canal is prepared following the traditional cleaning and shaping procedures, then the test material is applied into the root canal according to endodontic techniques. Radiographic analysis and histologic appraisal of the periapical tissues are used to assess the biocompatibility of the material and its effect on the surrounding tissue (Fouad et al., 1993; Ørstavik and Mjör, 1992).

8.6 Combining in vitro, animal, and usage tests together

For many years, it has been accepted that the use of a combination of primary, secondary, and usage tests is the most effective way to characterize the biocompatibility of a new material based on the idea that no single test can be used to evaluate the biocompatibility of a material. Primary tests are the first tests to be conducted on materials such as cytotoxicity and mutagenicity testing. They are usually performed in vitro, but may be carried out in animals as in cases of measuring systemic toxicity. Secondary tests are carried out in experimental animals. A few decades ago, a paradigm was proposed (Fig. 8.1) to predict the behavior of a newly introduced material that was endorsed by researchers and standards organizations. The paradigm presents a stepwise scheme which recommends initially subjecting the materials to primary tests and only materials deemed suitable are subjected to secondary tests. Likewise, only the materials that show satisfactory results during secondary testing are considered for usage tests. All the materials are tested with primary tests; however, not all the materials pass the tests to be used in secondary tests and even fewer materials reach the usage stage of testing. Accordingly, the paradigm assumes a pyramidal shape with all the materials at the bottom of the pyramid with the number decreasing as testing resumes towards the pyramid top. An important benefit of this methodology is that it allows the early screening of high-risk materials using tests that are quick and cheap while evading unnecessary pain and distress experienced by animals. However, this depends highly on the precision

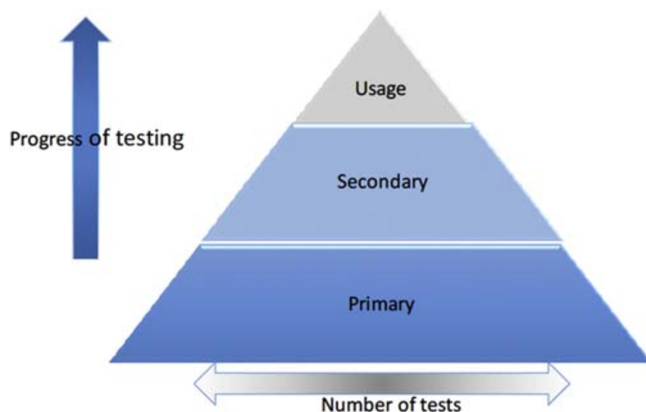


Figure 8.1 Diagram showing a pyramidal shape stepwise scheme in which the material is initially subjected to primary tests, then secondary and usage tests. The number of tests decreases as testing resumes towards the pyramid top.

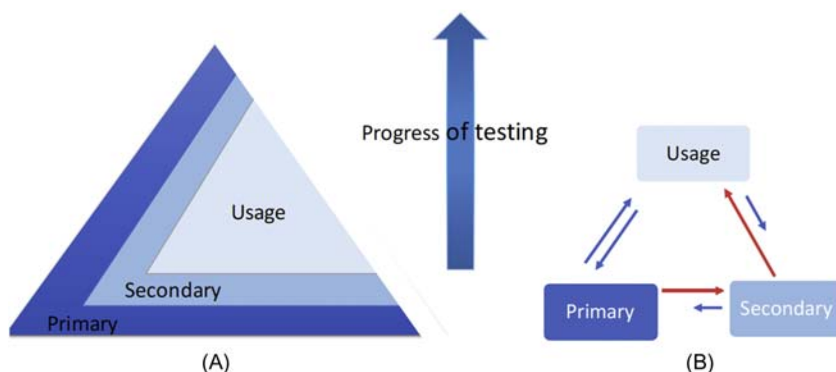


Figure 8.2 (A and B) Diagrams showing nonliner schemes for testing biocompatibility, where primary and secondary tests have a smaller, continuous part, and the usage test dominates.

of the tests as any false results can result in wasting money, and time and endangering animals and humans (Anusavice et al., 2013; De Souza Costa et al., 2014).

An important obstacle that was encountered during the use of this paradigm was the failure of in vitro and animal tests to satisfactorily simulate clinical environments. It was reported that these tests may fail to screen out materials that were clinically unsuccessful. On the other hand, materials that showed high convenience during clinical use may not display promising results during in vitro and animal testing (Wataha, 2012, 2001). For example, zinc oxide eugenol cements have shown great success when used clinically, yet when directly incubated with cell culture, all of the cells died. Recently newer nonlinear schemes have been introduced (Fig. 8.2A and B) that acknowledge the importance of the primary, secondary and

usage tests to evaluate the biocompatibility of materials. It was proposed that primary and secondary tests will have a smaller but continuous part as testing progresses and usage tests dominate (Fig. 8.2A). For instance, in vitro and animal tests may be used to investigate and understand a problem caused by the material during clinical assessment in addition to their primary role in the evaluation of the material. It was also proposed that it is common for testing to advance from primary to secondary to usage tests, yet, any tests can be done at any time according to the situation encountered and switching between different tests is acceptable (Anusavice et al., 2013; Sakaguchi and Powers, 2012; Wataha, 2012, 2001).

8.7 Biomaterials for tissue engineering

8.7.1 Polymeric biomaterials

Polymers are long-chain vast molecules consisting of minute repeated building units called "mers." Polymers are capable of assuming different conformations through their valence bonds' rotations (Ratner et al., 2004). Biocompatible polymeric biomaterials have many important applications; for examples, poly-methyl methacrylate polymer can be used as bone cement, poly-glycolic acid may be used as degradable sutures, and poly-glycolic-co-lactic acid is used as bone screws. Polyethylene glycol polymers are often used to prolong the dissemination half-life of some drugs (Seala et al., 2001).

For specific applications the material choice depends on the type of material needed, the tissues' characteristics, and regeneration time. Moreover, the extensive structures' diversity and the distinctive physical, biological or chemical functionalities of polymers can be allied, permitting the creation of fascinating materials such as hydrogels, membranes, and scaffolds. The natural polymeric materials can create new opportunities for simulating the microenvironment of tissues and can also motivate the suitable physiological reactions needed for cell regeneration. All these qualities simultaneous with a coordinated biodegradation rate and biocompatibility of natural polymers can be beneficial when paralleled to synthetic polymers (Silva et al., 2010).

High-density polymers are widely used in different medical applications. However, linear polymer chains possess high degrees of crystallinity, which results in increasing polymer rigidity, and decreased permeability. Linear polymers are manufactured through advanced processing technology, hence, polymer chains will be aligned linearly to produce high stiff and high strong materials (Callister et al., 2007).

Sometimes, biodegradation of implants is intended to occur after a certain period and after achieving the proposed function. Therefore biocompatible and biodegradable polymers can be used in these circumstances. It is highly important to select a biodegradable polymer that has a degradation rate that matches the new tissue growth rate (Ratner et al., 2004).

8.7.1.1 Natural origin polymers

Naturally derived polymers have repeatedly been utilized in tissue engineering purposes because they have comparable properties to natural extracellular matrix (ECMs). Natural origin polymers involve collagen/gelatin, chitin/chitosan, glycosaminoglycan/hyaluronic acid (HA)/chondroitin sulfate, alginate, and cellulose.

8.7.1.1.1 Collagen/gelatin

Collagen is the main constituent of natural ECM and the plentiful protein in mammalian tissues and is therefore an appealing material for biomedical applications (Tan et al., 2008). Collagen is constituted of triple polypeptide chains wrapped in a rope-like structure (Drury and Mooney, 2003). Collagen degradation occurs naturally by collagenase and serine proteases (Mueller et al., 1999), it is thus controlled locally by engineered tissue cells.

Gelatin is formed by hydrolysis of collagen into single-strand molecules (Tan et al., 2009b). As comparable to collagen, gelatin is less immunogenic and obviously recalls information as arginylglycylaspartic acid sequence, thus promoting cell adhesion, differentiation, and proliferation (Huang et al., 2005). Hydrogels can be produced from collagen and gelatin and an enhancement of their mechanical properties can be done by glutaraldehyde chemical crosslinking.

8.7.1.1.2 Chitin/chitosan

Chitin is a natural polymer highly abundant in nature; it is detected in shrimps' shell, insects' cuticles, and fungi cell walls. Chitin polymer has fungistatic and bacteriostatic actions, that are advantageous for promoting rapid regeneration (Kumar, 2000). Chitin is soluble in hexa fluoroiso-propanol, acetone, and dimethylacetamide containing 5% LiCl. Nevertheless the chitin insolubility in water and organic solvents have restricted its applications (Kumar, 2000).

Chitosan is a biocompatible polysaccharide polymer attained by deacetylation of chitin in alkaline media. Chitosan is efficient against fungi, bacteria, and viruses and has wound healing properties, film-forming ability and biodegradability (Rinaudo, 2006). Chitosan can be used in the biomedical field and can be prepared in the form of films, fibers, sponges, or hydrogels. Chitosan is simpler to process than chitin, but its stability is typically lower, due to its hydrophilicity and pH sensitivity (Rinaudo, 2006). With the upsurge of nanotechnology, chitosan has been molded into various bio-nanocomposites, providing unconventional functions in regenerative medicine (Lee et al., 2009). One study proposed that the mechanical properties of chitosan and alginate would be greater in acidic media where chitosan dissolves (Hamman, 2010).

Methodologies using injectable scaffolds formed of chitosan hydrogels for cartilage repair have also been researched. Hao et al. prepared a temperature-responsive, injectable scaffolds created from chitosan/hydrogels for cartilage repair (Hao et al., 2010), while (Tan et al., 2009a) have revealed that the chitosan hydrogel composite supported the chondrocytes' survival and preserved their morphology.

8.7.1.1.3 Glycosaminoglycan

Glycosaminoglycan (GAGs) are polysaccharides present in all vertebrates (ECM) (Kirker et al., 2002). One such example, hyaluronic acid or hyaluronan (HA) is a naturally occurring water soluble polysaccharide extensively dispersed in the connective tissue ECM of animals and has many significant biological functions (Kogan et al., 2007). HA and its derivatives have been investigated as hydrogels for tissue engineering, due to its biocompatibility, and biodegradability by hyaluronidase, in addition to excellent gel-forming properties (Fraser et al., 1997). Tang et al. employed a mixture of collagen/HA to stabilize and improve the performance of the materials (Tang et al., 2007).

Chondroitin sulfate can stimulate cell proliferation and matrix retention; thus it has been used in cartilaginous scaffolds in tissue engineering. Chang et al. revealed that the chondrocytes seeded on gelatin, chondroitin sulfate, and hyaluronan scaffolds were consistently dispersed and secreted ECM and collagen (Chang et al., 2003).

8.7.1.1.4 Alginate

Alginate is a copolymer of mannuronic acid and guluronic acid, derived from sea algae. It forms steady hydrogels at room temperature and in the presence of divalent cations as calcium or strontium (Seala et al., 2001). Successful utilization of alginate in skin, cartilage, and bone tissues indicates their favorable potential for repair and regenerative functions (Sun and Tan, 2013). Nonetheless the present alginate is incapable of gathering all the design considerations concurrently; for example, bioactivities, mechanical properties, or degradation (Boontheekul et al., 2005). Advanced studies are done to modify alginate in order to improve its properties and functions that support the growth of natural tissue. The nanoscaled alginate fiber matrix scaffold material is similar to fibrous ECM proteins, therefore it is an aspirant ECM mimetic material (Kang et al., 2012).

Alginate-based delivery systems for functional DNAs or small interfering RNAs (siRNAs) are of massive importance in constructing bioactive biomaterials. In this type of application, modified alginate with cationic properties is necessary to enhance the efficiency of DNAs or siRNAs to target cells (Sun and Tan, 2013).

8.7.1.1.5 Cellulose

Cellulose is an abundant organic polymer on earth, it is insoluble in the majority of solvents because of its strong intra/inter-molecular bonding. However, it can be used as wound dressing materials (Boateng et al., 2007), this is because of its effective hemostatic and antibacterial activity (Martina et al., 2009).

Cellulose and collagen composites can be produced as films, beads, and scaffolds by varying the composition and the precipitation technique (Pinkert et al., 2009). Bacterial or microbial cellulose may similarly be formed by *Acetobacter xylinum* (Czaja et al., 2007). While bacterial and plant cellulose are both identical regarding molecular formula, bacterial cellulose has astonishing mechanical and physical properties due to its crystalline nano- and microfibril structure, it also has

high purity, water holding ability, and biocompatibility (Czaja et al., 2007; Klemm et al., 2005).

Bacterial cellulose/chitosan composite can be used as a wound dressing due to its antibacterial and barrier properties; it also has optimum moisture conditions for fast healing without irritation (Ciechańska, 2004). Bacterial cellulose can also be used as scaffolds for cartilage repair, since it can support cell ingrowth and maintain chondrocytes' differentiation (Svensson et al., 2005).

8.7.1.2 Synthetic polymers

Synthetic polymers are more frequently selected for the manufacturing of hydrogels since their physical and chemical properties are predictably more manageable than those of natural polymers; however, their biological activities are limited. Incorporation of bioactive materials into the synthetic hydrogel can increase cellular activity. Synthetically derived materials involve poly ethylene glycol (PEG) and, poly ethylene oxide (PEO), poly-lactic acid (PLA), poly-glycolic acid (PGA), polyvinyl alcohol (PVA), polypropylene fumarate (PPF), and polyurethanes (PU) (Tan and Marra, 2010).

8.7.1.2.1 Poly ethylene glycol and poly ethylene oxide

PEG and PEO are currently FDA licensed and are used for several tissue engineering applications. Both PEG and PEO have chemical similarities and can be photo-cross-linked by modifying the polymer chain end with acrylates or methacrylates (Tan and Marra, 2010). Though many biocompatible polymers can produce hydrogels by chemical crosslinking, PEG holds its place as one of the extensively investigated systems (Guillaudeu et al., 2008), by a particular power to regulate the density of crosslinking that delivers the tractability for cell encapsulation and proliferation. PEG is a great hydrophilic material with an excellent solubility in a variety of solvents, it also has a very high ability to imbibe water (Place et al., 2009). PEG hydrogels can be utilized as scaffold materials for cell encapsulation, and as vehicles for vaccine delivery systems (Nicodemus et al., 2007; Zhou et al., 2003). Copolymerization with PLA and PGA degradable polymers can be used to achieve biodegradable PEG hydrogels (Hiemstra et al., 2007).

8.7.1.2.2 Poly-glycolic acid and poly-lactic acid

The desirability of PLA and PGA as biodegradable polymers in medical applications is that their degradation products, lactic and glycolic acids, are biological metabolites. Lactic acid is metabolized through the carboxylic acid cycle and excreted as CO_2 and H_2O. The glycolic acid may be directly excreted in urine or react to form glycine. Glycine is used to synthesize serine and then converted into pyruvic acid and enters the carboxylic acid cycle (Athanasiou et al., 1998).

PGA is a rigid, thermoplastic, highly crystalline, aliphatic polyester, made by a ring opening polymerization reaction of a cyclic glycolide. PGA has outstanding mechanical properties; however, its low solubility in organic solvents, faster degradation, and the high acidity of its degradation products have limited its biomedical

applications (Nair and Laurencin, 2007). Poly-lactic–glycolic acid copolymers and PGA have been approved by the FDA and used as degradable sutures for decades.

PLA is a semi-crystalline solid, more hydrophobic, and more resistant to hydrolytic attack than PGA. Concerns about the biocompatibility of PLA and PGA do exist because of the resultant toxic solutions from acidic degradation (Taylor et al., 1994). In sizeable orthopedic implants, this may result in high local acid concentrations. Likewise, it was noticed that no unfavorable biological responses appear, especially if the volume of material is quite small. Additional concern is the small particles released through degradation, which may initiate foreign-body reactions or osteolytic responses. It has been presented that the small particles that detach are phagocytized by multinucleated giant cells and macrophages (Gibbons, 1992).

8.7.1.2.3 Poly(caprolactone)

Poly(caprolactone) (PCL) is a semi-crystalline polymer that has a significantly lower degradation rate than PLA and can be used for evolving long-term/implantable/drug delivery systems. PCL with an average 50,000 molecular weight takes 2–3 years for complete degradation (Middleton and Tipton, 2000). To generate materials with fast degradation rates the hydrolysis rate may be changed by copolymerization with other lactones or with lactide (Middleton and Tipton, 2000). PCL is considered a nontoxic and a tissue compatible material. The blending of gelatin, PCL, and bone powder allows for better scaffolds for bone tissue engineering and can increase cells' growth, adhesion, and proliferation (Rong et al., 2016).

8.7.1.2.4 Polyvinyl alcohol

PVA is a polymer with a carbon backbone that is not liable to hydrolysis. It requires an oxidation process for its biodegradation. PVA biodegradability can occur by microorganisms in addition to enzymes through an oxidation or photo-oxidation process (Watanabe et al., 1976). Polyvinyl alcohol is commonly utilized because of its water solubility (Chandra and Rustgi, 1998). PVA can be modified into multifunctional macromers by substituting the pendant hydroxyl groups, with a variability of substituents (Drury and Mooney, 2003). PVA can be either physically cross-linked through repeated freezing/thawing techniques, or chemically cross-linked by glutaraldehyde to form hydrogels (Nuttelman et al., 2001).

8.7.1.2.5 Polypropylene fumarates

PPF is a linear polyester based on fumaric acid. Its degradation occurs by hydrolysis of the ester linkage and leads to fumaric acid that is located in the Krebs cycle. The copolymer backbone has unsaturated sites, which could be used in consequent crosslinking reactions (Gunatillake et al., 2003).

PPF can copolymerize with hydrophilic PEG to form hydrogels (Drury and Mooney, 2003). High molecular weight PPF is not easily achievable due to the presence of side reactions. To achieve good mechanical strength, ceramic materials such as tricalcium phosphate, calcium sulfate, or calcium carbonate may be incorporated. Compositions without ß-TCP reinforcement in the implant, disintegrated very early (Temenoff and Mikos, 2000). In addition to the mechanical properties' improvement, ß-tricalcium phosphate (ß-TCP) also acts as a buffer during the

degradation process minimizing the pH changes. PPF when subcutaneously implanted in rats did not elect an adverse inflammatory response. Only mild inflammatory response was initially detected, later on a fibrous capsule formation occurred at 12 weeks in the implant region (Peter et al., 1998).

8.7.1.2.6 Polyurethanes

PU is a synthetic elastomer that has excellent mechanical properties and good biocompatibility. PU is particularly used for medical long-term implants (Pinchuk, 1994). PU are made of alternating hard and soft segments. The hard segment is made from diisocyanate and a chain extender. The soft segment originates from polyol. The biodegradation of PU can be designed through proper selection of the chemical nature of the soft segment. For example, polyether-polyol is resistant to biodegradation, while polyester-polyol is readily biodegradable (Nakajima-Kambe et al., 1999).

Both in vitro and in vivo studies indicated that degradable PU have an acceptable biocompatibility. Animal studies disclosed fast cell ingrowth in addition to no unfavorable tissue reactions. Still the removal of degradation products from the body is not clearly understood (Gunatillake et al., 2003; Zhang et al., 2000).

8.7.2 Ceramic biomaterials

Ceramic materials are able to encourage direct bone-implant adhesion without fibrous interface, although they are incapable to be used as loaded devices.

Bioceramics are commonly categorized into two families: "bioinert" and "bioactive." In bioinert materials, no direct bone−material interface is created and a soft tissue layer shields the bone from the implant. Unfortunately, this shielding promotes micromotion and a subsequent implant loosening. Bioinert materials cannot be used as bone filler materials (Chevalier and Gremillard, 2009).

Bioactive ceramics provide constructive surfaces for bone adhesion and ingrowth (Hench, 1998). Furthermore, the requirements for load-bearing ability are less challenging. Mostly bioactive ceramics are based on calcium phosphate materials primarily hydroxyapatite (HA) and tricalcium phosphate, because of their semblance to the mineral portion of bone (Chevalier and Gremillard, 2009).

8.7.2.1 Hydroxyapatite

$(Ca_{10}(PO_4)6(OH)_2$ is the principal component of bones and teeth in vertebrates, with crystallographic and chemical properties similar to their carbonated apatite (Suchanek and Yoshimura, 1998). It has unique biological properties, as it is bioactive, osteoconductive, noninflammatory, nontoxic, biodegradable and has superior biocompatibility. Therefore HA is widely used to replace bone and teeth in musculoskeletal procedures. Moreover, HA is used in bone tissue engineering for many years as a successful bone repair and regeneration replacing material. On the other hand, HA is incapable to function as a load-bearing implant due to its inadequate mechanical properties.

HA is presented in numerous forms such as microporous blocks, solids blocks, and as granules. Nanohydroxyapatite (n-HA) has great biological efficacy and is capable of encouraging the attachment of osteoblast-like cells and their growth. HA coating supports mesenchymal stem cell (MSC) attachment, proliferation, and differentiation (Zhao et al., 2002). Nano-HA has a remarkable ultra-fine structure comparable to that of biological apatite and a high surface area to volume ratio. This has a huge influence on the cell—biomaterial interaction as it is biocompatible, resistant to infection, can attach to the living bone in implanted areas and can be used for the treatment of bone defects (Ayatollahi et al., 2015; Liuyun et al., 2008).

HA has been applied to an extensive diversity of biomedical devices including, dental and orthopedic implants coatings on hip implants and biodegradable scaffolds (Wilson and Hull, 2008). An ideal ceramic composite should comply to characteristic parameters, such as osteoconductivity; in order to guide bone around and inside the implant, macroporosity; to assist the bone cells ingrowth, the ability to carry growth factors and drugs to the target cells, biodegradability; to enable for bone remodeling, in addition to the mechanical stability and ease of handling (Khaled, 2012).

HA has a structure and composition resembling the natural bone mineral and consequently has been well-thought-out to be the idyllic material for bone tissue engineering scaffolds due to its osteoconductivity and osteoinductivity. Improved osteoclast-like cell functions (resorption pits' formation) have also been spotted on nano-HA. This is because of the presence of nanoporosity and nanofibrous polymer matrices. These materials can be manufactured via phase separation, electrospinning, chemical etching, particulate leaching, and 3-D printing techniques (Zhang et al., 2008).

8.7.2.2 Calcium phosphate (Ca-PO$_4$)

Calcium phosphate polycrystalline ceramic materials can be manufactured by solid-state reactions or by precipitation from aqueous solutions. Calcium-based ceramic materials could enthusiastically inspire bone regeneration at the implant surface. Consequently the calcium phosphate ceramic biomaterials may replace the use of bone grafts (Dorozhkin, 2009).

Calcium phosphates are extremely biocompatible because of their chemical similarity with mineralized hard tissues. This property is extensively utilized for biomaterials that are either completely made of calcium phosphate or covered with it. Calcium phosphate bone cements are helpful in bone repair as they can assist the bone ingrowth and improve mechanical strength (Huan and Chang, 2009).

In the field of bone tissue engineering, utmost scaffolds are being prepared from ceramic or ceramic derivatives. The chemically stable surface of sintered ceramics is a good substrate for seeding cells. Ceramics have positive influence on the differentiation and proliferation of bone-forming cells, so that cells can be introduced into these ceramics to increase the rate of tissue ingrowth (Habraken et al., 2007). Once cells are added, complications rise, possibly because of inadequate nutritional supply at the inner part of the implant. Growth factors like the transforming growth

factor, bone morphogenic protein-2, vascular endothelial growth factor and basic fibroblast growth factor are usually impregnated into the scaffolds due to their vascularization and osteoinductive properties (Habraken et al., 2007).

Chemically, bone-substitution materials are frequently made from HA, tri- and bi-calcium phosphates. An ideal bone substitute material would be porous to permit cell penetration and bone growth. Furthermore, the material ought to have an appropriate biodegradation rate equivalent to the bone tissue formation (few months and about 2 years). Also the material must have satisfactory mechanical permanence. Ideally a material degradation rate should occur concurrently with the new bone formation, i.e., the complete rebuilding of the defect with a biological material is anticipated. HA is more stable than TCP under physiological conditions, because of its slower resorption kinetics and its lower solubility (Dorozhkin, 2009).

8.7.2.3 Bioglass

Bioactive glasses represent unique systems as they can elicit an appropriate biological response. They are generally silicate based, containing calcium and phosphate (Bansode and Sakharkar, 2015). They can form bonds with soft and hard tissues by means of reactions' series. Therefore the formed interface between the glass and the tissue is strong and compliant. This makes these materials the superior choice in tissue engineering scaffolds. Furthermore, bioactive glasses have high tissue integration and regeneration quality (Jones, 2015).

If bioactive glasses are exposed to physiological fluids, tenacious bonds will be formed with bone. This is done by the creation of bone-like HA layers and the biological interaction of collagen with the material surface (Bansode and Sakharkar, 2015). These surface reactions lead to the increase in the critical concentrations of soluble ions such as Si, Ca, P, and Na. These ions induce favorable responses both intra and extracellular, that result in rapid bone formation. However, these materials have limitations and can only be used in a low stress bearing area, due to their weakness and low fracture resistance. Moreover, changing the chemical composition of the material could easily overcome this limitation (Jones, 2015).

The bioglass (45S5) is composed of 45 wt% silica, 24.5 wt% of both calcium oxide and sodium oxide, and 6 wt% P_2O_5. Bioglass acts as a miracle material, because these compositional oxides provide unique characteristic features. The surface of bioglass becomes highly reactive when exposed to physiological environments due to the presence of high amounts of Na_2O, CaO, and P_2O_5 (Bretcanu et al., 2009). Recently, additional elements such as magnesium, strontium, fluorine, iron, and silver have been intentionally incorporated in the silicate network composition to enhance the material properties (Krishnan and Lakshmi, 2013; Rabiee et al., 2015).

Massive bone defects caused by disease and trauma are required to be repaired through bone grafting procedures. Bioactive glass is a superior choice for hard-tissue prosthetic material because of its interconnected porosity which supports the biological tissue growth and improves implant stability (Van Gestel et al., 2015).

Bioactive glass is used successfully in management of hypersensitivity, through occluding the dentinal tubules and has the ability to induce tissue mineralization at the glass−tissue interface. Dentine treated with melt-driven bioglass showed an apatite layer, which was continuous and adherent (Miglani et al., 2010). Bioglass is also an efficient antibacterial agent due to its alkaline nature (Krishnan and Lakshmi, 2013).

Drug delivery systems are used for better and prolonged drug control action. This will protect the drug from decomposition or secondary reactions until it reaches the right place to perform its function efficiently. A drug delivery system should be inert, biologically compatible, mechanically strong and tolerated by the patient. It should have the ability to carry high doses of the drug, with no risk of accidental release. Moreover, it should exhibit ease of administration, removal, fabrication, and sterilization (Wu and Chang, 2012). Bioglass has been tried as a vehicle for drug delivery. One trial of modifying bioactive bioglass scaffolds for bone tissue engineering applications, blending biopolymer coating as a carrier of vancomycin to deliver antibacterial effects at the wound site with a further sustained release (Olalde et al., 2013).

Bioactive glasses together with other related bioactive composite materials are considered the most promising materials in building scaffolds using biocomposite, nanofibers, and nano-HA. These porous materials can ensure good cell occupancy, vascularity, the movement of nutrients, and metabolic waste products. Thus in turn enable the osteoblasts to adhere, migrate, proliferate, and mineralize into bone, which was a tremendous step ahead in bone defects' filling (Mosbahi et al., 2016).

8.7.3 Biocomposite

Unfortunately, ceramic materials are brittle materials with low fracture toughness, it is thus challenging to shape them in the exact forms needed for bone replacement. Therefore composites of ceramic materials and organic polymers, such as poly-lactic acid, chitosan, and polyethylene are introduced to compensate for the intrinsically weak mechanical properties of ceramics and to control bioactivity and biodegradability. The composite material consists of more than one material and has the structural properties of all the constituents that are superior to either solitary constituent material. The composites of ceramic integrated with natural degradable polymer become the superior material of choice as bone fillers (Yunus Basha et al., 2015). For example the self-setting calcium phosphate cement/chitosan/MSC composite scaffold is desired for dental, orthopedic, and maxillofacial repairs because it is strong, moldable, and can easily be shaped. The mixed calcium phosphate cement powder with liquid chitosan produces a paste to be injected in surgical sites to form a scaffold in situ via a minimally invasive technique (Moreau and Xu, 2009).

Collagen/HA composites are developed as a scaffold for hard-tissue regeneration. These composites have good mechanical properties and good bonding properties with surrounding host tissues (Wahl and Czernuszka, 2006). Composite biomaterials with smart effects were reviewed by Pérez et al. Composites refer to the combination of biomaterials with biological factors proposed for such smart purposes. The smart

actions trigger stimulating effects to the surrounding cells and tissues such as shape memory effects, maintainable, or sequential delivery of bio-factors. The smart composite biomaterials may be applied to a wide range of tissues; from hard to soft tissues that require precise composite designs (Pérez et al., 2013).

8.8 Conclusion

It is apparent that in the last decade, there has been an enormous amplification in the fields of biomaterials and tissue engineering, each carries its own sole features to the development of biocompatibility testing that will guarantee safety in clinical application. A comprehensive standpoint of biocompatibility assays' selection is not only to detect unfavorable reactions but also to ascertain indicative reactions of the successful biomaterial in its anticipated purpose. The biological requirements of a biomaterial demand the identification of its intended application, the type of contact tissues, and the duration of its function. After these considerations have been identified, appropriate in vitro and in vivo tests can be nominated to detect the success or failure of the material. Biocompatibility testing is specified by latest efforts to substitute the animal use methods and develop cell culture alternative assays. Finally it must be considered that relevant and satisfactory biocompatibility evaluation requires assessment of the material product at its end use, after manufacturing and sterilization processes have been deliberated.

References

Anderson, J., 2011. Biocompatibility and the Relationship to Standards: Meaning and Scope of Biomaterials Testing. Comprehensive Biomaterials. Elsevier Ltd, Cleveland, OH, USA, pp. 7−26.

Anusavice, K.J., Shen, C., Rawls, H.R., 2013. Phillips' Science of Dental Materials. Elsevier Health Sciences, St Louis, MO.

ASTM, F., 2000. 756-00. Standard practice for assessment of hemolytic properties of materials. Philadelphia Am. Soc. Test. Mater.

Athanasiou, K.A., Agrawal, C.M., Barber, F.A., Burkhart, S.S., 1998. Orthopaedic applications for PLA-PGA biodegradable polymers. Arthroscopy. 14, 726−737.

Ayatollahi, M.R., Yahya, M.Y., Asgharzadeh Shirazi, H., Hassan, S.A., 2015. Mechanical and tribological properties of hydroxyapatite nanoparticles extracted from natural bovine bone and the bone cement developed by nano-sized bovine hydroxyapatite filler. Ceram. Int. 41, 10818−10827.

Bansode, P., Sakharkar, R., 2015. Bioglass—a miracle material. IOSR J. Dent. Med. Sci. Ver. I. 14, 2279−2861.

Basketter, D.A., McFadden, J.P., Kimber, I., 2012. Assessing the severity of allergic reactions: a regulatory dilemma. Contact Dermat. 67, 3−8.

Boateng, J.S., Matthews, K.H., Stevens, H.N., Eccleston, G.M., 2007. Wound healing dressings and drug delivery systems: a review. J. Pharm. Sci. 97 (8), 2892−2923.

Boontheekul, T., Kong, H.J., Mooney, D.J., 2005. Controlling alginate gel degradation utilizing partial oxidation and bimodal molecular weight distribution. Biomaterials. 26, 2455–2465.

Bretcanu, O., Misra, S., Roy, I., Renghini, C., Fiori, F., Boccaccini, A.R., et al., 2009. In vitro biocompatibility of 45S5 Bioglass®-derived glass-ceramic scaffolds coated with poly(3-hydroxybutyrate). J. Tissue Eng. Regen. Med. 3, 139–148.

Callister, W.D., Rethwisch, D.G., Wiley, J., 2007. Materials science and engineering: an introduction. Mater. Sci. Eng. 7, 266–267.

Chandra, R., Rustgi, R., 1998. Pergamon biodegradable polymers. Prog. Polym. Sci. 23, 1273–1335.

Chang, C.H., Liu, H.C., Lin, C.C., Chou, C.H., Lin, F.H., 2003. Gelatin-chondroitin-hyaluronan tri-copolymer scaffold for cartilage tissue engineering. Biomaterials. 24, 4853–4858.

Chevalier, J., Gremillard, L., 2009. Ceramics for medical applications: a picture for the next 20 years. J. Eur. Ceram. Soc. 29, 1245–1255.

Cho, M.-H., Niles, A., Huang, R., Inglese, J., Austin, C.P., Riss, T., et al., 2008. A bioluminescent cytotoxicity assay for assessment of membrane integrity using a proteolytic biomarker. Toxicol. In Vitro. 22, 1099–1106.

Ciechańska, D., 2004. Multifunctional bacterial cellulose/chitosan composite materials for medical applications. Fibres Text. East. Eur. 12, 69–72.

Czaja, W.K., Young, D.J., Kawecki, M., Brown, R.M., 2007. The future prospects of microbial cellulose in biomedical applications. Biomacromolecules. 8 (1), 1–12.

De Souza Costa, C.A., Hebling, J., Scheffel, D.L.S., Soares, D.G.S., Basso, F.G., Ribeiro, A. P.D., 2014. Methods to evaluate and strategies to improve the biocompatibility of dental materials and operative techniques. Dent. Mater. 30, 769–784.

Dorozhkin, S.V., 2009. Calcium orthophosphates in nature, biology and medicine. Materials (Basel). 2, 399–498.

Drury, J.L., Mooney, D.J., 2003. Hydrogels for tissue engineering: Scaffold design variables and applications. Biomaterials. 24, 4337–4351.

Fenech, M., 2000. The in vitro micronucleus technique. Mutat. Res. Mol. Mech. Mutagen. 455, 81–95.

Fouad, A.F., Walton, R.E., Rittman, B.R., 1993. Healing of induced periapical lesions in ferret canines. J. Endod. 19, 123–129.

Fraser, J.R., Laurent, T.C., Laurent, U.B., 1997. Hyaluronan: its nature, distribution, functions and turnover. J. Intern. Med. 242, 27–33.

Gad, S.C., 2010. Genotoxicity. Drug Safety Evaluation. John Wiley & Sons, Inc, Hoboken, NJ, pp. 234–303.

Galler, K., Hiller, K.-A., Ettl, T., Schmalz, G., 2005. Selective influence of dentin thickness upon cytotoxicity of dentin contacting materials. J. Endod. 31, 396–399.

Gerberick, G.F., Ryan, C.A., Dearman, R.J., Kimber, I., 2007. Local lymph node assay (LLNA) for detection of sensitization capacity of chemicals. Methods. 41, 54–60.

Gerberick, G.F., Ryan, C.A., Kern, P.S., Dearman, R.J., Kimber, I., Patlewicz, G.Y., et al., 2004. A chemical dataset for evaluation of alternative approaches to skin-sensitization testing. Contact Dermat. 50, 274–288.

Gibbons, D., 1992. Tissue response to resorbable synthetic polymers. In: Plank, H. D.M.R.M. (Ed.), Degradation Phenomena on Polymeric Biomaterials. Springer, New York, pp. 97–104.

Guillaudeu, S.J., Fox, M.E., Haidar, Y.M., Dy, E.E., Szoka, F.C., Fréchet, J.M.J., 2008. PEGylated dendrimers with core functionality for biological applications. Bioconjug. Chem. 19, 461–469.

Gunatillake, P.A., Adhikari, R., Gadegaard, N., 2003. Biodegradable synthetic polymers for tissue engineering. Eur. Cells Mater. 5, 1−16.

Habraken, W.J.E.M., Wolke, J.G.C., Jansen, J.A., 2007. Ceramic composites as matrices and scaffolds for drug delivery in tissue engineering. Adv. Drug Deliv. Rev. 59, 234−248.

Hamman, J.H., 2010. Chitosan based polyelectrolyte complexes as potential carrier materials in drug delivery systems. Mar. Drugs. 8, 1305−1322.

Hao, T., Wen, N., Cao, J.K., Wang, H.B., Lü, S.H., Liu, T., et al., 2010. The support of matrix accumulation and the promotion of sheep articular cartilage defects repair in vivo by chitosan hydrogels. Osteoarthr. Cartil. 18, 257−265.

Hench, L.L., 1998. Bioceramics. J. Am. Ceram. Soc. 28, 1705−1728.

Henkelman, S., Rakhorst, G., Blanton, J., van Oeveren, W., 2009. Standardization of incubation conditions for hemolysis testing of biomaterials. Mater. Sci. Eng. C. 29, 1650−1654.

Hiemstra, C., Zhong, Z., Van Tomme, S.R., van Steenbergen, M.J., Jacobs, J.J.L., Otter, W., et al., 2007. In vitro and in vivo protein delivery from in situ forming poly(ethylene glycol)-poly(lactide) hydrogels. J. Control. Release. 119, 320−327.

Huan, Z., Chang, J., 2009. Novel bioactive composite bone cements based on the β-tricalcium phosphate-monocalcium phosphate monohydrate composite cement system. Acta Biomater. 5, 1253−1264.

Huang, Y., Onyeri, S., Siewe, M., Moshfeghian, A., Madihally, S.V., 2005. In vitro characterization of chitosan-gelatin scaffolds for tissue engineering. Biomaterials. 26, 7616−7627.

Idehara, K., Yamagishi, G., Yamashita, K., Ito, M., 2008. Characterization and evaluation of a modified local lymph node assay using ATP content as a non-radio isotopic endpoint. J. Pharmacol. Toxicol. Methods. 58, 1−10.

ISO, 2009. 10993-5. Biological evaluation of medical devices-Part 5: Tests for in vitro cytotoxicity. Geneva Int. Organ. Stand.

Jack, H.D., 1994. Immunotoxicology and Immunopharmacology. CRC Press, New York.

Jones, J.R., 2015. Reprint of: Review of bioactive glass: From Hench to hybrids. Acta Biomater. 23, S53−S82.

Jong, W.H.D., Carraway, J.W., Geertsma, R.E., 2012. 7—In vivo and in vitro testing for the biological safety evaluation of biomaterials and medical devices. In: Boutrand, J.-P. (Ed.), Biocompatibility and Performance of Medical Devices, Woodhead Publishing Series in Biomaterials. Woodhead Publishing, Cambridge, UK, pp. 120−158.

Kang, E., Choi, Y.Y., Chae, S.K., Moon, J.H., Chang, J.Y., Lee, S.H., 2012. Microfluidic spinning of flat alginate fibers with grooves for cell-aligning scaffolds. Adv. Mater. 24, 4271−4277.

Khaled, M., 2012. Composites and their applications. In: Ning, H. (Ed.), Biocomposite Materials. InTech, Rijeka, Croatia.

Kimber, I., Basketter, D.A., Gerberick, G.F., Ryan, C.A., Dearman, R.J., 2011. Chemical allergy: translating biology into hazard characterization. Toxicol. Sci. 120, S238−S268.

Kirker, K.R., Luo, Y., Nielson, J.H., Shelby, J., Prestwich, G.D., 2002. Glycosaminoglycan hydrogel films as bio-interactive dressings for wound healing. Biomaterials. 23, 3661−3671.

Klemm, D., Heublein, B., Fink, H.P., Bohn, A., 2005. Cellulose: Fascinating biopolymer and sustainable raw material. Angew. Chemie-Int. Ed. 44, 3358−3393.

Klötzer, W.T., 1978. Oral mucosa usage tests. J. Endod. 4, 312−315.

Kogan, G., Šoltés, L., Stern, R., Gemeiner, P., 2007. Hyaluronic acid: A natural biopolymer with a broad range of biomedical and industrial applications. Biotechnol. Lett. 29, 17−25.

Krishnan, V., Lakshmi, T., 2013. Bioglass: a novel biocompatible innovation. J. Adv. Pharm. Technol. Res. 4, 78–83.

Kumar, M., 2000. A review of chitin and chitosan applications. React. Funct. Polym. 46, 1–27.

Lee, K.Y., Jeong, L., Kang, Y.O., Lee, S.J., Park, W.H., 2009. Electrospinning of polysaccharides for regenerative medicine. Adv. Drug Deliv. Rev. 61, 1020–1032.

Liuyun, J., Yubao, L., Li, Z., Jianguo, L., 2008. Preparation and properties of a novel bone repair composite: nano-hydroxyapatite/chitosan/carboxymethyl cellulose. J. Mater. Sci. Mater. Med. 19, 981–987.

Martina, B., Kateřina, K., Miloslava, R., Jan, G., Ruta, M., 2009. Oxycellulose: significant characteristics in relation to its pharmaceutical and medical applications. Adv. Polym. Technol. 28, 199–208.

Middleton, J.C., Tipton, A.J., 2000. Synthetic biodegradable polymers as orthopedic devices. Biomaterials. 21, 2335–2346.

Miglani, S., Aggarwal, V., Ahuja, B., 2010. Dentin hypersensitivity: recent trends in management. J. Conserv. Dent. 13, 218–224.

Moreau, J.L., Xu, H.H.K., 2009. Mesenchymal stem cell proliferation and differentiation on an injectable calcium phosphate-chitosan composite scaffold. Biomaterials. 30, 2675–2682.

Mosbahi, S., Oudadesse, H., Wers, E., Trigui, M., Lefeuvre, B., Roiland, C., et al., 2016. Study of bioactive glass ceramic for use as bone biomaterial in vivo: investigation by nuclear magnetic resonance and histology. Ceram. Int. 42, 4827–4836.

Mueller, S.M., Shortkroff, S., Schneider, T.O., Breinan, H.A., Yannas, I.V., Spector, M., 1999. Meniscus cells seeded in type I and type II collagen-GAG matrices in vitro. Biomaterials. 20, 701–709.

Murray, P.E., Garcia-Godoy, F., 2007. Comparison of the clinical and preclinical biocompatibility testing of dental materials: are the ISO usage tests meaningful? J. Biomed. Mater. Res. A. 81, 51–58.

Nair, L.S., Laurencin, C.T., 2007. Biodegradable polymers as biomaterials. Prog. Polym. Sci. 32, 762–798.

Nakajima-Kambe, T., Shigeno-Akutsu, Y., Nomura, N., Onuma, F., Nakahara, T., 1999. Microbial degradation of polyurethane, polyester polyurethanes and polyether polyurethanes. Appl. Microbiol. Biotechnol. 51, 134–140.

Nicodemus, G.D., Villanueva, I., Bryant, S.J., 2007. Mechanical stimulation of TMJ condylar chondrocytes encapsulated in PEG hydrogels. J. Biomed. Mater. Res. A. 83, 323–331.

Nuttelman, C.R., Mortisen, D.J., Henry, S.M., Anseth, K.S., 2001. Attachment of fibronectin to poly(vinyl alcohol) hydrogels promotes NIH3T3 cell adhesion, proliferation, and migration. J. Biomed. Mater. Res. 57, 217–223.

Olalde, B., Garmendia, N., Sáez-Martínez, V., Argarate, N., Nooeaid, P., Morin, F., et al., 2013. Multifunctional bioactive glass scaffolds coated with layers of poly(d,l-lactide-co-glycolide) and poly(n-isopropylacrylamide-co-acrylic acid) microgels loaded with vancomycin. Mater. Sci. Eng. C. 33, 3760–3767.

Ørstavik, D., Mjör, I.A., 1992. Usage test of four endodontic sealers in Macaca fascicularis monkeys. Oral Surg. Oral Med. Oral Pathol. 73, 337–344.

Pérez, R.A., Won, J.E., Knowles, J.C., Kim, H.W., 2013. Naturally and synthetic smart composite biomaterials for tissue regeneration. Adv. Drug Deliv. Rev. 65, 471–496.

Peter, S.J., Miller, S.T., Zhu, G., Yasko, A.W., Mikos, A.G., 1998. In vivo degradation of a poly(propylene fumarate)/beta-tricalcium phosphate injectable composite scaffold. J. Biomed. Mater. Res. 41, 1–7.

Phillips, D.H., Arlt, V.M., 2009. Genotoxicity: damage to DNA and its consequences. In: Luch, A. (Ed.), Molecular, Clinical and Environmental Toxicology: Volume 1: Molecular Toxicology. Birkh{ä}user Basel, Basel, pp. 87–110.

Pinchuk, L., 1994. A review of the biostability and carcinogenicity of polyurethanes in medicine and the new generation of "biostable" polyurethanes. J. Biomater. Sci. Polym. Ed. 6, 225–267.

Pinkert, A., Marsh, K.N., Pang, S., Staiger, M.P., 2009. Ionic liquids and their interaction with cellulose. Chem. Rev. 109, 6712–6728.

Place, E.S., George, J.H., Williams, C.K., Stevens, M.M., 2009. Synthetic polymer scaffolds for tissue engineering. Chem. Soc. Rev. 38, 1139–1151.

Rabiee, S.M., Nazparvar, N., Azizian, M., Vashaee, D., Tayebi, L., 2015. Effect of ion substitution on properties of bioactive glasses: A review. Ceram. Int. 41, 7241–7251.

Ratner, B.D., Hoffman, A.S., Schoen, F.J., Lemons, J.E., 2004. Biomaterial Science—An Introduction to Materials in Medicine, Biomaterials Science. Elsevier Academic Press, San Diego, CA.

Repetto, G., del Peso, A., Zurita, J.L., 2008. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. Nat. Protoc. 3, 1125–1131.

Rinaudo, M., 2006. Chitin and chitosan: properties and applications. Prog. Polym. Sci. 31, 603–632.

Rong, D., Chen, P., Yang, Y., Li, Q., Wan, W., Fang, X., et al., 2016. Fabrication of gelatin/PCL electrospun fiber mat with bone powder and the study of its biocompatibility. J. Funct. Biomater. 7, 6.

Sakaguchi, R.L., Powers, J.M., 2012. Craig's Restorative Dental Materials. Elsevier Health Sciences, Philadelphia, PA.

Schmalz, G., Arenholt-Bindslev, D., 2009. Biocompatibility of Dental Materials. Springer-Verlag GmbH, Heidelberg, Germany.

Schmalz, G., Arenholt-Bindslev, D., 2009. Biocompatibility of Dental Materials.

Schmalz, G., Schweikl, H., 1994. Characterization of an in vitro dentin barrier test using a standard toxicant. J. Endod. 20, 592–594.

Seala, B.L., Oterob, T.C., Panitch, A., 2001. Polymeric biomaterials for tissue and organ regeneration. Mater. Sci. Eng. 34, 147–230.

Seyfert, U.T., Biehl, V., Schenk, J., 2002. In vitro hemocompatibility testing of biomaterials according to the ISO 10993-4. Biomol. Eng. 19, 91–96.

Silva, S.S., Mano, J.F., Reis, R.L., 2010. Potential applications of natural origin polymer-based systems in soft tissue regeneration. Crit. Rev. Biotechnol. 30, 200–221.

Sjögren, G., Sletten, G., Dahl, J.E., 2000. Cytotoxicity of dental alloys, metals, and ceramics assessed by Millipore filter, agar overlay, and MTT tests. J. Prosthet. Dent. 84, 229–236.

Suchanek, W., Yoshimura, M., 1998. Processing and properties of hydroxyapatite-based biomaterials for use as hard tissue replacement implants. J. Mater. Res. 13, 94–117.

Sun, J., Tan, H., 2013. Alginate-based biomaterials for regenerative medicine applications. Materials (Basel). 6, 1285–1309.

Svensson, A., Nicklasson, E., Harrah, T., Panilaitis, B., Kaplan, D.L., Brittberg, M., et al., 2005. Bacterial cellulose as a potential scaffold for tissue engineering of cartilage. Biomaterials. 26, 419–431.

Tan, H., Chu, C.R., Payne, K.A., Marra, K.G., 2009a. Injectable in situ forming biodegradable chitosan-hyaluronic acid based hydrogels for cartilage tissue engineering. Biomaterials. 30, 2499–2506.

Tan, H., Huang, D., Lao, L., Gao, C., 2009b. RGD modified PLGA/gelatin microspheres as microcarriers for chondrocyte delivery. J. Biomed. Mater. Res. B: Appl. Biomater. 91, 228−238.

Tan, H., Marra, K.G., 2010. Injectable, biodegradable hydrogels for tissue engineering applications. Materials (Basel). 3, 1746−1767.

Tan, H., Wan, L., Wu, J., Gao, C., 2008. Microscale control over collagen gradient on poly(l-lactide) membrane surface for manipulating chondrocyte distribution. Colloids Surf. B: Biointerfaces. 67, 210−215.

Tang, S., Vickers, S.M., Hsu, H.P., Spector, M., 2007. Fabrication and characterization of porous hyaluronic acid-collagen composite scaffolds. J. Biomed. Mater. Res. A. 82, 323−335.

Taylor, M.S., Daniels, A.U., Andriano, K.P., Heller, J., 1994. Six bioabsorbable polymers: in vitro acute toxicity of accumulated degradation products. J. Appl. Biomater. 5, 151−157.

Temenoff, J.S., Mikos, A.G., 2000. Injectable biodegradable materials for orthopedic tissue engineering. Biomaterials. 21, 2405−2412.

Tziafas, D., Smith, A.J., Lesot, H., 2000. Designing new treatment strategies in vital pulp therapy. J. Dent. 28, 77−92.

Van Gestel, N.A.P., Geurts, J., Hulsen, D.J.W., Van Rietbergen, B., Hofmann, S., Arts, J.J., 2015. Clinical applications of S53P4 bioactive glass in bone healing and osteomyelitic treatment: a literature review. Biomed. Res. Int.1−12.

van Meerloo, J., Kaspers, G.J.L., Cloos, J., 2011. Cell sensitivity assays: the MTT assay. In: Cree, I.A. (Ed.), Cancer Cell Culture: Methods and Protocols. Humana Press, Totowa, NJ, pp. 237−245.

Wahl, D.A., Czernuszka, J.T., 2006. Collagen-hydroxyapatite composites for hard tissue repair. Eur. Cells Mater. 11, 43−56.

Wataha, J.C., 2001. Principles of biocompatibility for dental practitioners. J. Prosthet. Dent. 86, 203−209.

Wataha, J.C., 2012. Predicting clinical biological responses to dental materials. Dent. Mater. 28, 23−40.

Watanabe, Y., Hamada, N., Morita, M., Tsujisaka, Y., 1976. Purification and properties of a polyvinyl alcohol-degrading enzyme produced by a strain of Pseudomonas. Arch. Biochem. Biophys. 174, 575−581.

Wilson, O.C., Hull, J.R., 2008. Surface modification of nanophase hydroxyapatite with chitosan. Mater. Sci. Eng. C. 28, 434−437.

Wu, C., Chang, J., 2012. Mesoporous bioactive glasses: structure characteristics, drug/growth factor delivery and bone regeneration application. Interface Focus. 2, 292−306.

Yunus Basha, R., Sampath, S.K., Doble, M., 2015. Design of biocomposite materials for bone tissue regeneration. Mater. Sci. Eng. C. 57, 452−463.

Zhang, J.Y., Beckman, E.J., Piesco, N.P., Agarwal, S., 2000. A new peptide-based urethane polymer: synthesis, biodegradation, and potential to support cell growth in vitro. Biomaterials. 21, 1247−1258.

Zhang, Y., Venugopal, J.R., El-Turki, A., Ramakrishna, S., Su, B., Lim, C.T., 2008. Electrospun biomimetic nanocomposite nanofibers of hydroxyapatite/chitosan for bone tissue engineering. Biomaterials. 29, 4314−4322.

Zhao, F., Yin, Y., Lu, W.W., Leong, J.C., Zhang, W., Zhang, J., et al., 2002. Preparation and histological evaluation of biomimetic three-dimensional hydroxyapatite/chitosan-gelatin network composite scaffolds. Biomaterials. 23, 3227−3234.

Zhou, S., Liao, X., Li, X., Deng, X., Li, H., 2003. Poly-D,L-lactide-co-poly(ethylene glycol) microspheres as potential vaccine delivery systems. J. Control. Release. 86, 195−205.

Processing and preservation of biomaterials and regulatory issues

9

Keyvan Moharamzadeh
University of Sheffield, Sheffield, United Kingdom

9.1 Introduction

Biomaterials used in oral and dental tissue engineering and regeneration can be classified based on their original sources into different categories: (1) human-derived biomaterials such as human acellular dermal matrix (Chen and Liu, 2016); (2) animal-derived biomaterials including processed bovine bone materials (Anderson et al., 2000) and porcine-based collagen membranes (Herford et al., 2010); (3) other natural biomaterials such as polysaccharide-based biomaterials (cellulose, chitin/chitosan) (Oliveira and Reis, 2011); (4) synthetic biomaterials such as polymers, composites, and ceramic-based dental biomaterials (Rojo and Deb, 2015; Deb and Chana, 2015).

Different processing techniques have been developed aiming to convert the original material into a suitable biocompatible biomaterial that can be clinically transplanted into the body to promote tissue repair and regeneration without the risk of adverse reactions such as infection, disease transmission, local or systemic toxicity, and graft rejection. Essential processing steps to achieve this aim include the removal of cells and other antigenic components, maintaining nonantigenic bioactive structures, optimization of biological properties such as biodegradability and customization of the physical properties such as shape, density, and porosity of the final product (Keane et al., 2015; Tabatabaei et al., 2016; Dutta et al., 2017).

In order to minimize the risk of disease transmission and microbial infection an appropriate sterilization method should be used to produce a sterile biomaterial without compromising its physical and biological properties during sterilization process (Dai et al., 2016).

Preservation and achieving optimal bio-stability for the final product can be challenging especially if the biomaterial is used as a carrier for cells and packaged and stored in combination with cells (Almela et al., 2016b).

There is a large number of ethical and regulatory issues surrounding the development and the clinical use of biomaterials with different origins on patients (Williams, 2015). These include regulatory and ethical issues regarding obtaining of the source material, treatment, and processing of the material, testing of the developed products on animals and human, marketing, and postmarket surveillance of biomaterials.

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00009-8
© 2017 Elsevier Ltd. All rights reserved.

9.2 Processing of biomaterials

Processing techniques differ significantly for some animal and human-derived biomaterials used in dentistry compared to the other biomaterials. Important aspects of the processing methods for different types of biomaterials are discussed in the following sections.

9.2.1 Animal and human-derived biomaterials

Since the source material for animal and human-derived biomaterials contain cells, proteins, and biological components that can act as antigens, decellularization and deproteinization of the original hard and soft tissues have been investigated in the literature (Keane et al., 2015; Broz et al., 1997; Castro-Cesena et al., 2013) and are currently used in the manufacturing of commercially available products.

9.2.1.1 Soft tissue biomaterials

Acellularized human dermal matrix (e.g., AlloDerm, Lifecell Corp.) and processed porcine collagen membranes (e.g., Bio-Gide and Mucograft, Geistlich) have been widely used for regeneration of oral soft tissues and guided tissue regeneration procedures in dentistry (Shridharani and Tufaro, 2012; Herford et al., 2010; Bottino et al., 2012).

Acellular human dermis is prepared by first separation and removing the epidermal component of cadaver skin by storage in sodium chloride solution (Lamb and Ambler, 2014). The dermal component of the skin is then treated with an appropriate detergent to dissolve and remove the remaining cellular components of the dermis. The decellularized dermis is then sterilized and freeze-dried to maintain the structural integrity and the porosity of the dermal scaffold which would allow infiltration of the cells into the membrane once grafted into the body. It is important to rehydrate the dried matrix in a physiologically balanced solution for adequate time prior to surgical transplantation or cell seeding if the intended application is for in vitro tissue engineering (Bottino et al., 2009).

Other examples of clinically used decellularized dermal matrices include: NeoForm (Mentor Worldwide LLC) and GraftJacket (Wright Medical Technology Inc.) derived from human dermis, Strattice (Lifecell Corp.) and Zimmer Collagen Repair Patch (Zimmer Inc.) derived from porcine dermis, and TissueMend (Stryker Corp.) derived from bovine dermis. Nondermal sources of soft tissue for decellularized biomaterials include human, equine, and bovine pericardium, porcine small intestine, mesothelium, urinary bladder, and heart valve (Crapo et al., 2011).

Decellularization techniques can be categorized into three different groups (Crapo et al., 2011; Keane et al., 2015):

- Physical methods including freeze–thaw cycles, direct application of force and pressure, and electroporation.

- Chemical treatment with acids and bases, hypotonic and hypertonic solutions, detergents such as triton X-100, triton X-200, sodium dodecyl sulfate, sodium deoxycholate, sulfobetaine-10 and -16, and solvants such as alcohols, acetone, and tributyl phosphate.
- Treatment with chelating agents such as ethylenediaminetetraacetic acid.
- Enzyme treatment including nucleases, trypsin, and dispase.

Animal-derived collagen-based membranes are produced by first extraction of collagen from the body parts of the animal that contain high amounts of collagen such as skin, cartilage, or tendon (Yarat et al., 1996). A common source of collagen for commercially available products is calf skin or rat tail. Collagen is extracted by incubation of the minced tissue in acids such as acetic acid. Soluble collagen is then neutralized using alkaline buffer solutions to produce the collagen pellets. The final stages include sterilization of collagen using ethanol and lyophilization. Collagen can also be cross-linked using glutaraldehyde to increase its mechanical strength and reduce its biodegradability (Dunn, 2012).

Fibrin-based adhesive biomaterials have been used clinically as hemostatic plug and for tissue approximation and fixation as alternative to suturing (Spotnitz and Prabhu, 2005). In vitro applications include tissue engineering of oral mucosa (Llames et al., 2014; Peña et al., 2010) and complex 3D oral osteo-mucosal model (Almela et al., 2016a). Fibrin gels can be obtained from the patient's serum or can be prepared by combining its separate components including fibrinogen, thrombin, and $CaCl_2$. Fibrin-based biomaterials show excellent biocompatibility and biodegradability and continue to be used both in vivo and in vitro (Bao Ha et al., 2013).

9.2.1.2 Hard tissue biomaterials

Processed bovine bone has been widely used as a bone graft material for alveolar bone augmentation and guided bone regeneration around dental implants (Jensen et al., 2012; Tolstunov and Chi, 2011; Esposito et al., 2006). Commonly used manufacturing process involves separation of soft tissue from the bone, grinding the bone into small particles, boiling of the bone fragments in various organic solvents to remove the cellular components, boiling the material in water several times to eliminate residual organic solvents, freeze drying, and sterilization (Lytle, 1994).

Animal dentin has also been processed using different methods for potential applications as bone substitute (Tabatabaei et al., 2016). The author's research group developed a bovine dentin-based bone material by processing the extracted bovine teeth according to the following protocol (Moharamzadeh et al., 2008):

Extracted young bovine teeth with open apices were washed with water, cleaned, and all soft tissues including the pulp and periodontal ligament were removed from the root. After the enamel had been removed with a high-speed diamond bur the dentine was broken into small pieces (5−10 mm) using a mortar and pestle. After boiling the dentine fragments in distilled water for 2 hours, they were refluxed in isopropanol for 2 hours to remove any remaining soft tissue or fat. Following further washing with distilled boiling water several times to eliminate the organic solvent the fragments were air dried at 100°C. Final stages involved grinding pieces of

dentine into small particle powder using a high-speed mechanical blender, packaging, and sterilization by gamma-irradiation.

In vivo implantation testing of the dentine into rat femur showed good biocompatibility without eliciting any inflammatory reaction or infiltration of foreign body giant cells (Moharamzadeh et al., 2008). Processed dentin had the ability to be replaced by the new bone, indicate that the developed material had the potential to be used as an osteoconductive bone substitute. Further animal studies using rabbit calvarial defect model showed the processed bovine dentin was a biostable material but its use may be limited to repairing small 5-wall defects rather than large 4-wall defects in the absence of barrier membranes (Hussain et al., 2012).

Extracted human tooth dentin has been processed and used as an autologous bone graft material at the same time of bone augmentation surgery. Some recent studies show promising results for this approach (Kim et al., 2017; Schwarz et al., 2016).

Extracted human tooth dentin processing devices have been developed to facilitate the production of autologous dentin-based graft materials. However, the major limitations of these devices are the issues with cross infection control between the patients as it would be difficult to sterilize the parts of the equipment that come to contact with human teeth and it may be necessary to discard some parts after single use which can increase the costs significantly. Effective elimination of the chemicals used by the device to treat dentin poses another challenge due to the limited time available from processing of the material to its clinical use at the same time in the surgery.

9.2.2 Other natural scaffolds and synthetic biomaterials

A wide range of processing techniques have been used for the development of synthetic biomaterials and natural scaffolds with ideal physical, chemical, and structural properties (Dutta et al., 2017). The focus has been on the optimization of the mechanical strength, shape and morphology, and cell interactivity of the biomaterial.

Mechanical strength is particularly important for the biomaterials to be used as load bearing hard tissue structures and is affected by microstructure, and the material properties, such as elasticity and hardness.

The shape of the scaffold should match the size of the defect if the material is intended for clinical use. In addition, creating a 3D scaffold with optimal porosity including pore size, distribution, and connectivity to allow adequate and efficient cell and tissue growth is another crucial aspect of processing biomaterials.

Cell interactivity of the biomaterial can be enhanced by optimizing the 3D molecular architecture and arrangement in the scaffold. Integration of the molecules which interact with cells and promote cell adhesion and differentiation in a controlled manner within the scaffold can be a helpful approach to achieve desirable regeneration outcome.

Processing techniques used to date for biomaterials to achieve the above goal can be divided into conventional material processing techniques and advanced manufacturing techniques (Dutta et al., 2017).

Examples of conventional methods for processing of biomaterials and scaffolds that can be used in tissue engineering of oral and dental tissues are listed below:

- Solvent casting with particulate leaching (Marei et al., 2003)
- Extrusion (Danoux et al., 2014)
- Molding including compression molding, melt molding/blowing and injection molding (Haugen et al., 2006; Conrad et al., 2013)
- Thermally induced gelation/freeze drying (Li et al., 2015)
- Gas foaming (Chen et al., 2012)
- Supercritical fluid processing (Garcia-Gonzalez et al., 2015)
- Microsphere sintering (Rasoulianboroujeni et al., 2016, Khoshroo et al., 2017)
- Fiber bonding (Tang et al., 2011)
- Sponge matrix embedding (Polo-Corrales et al., 2014)

Advanced manufacturing techniques that have the potential to be used for processing of biomaterials for oral and dental tissue engineering include:

- Rapid prototyping techniques including 3D printing (Obregon et al., 2015), stereolithography (Kim et al., 2010), fused deposition modeling (Ramanath et al., 2008), and selective laser sintering (Mangano et al., 2013).
- Electro-spinning (Ji et al., 2011; Sajkiewicz and Kolbuk, 2014; Ohkawa, 2015)
- In situ photopolymerization (Nguyen and West, 2002; Wang et al., 2012)
- High internal phase emulsion (Kovacic et al., 2014)
- Self-assembling peptides (Pugliese and Gelain, 2017)

9.3 Sterilization of biomaterials

The scaffolds and biomaterials must be sterilized prior to clinical use or before cell seeding if the intended application is for in vitro tissue engineering. The aim of sterilization is to eliminate the risk of disease transmission to the patients and also to reduce the risk of infection of the implanted material in vivo or the scaffold-tissue culture system in vitro. Different techniques that have been used for sterilization of biomaterials are thoroughly reviewed in a recent publication by Dai et al. (2016) including their mechanism of action, efficiencies, effects on biomaterials, and their relative advantages and disadvantages. Sterilization methods for biomaterials have been listed below:

- Heat sterilization using steam under pressure or dry heat
- Irradiation with gamma ray, electron-beam, or ultraviolet (UV) light
- Plasma sterilization using inert gas plasma
- Chemical sterilization using ethylene oxide, ethanol, peracetic acid, or iodine.
- Other new techniques including the use of supercritical carbon dioxide, antibiotics, and freeze drying.

Important considerations in the choice of sterilization technique for a particular biomaterial include the effects of various sterilization techniques on the biochemical, structural, and physical properties of the scaffolds such as mechanical strength,

porosity, and morphology as well as the effects of residual toxic chemical sterilization agents on biocompatibility of the biomaterial.

Despite the availability of a wide range of sterilization methods, systematic reviews show bovine-derived graft biomaterials may carry a risk of bovine spongiform encephalopathy (BSE) prion transmission to patients (Kim et al., 2013).

Mycoplasma infection of biomaterials and tissue cultures has a high incidence rate and is particularly problematic due to being resistant to antibiotics and visually undetectable. Practical antibiotic protocols have been developed for the elimination of mycoplasma contaminations from cell cultures (Uphoff and Drexler, 2014).

9.4 Preservation and storage

Biomaterials are often stored for a period of time prior to being used clinically on patients or in the laboratory before implantation in vivo or cell seeding in vitro. Storage conditions such as temperature and preservation media can have significant effects on the quality of the biomaterial over long period of time. Preservation of cell-containing products can be more challenging than the preservation of acellular products due to presence of living cells that need to be maintain vital and functional without compromising their biological regenerative potential. Techniques and challenges for preservation of both types are discussed below.

9.4.1 Cell-containing products

Commercially available fibroblast- and keratinocyte-populated skin substitutes are the examples of cell-containing biomaterials with potential applications in oral and dental tissue engineering and regeneration (Moharamzadeh et al., 2007) that have been discussed in a separate chapter on oral mucosa tissue engineering.

Cryopreservation is one of the most commonly used methods of preserving living cells and tissues by using very low temperatures to preserve the cells and their structures intact (Pegg, 2015). Cooling can be lethal to cells due to freezing water and formation of ice crystals inside cells. Cryoprotectants such as dimethyl sulfoxide, glycerol, ethanediol, and propanediol are often used to minimize the risk of ice formation during temperature change. However, current washing protocols may not ensure complete elimination of these chemicals and the clinical adverse effects of some of these cryoprotectants have been documented (Almela et al., 2016b). The hazard of formation of ice crystals can be further prevented by vitrification which is the process of ultra-rapid cooling of the tissue at an optimal rate to cryogenic temperatures to produce an amorphous ice-free solid (Fahy and Wowk, 2015).

Vitrification demonstrates a higher cell recovery rate than conventional freeze−thawing technique. Limitations of vitrification include increased contamination risk due to direct exposure of cells to liquid nitrogen, labor intensiveness, and inefficiency in large-scale cell production. Microencapsulation techniques have been

introduced to overcome some of these limitations but they would be extra steps that adds to the complexity of the preservation process (Serra et al., 2011).

Cell storage also carries the risk of misidentification and cross-contamination of cell lines, contamination with environmental and bacterial organisms and therefore, requires stringent methodology and quality control testing (Almela et al., 2016b).

9.4.2 Acellular biomaterials

Freeze drying, or lyophilization, is the most commonly used method for preservation of acellular protein-containing biomaterials such as decellularized dermal matrices, collagen-based membranes, demineralized bone substitutes. Lyophilization is a dehydration storage technique that involves the vacuum removal of water from the material after it is frozen. This process allows the solid ice to change directly to vapor phase without passing through a liquid phase. Carbohydrates can be added as lyoprotectant for proteins. The lyophilized biomaterials are often reconstituted prior to their clinical use with the addition of an appropriate solvent which is usually a balanced fluid.

Freeze drying has also been used for processing and preservation of chitosan-based (Aranaz et al., 2014) and other biodegradable polymer-based biomaterials (Janik and Marzec, 2015).

The quality of the freeze-dried product depends on the material freezing protocol, the size and distribution of the produced ice crystals and amorphous areas, the degree of heterogeneity, and the final drying conditions (Coger and Toner, 2000).

9.5 Regulatory issues

Regulations surrounding the safety of biomaterials can differ from a country to another country and can be very confusing when it comes to conducting research that involves the animals and human subjects. Obtaining permission and license for commercializing the biomaterial products can also be a very challenging process. The relevant regulatory bodies in the United States, and the United Kingdom as well as the European and international regulators have been discussed below.

9.5.1 International regulations

The International Standards Organization (ISO) is the main international body responsible for ensuring the safety and quality assurance of products. Biological evaluation of medical devices and biomaterials are described by ISO 10993. Part 1 to part 18 set out standards for various aspects of biological assessment of biomaterials including guidance on test selection, animal welfare requirements, different relevant in vitro and in vivo biological tests, sterilization standards, clinical trials, evaluation and quantification of biodegradation, and characterization of materials (ISO, 2009).

ISO tests are mainly based on the traditional biological tests that purely assess the safety and toxicity of the material when it comes to contact with the living tissues. However, these tests do not assess the intended biological performance of the biomaterials used in tissue engineering in terms of the ability of the material to actively signal the target cells. Therefore relying only on ISO tests may permit the use of some inert and harmless biomaterials which have no appropriate biological activity and may not benefit the patients (Williams, 2015).

9.5.2 United States regulatory agencies

The US Food and Drug Administration (FDA) is the regulator for all the medical devices in the United States (Sall, 2013). FDA classifies medical devices based on their associated risks into three risk categories including Class I (low risk), Class II (medium risk), and Class III (high risk). The important determining factors are used according to the ISO standards including the type of body contact (such as surface contact, external communicating devices, and implanted devices) and the duration of contact (limited, prolonged, and permanent exposure). Class I devices are subject to the least regulatory controls (general controls). Class II devices require greater regulatory control (general controls and special controls) to ensure their safety and efficacy. Class III devices are subject to the highest level of regulatory control (general controls and premarket approval (PMA)) (FDA, 2002). Preclinical testing is an essential part of the biomaterial development process. The types of required preclinical tests depend on the nature of the device and the intended application and may include animal and bench studies. All nonclinical laboratory tests must be performed under the Good Laboratory Practices regulation (FDA, 2016). Research involving human subjects and clinical trials require Institutional Review Board (IRB) review prior to study initiation.

Submission process for the Class I devices may include only registration of the product. However, 510(k) application will be normally required for Class II devices including clinical or product testing. Class III devices require clinical trial protocol development and approval by the FDA as well as preparation and submission of PMA application to the FDA.

9.5.3 European regulators

Introducing medical devices to the EU market required compliance with the EU's Medical Devices Directive (MDD) or Active Implantable Medical Devices Directive (AIMDD) requirements and regulations. MDD classified the medical devices into four classes (Class I, IIa, IIb, and III) based on their risk (low, medium, medium to high, and high) and intended usage and degree of invasiveness (sterile, nonsterile, implantable, active implantable) and duration of contact with the patient (transient, short term, and long term). Following the establishment of the classification the manufacturer must put into effect a quality management system (QMS) and prepare a design dossier and submit a technical file demonstrating compliance with MDD to the notified body. The manufacturer's QMS and technical file would then be audited

by the notified body. Once the device passed the audit the device must be registered with the competent authority and the manufacturer must prepare a declaration of conformity so that the CE Mark to be granted (Talary and Huang, 2015).

The Medical Devices Directive is being revised and replaced by a Medical Device Regulation (MDR). Final formal adoption is expected during the first semester 2017.

9.5.4 United Kingdom regulatory requirements

The Medicines and Healthcare products Regulatory Agency (MHRA) is responsible to ensure the safety and the quality of medical devices marketed in the United Kingdom. The MHRA conducts postmarketing surveillance of medicines and medical devices, evaluates and authorizes the drugs and medical devices for marketing in the United Kingdom and ensures manufacturer's compliance with the regulatory requirements. It operates a quality surveillance system including sampling and testing medicines to identify quality defects and monitors the quality and safety of unlicensed products. MHRA regulates clinical trials of the products, monitors and ensures compliance with statutory obligations, and promotes safe use of medicines and devices (MHRA, 2017).

Research using animal in the United Kingdom is strictly regulated and would require prior permission and appropriate personal, project, and establishment licenses from the Home Office. The laws regarding animal research are set out in the Animals (Scientific Procedures) Act 1986, or "ASPA" which are enforced by the Home Office and include regulations on animal care, housing, welfare, environment, and health.

The Human Tissue Authority (HTA) regulates the use of human tissue for research and transplantation and ensures compliance with the Human Tissue Act 2004. In addition to the MHRA approval, permission from the HTA would be required for the development of biomaterials that their origin is from human tissue. Additional HTA approval would also be required for the studies of clinical transplantation of tissue-engineered human organs.

Research involving human subjects will also require prior ethical approval from an appropriate research ethics committee in the United Kingdom. Applications to the National Research Ethics Service (NRES) which is now part of the Health Research Authority (HRA) can be made online through the Integrated Research Application System (IRAS) which is a web-based system for preparing regulatory and governance applications for health and social care research. All clinical research must be performed under Good Clinical Practice regulations (NIHR, 2016).

9.6 Conclusions

It is essential to be aware of the complex and constantly changing regulatory requirements and consider these issues in the planning stages to facilitate the entire development process for biomaterials. All stages including obtaining the source

material, processing, characterization, sterilization, preservation, in vitro and in vivo biocompatibility testing, clinical trials, and efficacy studies of biomaterials must be tailored to fulfill the regulatory requirements from the outset. This will speed up the marketing process and allow rapid access to innovative technology for the general population. Postmarket surveillance plays an important role in identifying the products with the risk of adverse effects that their risks had not been detected during premarket evaluation phase.

References

Almela, T., Brook, I.M., Moharamzadeh, K., 2016a. Development of three-dimensional tissue engineered bone-oral mucosal composite models. J. Mater. Sci. Mater. Med. 27, 65.

Almela, T., Brook, I.M., Moharamzadeh, K., 2016b. The significance of cell-related challenges in the clinical application of tissue engineering. J. Biomed. Mater. Res. A. 104, 3157–3163.

Anderson, I.A., Mucalo, M.R., Johnson, G.S., Lorier, M.A., 2000. The processing and characterization of animal-derived bone to yield materials with biomedical applications. Part III: material and mechanical properties of fresh and processed bovine cancellous bone. J. Mater. Sci. Mater. Med. 11, 743–749.

Animals (Scientific Procedures) Act 1986, United Kingdom.

Aranaz, I., Gutierrez, M.C., Ferrer, M.L., Del Monte, F., 2014. Preparation of chitosan nanocomposites with a macroporous structure by unidirectional freezing and subsequent freeze-drying. Mar. Drugs. 12, 5619–5642.

Bao Ha, T.L., Quan, T.M., Nguyen, V.D., Si, D.M., 2013. Naturally derived biomaterials: preparation and application. Regener. Med. Tissue Eng. 1, 247–274.

Bottino, M.C., Jose, M.V., Thomas, V., Dean, D.R., Janowski, G.M., 2009. Freeze-dried acellular dermal matrix graft: effects of rehydration on physical, chemical, and mechanical properties. Dent. Mater. 25, 1109–1115.

Bottino, M.C., Thomas, V., Schmidt, G., Vohra, Y.K., Chu, T.M., Kowolik, M.J., et al., 2012. Recent advances in the development of GTR/GBR membranes for periodontal regeneration—a materials perspective. Dent. Mater. 28, 703–721.

Broz, J.J., Simske, S.J., Corley, W.D., Greenberg, A.R., 1997. Effects of deproteinization and ashing on site-specific properties of cortical bone. J. Mater. Sci. Mater. Med. 8, 395–401.

Castro-Cesena, A.B., Sanchez-Saavedra, M.P., Novitskaya, E.E., Chen, P.Y., Hirata, G.A., Mckittrick, J., 2013. Kinetic characterization of the deproteinization of trabecular and cortical bovine femur bones. Mater. Sci. Eng. C: Mater. Biol. Appl. 33, 4958–4964.

Chen, F.M., Liu, X., 2016. Advancing biomaterials of human origin for tissue engineering. Prog. Polym. Sci. 53, 86–168.

Chen, W., Zhou, H., Tang, M., Weir, M.D., Bao, C., Xu, H.H., 2012. Gas-foaming calcium phosphate cement scaffold encapsulating human umbilical cord stem cells. Tissue Eng. A. 18, 816–827.

Coger, R., Toner, M., 2000. Preservation Techniques for Biomaterials. In: Bronzino, J.D. (Ed.), The Biomedical Engineering Handbook, second ed. CRC Press, Boca Raton, FL.

Conrad, T.L., Jaekel, D.J., Kurtz, S.M., Roeder, R.K., 2013. Effects of the mold temperature on the mechanical properties and crystallinity of hydroxyapatite whisker-reinforced

polyetheretherketone scaffolds. J. Biomed. Mater. Res. B: Appl. Biomater. 101, 576–583.

Crapo, P.M., Gilbert, T.W., Badylak, S.F., 2011. An overview of tissue and whole organ decellularization processes. Biomaterials. 32, 3233–3243.

Dai, Z., Ronholm, J., Tian, Y., Sethi, B., Cao, X., 2016. Sterilization techniques for biodegradable scaffolds in tissue engineering applications. J. Tissue Eng. 7, 2041731416648810.

Danoux, C.B., Barbieri, D., Yuan, H., De Bruijn, J.D., Van Blitterswijk, C.A., et al., 2014. In vitro and in vivo bioactivity assessment of a polylactic acid/hydroxyapatite composite for bone regeneration. Biomatter. 4, e27664.

Deb, S., Chana, S., 2015. Biomaterials in relation to dentistry. Front Oral. Biol. 17, 1–12.

Dunn, R.M., 2012. Cross-linking in biomaterials: a primer for clinicians. Plast. Reconstr. Surg. 130, 18S–26S.

Dutta, R.C., Dey, M., Dutta, A.K., Basu, B., 2017. Competent processing techniques for scaffolds in tissue engineering. Biotechnol. Adv. 35 (2), 240–250.

Esposito, M., Grusovin, M.G., Coulthard, P., Worthington, H.V., 2006. The efficacy of various bone augmentation procedures for dental implants: a Cochrane systematic review of randomized controlled clinical trials. Int. J. Oral Maxillofac. Implants. 21, 696–710.

Fahy, G.M., Wowk, B., 2015. Principles of cryopreservation by vitrification. Methods Mol. Biol. 1257, 21–82.

FDA 2002. Food and Drug Administration PreMarket Approval Regulation, 21CFR 814.

FDA 2016. Food and Drug Administration Good Laboratory Practices Regulation, 21CFR 58.

Garcia-Gonzalez, C.A., Concheiro, A., Alvarez-Lorenzo, C., 2015. Processing of materials for regenerative medicine using supercritical fluid technology. Bioconjug. Chem. 26, 1159–1171.

Haugen, H., Will, J., Fuchs, W., Wintermantel, E., 2006. A novel processing method for injection-molded polyether-urethane scaffolds. Part 1: processing. J. Biomed. Mater Res. B: Appl. Biomater. 77, 65–72.

Herford, A.S., Akin, L., Cicciu, M., Maiorana, C., Boyne, P.J., 2010. Use of a porcine collagen matrix as an alternative to autogenous tissue for grafting oral soft tissue defects. J. Oral Maxillofac. Surg. 68, 1463–1470.

Home Office, 2017. Research and Testing Using Animals. Home Office, United Kingdom.

Human Tissue Act 2004, United Kingdom.

Hussain, I., Moharamzadeh, K., Brook, I.M., Jose De Oliveira Neto, P., Salata, L.A., 2012. Evaluation of osteoconductive and osteogenic potential of a dentin-based bone substitute using a calvarial defect model. Int. J. Dent.396316.

ISO 2009. International Standards Organization, biological evaluation of medical devices, ISO 10993.

Janik, H., Marzec, M., 2015. A review: fabrication of porous polyurethane scaffolds. Mater. Sci. Eng. C: Mater. Biol. Appl. 48, 586–591.

Jensen, T., Schou, S., Stavropoulos, A., Terheyden, H., Holmstrup, P., 2012. Maxillary sinus floor augmentation with Bio-Oss or Bio-Oss mixed with autogenous bone as graft: a systematic review. Clin. Oral. Implants Res. 23, 263–273.

Ji, W., Sun, Y., Yang, F., Van Den Beucken, J.J., Fan, M., Chen, Z., et al., 2011. Bioactive electrospun scaffolds delivering growth factors and genes for tissue engineering applications. Pharm. Res. 28, 1259–1272.

Keane, T.J., Swinehart, I.T., Badylak, S.F., 2015. Methods of tissue decellularization used for preparation of biologic scaffolds and in vivo relevance. Methods. 84, 25–34.

Khoshroo, K., Jafarzadeh Kashi, T.S., Moztarzadeh, F., Tahriri, M., Jazayeri, H.E., Tayebi, L., 2017. Development of 3D PCL microsphere/TiO_2 nanotube composite scaffolds for bone tissue engineering. Mater. Sci. Eng. C: Mater. Biol. Appl. 70, 586−598.

Kim, K., Yeatts, A., Dean, D., Fisher, J.P., 2010. Stereolithographic bone scaffold design parameters: osteogenic differentiation and signal expression. Tissue Eng. B: Rev. 16, 523−539.

Kim, Y.K., Pang, K.M., Yun, P.Y., Leem, D.H., Um, I.W., 2017. Long-term follow-up of autogenous tooth bone graft blocks with dental implants. Clin. Case Rep. 5, 108−118.

Kim, Y., Nowzari, H., Rich, S.K., 2013. Risk of prion disease transmission through bovine-derived bone substitutes: a systematic review. Clin. Implant Dent. Relat. Res. 15, 645−653.

Kovacic, S., Matsko, N.B., Ferk, G., Slugovc, C., 2014. Nanocomposite foams from iron oxide stabilized dicyclopentadiene high internal phase emulsions: preparation and bromination. Acta Chim. Slov. 61, 208−214.

Lamb, R., Ambler, C.A., 2014. Modified methods for growing 3-D skin equivalents: an update. Methods Mol. Biol. 1195, 171−182.

Li, X., Qin, J., Ma, J., 2015. Silk fibroin/poly (vinyl alcohol) blend scaffolds for controlled delivery of curcumin. Regen. Biomater. 2, 97−105.

Llames, S., García, E., Meana, Á., Larcher, F., Del Río, M., Recuero, I., et al., 2014. Tissue-engineered oral mucosa for mucosal reconstruction in a pediatric patient with hemifacial microsomia and ankyloglossia. Cleft Palate-Craniofac. J. 51, 246−251.

Lytle, J.L., 1994. Freeze-dried demineralized bone in dental implant reconstruction. J. Calif. Dent. Assoc. 22, 47−51.

Mangano, F., Bazzoli, M., Tettamanti, L., Farronato, D., Maineri, M., Macchi, A., et al., 2013. Custom-made, selective laser sintering (SLS) blade implants as a non-conventional solution for the prosthetic rehabilitation of extremely atrophied posterior mandible. Lasers Med. Sci. 28, 1241−1247.

Marei, M.K., Nouh, S.R., Fata, M.M., Faramawy, A.M., 2003. Fabrication of polymer root form scaffolds to be utilized for alveolar bone regeneration. Tissue Eng. 9, 713−731.

MHRA, 2017. Regulatory Guidance for Medical Devices. Medicines and Healthcare Products Regulatory Agency, UK.

Moharamzadeh, K., Brook, I.M., Van Noort, R., Scutt, A.M., Thornhill, M.H., 2007. Tissue-engineered oral mucosa: a review of the scientific literature. J. Dent. Res. 86, 115−124.

Moharamzadeh, K., Freeman, C., Blackwood, K., 2008. Processed bovine dentine as a bone substitute. Br. J. Oral Maxillofac. Surg. 46, 110−113.

Nguyen, K.T., West, J.L., 2002. Photopolymerizable hydrogels for tissue engineering applications. Biomaterials. 23, 4307−4314.

NIHR, 2016. Good Clinical Practice (GCP) Reference Guide Version 3.1. National Institute for Health Research, Clinical Research Network (NIHR CRN), UK.

Obregon, F., Vaquette, C., Ivanovski, S., Hutmacher, D.W., Bertassoni, L.E., 2015. Three-dimensional bioprinting for regenerative dentistry and craniofacial tissue engineering. J. Dent. Res. 94, 143S−152SS.

Ohkawa, K., 2015. Nanofibers of cellulose and its derivatives fabricated using direct electrospinning. Molecules. 20, 9139−9154.

Oliveira, J.T., Reis, R.L., 2011. Polysaccharide-based materials for cartilage tissue engineering applications. J. Tissue Eng. Regen. Med. 5, 421−436.

Pegg, D.E., 2015. Principles of cryopreservation. Methods Mol. Biol. 1257, 3−19.

Peña, I., Junquera, L.M., Meana, Á., García, E., García, V., De Vicente, J.C., 2010. In vitro engineering of complete autologous oral mucosa equivalents: characterization of a novel scaffold. J. Periodont. Res. 45, 375–380.
Polo-Corrales, L., Latorre-Esteves, M., Ramirez-Vick, J.E., 2014. Scaffold design for bone regeneration. J. Nanosci. Nanotechnol. 14, 15–56.
Pugliese, R., Gelain, F., 2017. Peptidic biomaterials: from self-assembling to regenerative medicine. Trends Biotechnol. 35, 145–158.
Ramanath, H.S., Chua, C.K., Leong, K.F., Shah, K.D., 2008. Melt flow behaviour of poly-epsilon-caprolactone in fused deposition modelling. J. Mater. Sci. Mater. Med. 19, 2541–2550.
Rasoulianboroujeni, M., Yazdimamaghani, M., Khoshkenar, P., Pothineni, V.R., Kim, K.M., Murray, T.A., et al., 2016. From solvent-free microspheres to bioactive gradient scaffolds. Nanomedicine. 13 (3), 1157–1169.
Rojo, L., Deb, S., 2015. Polymer therapeutics in relation to dentistry. Front. Oral Biol. 17, 13–21.
Sajkiewicz, P., Kolbuk, D., 2014. Electrospinning of gelatin for tissue engineering—molecular conformation as one of the overlooked problems. J. Biomater. Sci. Polym. Ed. 25, 2009–2022.
Sall, B., 2013. Regulation of Medical Devices. Madame Curie Bioscience Database.
Schwarz, F., Schmucker, A., Becker, J., 2016. Initial case report of an extracted tooth root used for lateral alveolar ridge augmentation. J. Clin. Periodontol. 43, 985–989.
Serra, M., Correia, C., Malpique, R., Brito, C., Jensen, J., Bjorquist, P., et al., 2011. Microencapsulation technology: a powerful tool for integrating expansion and cryopreservation of human embryonic stem cells. PLoS One. 6, e23212.
Shridharani, S.M., Tufaro, A.P., 2012. A systematic review of acelluar dermal matrices in head and neck reconstruction. Plast Reconstr. Surg. 130, 35S–43S.
Spotnitz, W.D., Prabhu, R., 2005. Fibrin sealant tissue adhesive—review and update. J. Long Term Eff. Med. Implants. 15, 245–270.
Tabatabaei, F.S., Tatari, S., Samadi, R., Moharamzadeh, K., 2016. Different methods of dentin processing for application in bone tissue engineering: a systematic review. J. Biomed. Mater. Res. A. 104, 2616–2627.
Talary, M.S., Huang, Y., 2015. Bringing an ophthalmic device to market. Is it now easier in the United States than in Europe? New Retina MD. 6, 6–9.
Tang, Y., Wong, C., Wang, H., Sutti, A., Kirkland, M., Wang, X., et al., 2011. Three-dimensional tissue scaffolds from interbonded poly(epsilon-caprolactone) fibrous matrices with controlled porosity. Tissue Eng. C: Methods. 17, 209–218.
Tolstunov, L., Chi, J., 2011. Alveolar ridge augmentation: comparison of two socket graft materials in implant cases. Compend. Contin. Educ. Dent. 32, E146–E155.
Uphoff, C.C., Drexler, H.G., 2014. Eradication of Mycoplasma contaminations from cell cultures. Curr. Protoc. Mol. Biol. 106 (285), 1–12.
Wang, H., Feng, Y., An, B., Zhang, W., Sun, M., Fang, Z., et al., 2012. Fabrication of PU/PEGMA crosslinked hybrid scaffolds by in situ UV photopolymerization favoring human endothelial cells growth for vascular tissue engineering. J. Mater. Sci. Mater. Med. 23, 1499–1510.
Williams, D.F., 2015. Regulatory biocompatibility requirements for biomaterials used in regenerative medicine. J. Mater. Sci. Mater. Med. 26, 89.
Yarat, A., Ozcelik, F., Emekli, N., 1996. A method for preparing collagen graft materials. J. Marmara Univ. Dent. Fact. 2, 527–529.

Part II

Tissue Engineering Strategies

This page intentionally left blank

Specific considerations in scaffold design for oral tissue engineering 10

Amir Yadegari[1,*], *Farahnaz Fahimipour*[1,*],
Morteza Rasoulianboroujeni[1], *Erfan Dashtimoghadarm*[1],
Maysam Omidi[1,2], *Hossein Golzar*[3], *Mohammadreza Tahriri*[1]
and Lobat Tayebi[1]
[1]Marquette University School of Dentistry, Milwaukee, WI, United States,
[2]Shahid Beheshti University, Tehran, Iran, [3]University of Tehran, Tehran, Iran

10.1 Introduction

The recent advances in tissue engineering and regenerative medicine have great impact on medical practice and changing therapeutic strategies. Tissue engineering is known as a multidisciplinary field which applies engineering and life sciences to treat tissue or organ damages (Vacanti and Langer, 1999; Yazdimamaghani et al., 2014c). Three main approaches are generally used in tissue engineering (Liu et al., 2005). In the first approach, isolated cells are employed to replace functional cells (Peterson et al., 2002). Tissue delivery to targeted locations is known as the second method (Bloch et al., 2001) and the third approach is based on growing cells in 3D scaffolds (King et al., 1997; Yazdimamaghani et al., 2014a, 2014b; Mozafari et al., 2012). The first and second approaches can be applied when the defects are too small; hence, their applications in practical size and determined shapes are extremely limited. Thus, the third approach based on 3D scaffold has attracted significantly greater attention (Tahmasbi Rad et al., 2014; Shabafrooz et al., 2014; Yazdimamaghani et al., 2013, 2015; Heidari et al., 2015). Scaffolds are known as the key components in this approach which facilitate the formation of functional tissues by simulating the extracellular matrix (ECM) (Liu et al., 2005). Using biomaterials in dentistry to replace lost tissues has long history, but regeneration of oral and dental tissues faces challenging issues due to their structural and multi-tissue complexity. Thus, oral and dental tissue engineering approaches are becoming more popular recently.

Such strategies in the fields of oral surgery and periodontology already have been applied in practice. Different commercially available scaffolds for periodontal and bone tissue regeneration are used by clinicians (Costello et al., 2010; Villar and Cochran, 2010). Biomaterial selections and scaffold fabrication technologies play important roles in this process (Cordeiro et al., 2008; Patrick Jr et al., 1998). Fabrication technique is specifically vital due to the multi-tissue nature of many oral defects. This chapter will first introduce different scaffold fabrication

[*] These authors contributed equally.

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00010-4
© 2017 Elsevier Ltd. All rights reserved.

techniques and then focus on the specific considerations on interfacial tissues in oral cavity and discuss the multi-tissue scaffolds for oral regeneration. Considering the wide range of available scaffold fabrication techniques and biomaterials, the question is how far the strategies can be optimized for the purpose of engineering complex tissues. Herein, we describe scaffold fabrication techniques and their application in periodontal and oral tissue engineering. Explicit applications of scaffolds in oral tissue engineering will be discussed in the last section.

10.2 Scaffold fabrication techniques

Up to now, many fabrication techniques have been applied for developing scaffolds. Generally, the scaffolds fabrication techniques can be divided into two categories including nondesigned and designed manufacturing methods. The majority of available manufacturing techniques such as solvent casting, gas foaming, freeze drying, and electrospinning belong to the nondesigned manufacturing techniques. The rapid prototyping (RP) methods that have been represented recently are grouped in designed manufacturing technique category. The conventional methods for fabrication of scaffolds are briefly described in the following sections (Li and Xia, 2004; Ma, 2004).

10.2.1 Solvent casting and leaching

In this method, at the first step, mineral and organic particles are dispersed in polymer solution. After that, the solvent is evaporated by applying solvent casting or freeze drying techniques. Finally, in order to produce the polymeric matrix, solid particles are leached out by selective dissolution. One of the main disadvantages of solvent casting method is related to the removal of dissolved particles in polymer matrix which restricts this technique only for producing thin membranes. However, highly porous structures (more than 93%) and facile tailoring the crystallinity of matrix are the main advantages of solvent casting and leaching particles technique. Hence, this method can be applied for the preparation of high porous constructs by using biodegradable polymers such as PLLA and PLGA. It should also be noted that this method is feasible to any polymer which is soluble in organic solvents (Mooney et al., 1991).

10.2.2 Phase separation

Phase separation is another solvent-based technique which is widely used in scaffold preparation and generally divided into two main parts including liquid−solid and liquid−liquid phase separation. Technically, phase separation can proceed through either thermal or nonsolvent process. In fact, phase separation can be conducted by reducing the solution temperature and extraction of solvent phase till reaching a porous polymer scaffold. Phase separation technique is extremely affected by variation of main parameters such as temperature reduction procedure,

type and concentration of polymer, and the amount of solvent. For instance, a small change of thermal quenching procedure may lead to significant morphology alteration of the produced scaffold. In this method, facile incorporation of biomolecules into porous polymeric matrix can be attributed to high activity of molecules owing to extreme driving forces emanating from thermal and chemical environment (Lo et al., 1995).

10.2.3 Freeze drying

Freeze drying has been recently used as an appropriate method for the fabrication of 3D porous structures in tissue engineering. This method is commonly used as an applicable process in pharmaceutical, enzyme stabilization, and food sciences to convert solutions into solid materials. There are three main steps in freeze-drying process. The solution is firstly frozen at extremely low temperature ~ -70 to $-80°C$. Then the solution is partially dried by reducing the pressure which is known as the initial drying. Finally, in the secondary drying process, the residual water is extracted. Using water instead of organic solvent is considered as the main advantage of freeze drying. Therefore, this method is more biocompatible versus other methods for fabrication of scaffolds. However, producing high ordered and hierarchical porous structure by freeze drying needs further investigation. For example, Whang et al. reported the preparation of porous scaffolds through freeze-drying method. They used polysaccharide for the fabrication of scaffolds and investigated the proliferation and adhesion of mesenchymal stem cells on the scaffolds (Whang et al., 1995).

10.2.4 Gas foaming

Gas foaming is a solvent-free method for the fabrication of porous three-dimensional scaffolds. In this method, at the first step, biodegradable polymers such as PLLA, PLGA, and poly glycolic acid (PGA) are formed as a solid disc by pouring molding techniques and heated up in CO_2 atmosphere at high pressure. The molds should be kept in this condition for about 3 days; then, the pressure reduces to ambient pressure. The produced scaffolds by gas foaming usually contain pore size about 100 μm with more than 90% porosity. However, the porosities are not uniform and disconnected on the surface of scaffolds which result in difficulties for cell seeding and proliferation. Furthermore, high temperature treatment in this method may hinder biomolecule and cell incorporation within scaffolds (Hile et al., 2000).

10.2.5 Electrospinning

Electrospinning is a method for the fabrication of porous scaffolds with an ability to mimic ECM. This is a versatile method which makes a wide range of nanofiber diameters from submicron to nanoscale (Arras et al., 2012). The small diameter of fibers may provide a large ratio of surface area to volume which is useful in separation membranes, wound dressing material, and other applications (Ghorani and

Tucker, 2015). Additionally, research showed that cell adhesion on porous scaffolds leads to higher rate of proliferation and differentiation (Zafar et al., 2016). As a matter of fact, fiber formation in electrospinning is based on electrical forces not mechanical forces and it occurs by repulsive electrostatic forces which is an odd self-organization process (Wendorff et al., 2012). Preparing ultra-long fibers with unique structures and assembling large-scale of nanofibers is difficult in other fabrication methods. Thus, facile preparation of long fiber while keeping desirable features is one of the most important advantages of electrospinning method (Zhang and Yu, 2014). Formation of fibers might be affected by many parameters such as physicochemical properties of solution including viscosity, conductivity, surface tension, and concentration. Also, controlling variables such as hydrostatic pressure in the syringe, voltage, tip to collector distance, flow rate, and angle can affect the performance of electrospinning. Alteration of ambient parameters such as temperature, humidity, and air velocity may also influence the formation of fibers (Zafar et al., 2016). Producing different morphology, the possibility of using various kinds of polymers, different surface topology, diameter, and porosity are the main characteristics of fiber formation through electrospinning (Wendorff et al., 2012). However, rotational speed, electrospinning jet bending, and buckling instabilities are known as the limitation factors in fiber formation which lead to unexpected deposition and make nonaligned fiber (Arras et al., 2012). Although random orientation of fibers is suitable and simulate the ECM, the impact of aligned and controlled fiber formation on cell culture, tissue engineering, and mechanical properties is undeniable. Electrospinning is a well-known method to fabricate fibers and mimic ECM in tissue engineering. Owing to high impact of different parameters on diameter and morphology of fibers, many attempts have been devoted to investigate how parameters can alter cell growth in tissue engineering and dental regeneration.

10.2.5.1 Important principles and methods

In a brief description, polymer solution holds by its surface tension at the end of the fluid jet; meanwhile, electric field induces on solution surface tension. By increasing the electric field and reaching to critical value, polymer moves because of the attached charges in a way that Coulomb interaction energy gets minimized (Wendorff et al., 2012). Polymer-based materials either natural or synthetic are generally used for making nanofibers. It should be noted that ceramic, metallic, and glass-based fibers can be produced by this approach. It is worth mentioning that a simple syringe with a metal tip and different diameters is used for polymer injection. The polymer solution is kept in a special container which holds syringe. An aluminum foil and collector are used to collect the fibers and adjust the desired fiber orientation, respectively. Finally, a high-voltage power supply is applied to create the required force for overcoming the surface tension of solution to produce nanofiber by evaporation of solvent. The voltage is applied between the tip of syringe and aluminum foil.

10.2.5.2 Materials properties

Diameter and orientation of nanofibers play an important role in microscopic and macroscopic properties of scaffolds which are measured by scanning electronic microscope (SEM) and image analyzing software like ImageJ (Jiang et al., 2015). Nanofibers diameter and surface area have inverse relationship. Zafar et al. reported that by adding sodium chloride to a polymeric solution charge density can be increased which results in smooth and uniform nanofibers formation (Zafar et al., 2016). Also, adding salts to PDLLA solution may produce smooth and bead-free nanofibers. Lower cellular adhesion is due to hydrophobic nature of some polymers which can be modified by treating the hydrophobic polymers to obtain hydrophilic structures (Zafar et al., 2016). In tissue engineering, degradation rate of scaffold should be proportional to the pace of tissue regeneration. This feature is measured by using enzymes such as lysozyme. Hydrophilicity is one of the most important characteristics that increases biodegradability obtaining by copolymers instead of homo-polymers (Zafar et al., 2016). It should be noted that all polymers are not suitable for electrospinning, and polymers must be selected according to the properties of desired tissue, mechanical strength, and hydrophilicity. For example, alginate has a high viscosity which is not appropriate for fiber formation and electrospinning. Using copolymers and cosolvents has been proposed to overcome this limitation (Zhang and Yu, 2014). Also, using cross-linking or even conformational changing has been suggested for improving the mechanical properties of nanofibers. For instance, using gene-pin as an applicable cross-linking material results in better morphology (Zafar et al., 2016).

10.2.5.3 The effect of parameters

Varying and optimizing parameters can improve fiber formation. Increasing applied voltage accelerates more volume of solution which leads to smaller diameter of fibers, whereas increasing the rate of injection produces fiber with larger diameter and vice versa. Also, high conductivity of solution leads to a great tensile force and reduction in nanofiber diameter (Zafar et al., 2016). One of the major difficulties in electrospinning is the formation of bead and droplet. The optimization of surface tension and viscosity prevents the creation of jet bubbles before solvent evaporation which is a undesired phenomenon in electrospinning (Zafar et al., 2016). Concentration of solution is also considered as an important characteristic in electrospinning. In low concentration of solution, solvent molecules tend to form a spherical shape or bead formation. However, high concentration results in viscous solution with processing problems and difficulties for pumping. Solution viscosity may affect by relative humidity, temperature, molecular weight, and concentration of polymer. It should be noted that in concentration of less than threshold the formation of droplet instead of fiber is more probable. It has been shown that physical properties of solution such as viscosity, bead size, and density are firmly related to each other. Low viscous polymer solution jet may break up into small droplets and

forms bead structure. Also, high viscosity leads to less spherical and more spindle beads (Zafar et al., 2016). Additionally, if the molecular weight of polymer is too low even with large concentration, polymer may break up into droplets during electrospinning (Zhang and Yu, 2014). In order to minimize the bead formation versus fiber formation, main parameters of electrospinning should be optimized. For instance, optimization of applied voltage, low surface tension solvents, amount of added surfactant to the solution, constant and stable flow rate of solution may provide better fiber formation in electrospinning technique (Zhang and Yu, 2014; Zafar et al., 2016).

10.2.6 Microsphere sintering

Microsphere sintering, first developed by Kumbar et al. (2014), offers a fabrication strategy based on the sintering of polymeric microspheres. Various natural or synthetic polymers such as polylactide (PLA) (Rasoulianboroujeni et al., 2016), Poly (Lactide-co-Glycolide) (PLGA) (Wang et al., 2009), Polycaprolactone (PCL) (Luciani et al., 2008), and chitosan (Abdel-Fattah et al., 2007) incorporating different fillers like titanium dioxide (Wang et al., 2010b), hydroxyapatite (Nukavarapu et al., 2008; Lv et al., 2009), and bioactive glass (Yao et al., 2005) have been used to fabricate plain or composite scaffolds. While heat sintering is the most popular approaches to prepare the scaffolds (Luciani et al., 2008; Abdel-Fattah et al., 2007; Yao et al., 2005), some researchers have used other sintering agents such as solvents (Jaklenec et al., 2008) or subcritical carbon dioxide (Singh et al., 2010). Fig. 10.1 shows the architecture of microsphere-based scaffolds and cell growth on them. The obtained scaffolds through this method represent sufficient mechanical properties, while the pore size and pore volume could be controlled by altering the fabrication parameters such as sintering time and temperature as well as microsphere size (Borden et al., 2002). In fact, one of the most important advantages of microsphere-based scaffolds is that any section of scaffold can be altered by altering the microspheres of that section. This feature allows someone to easily control

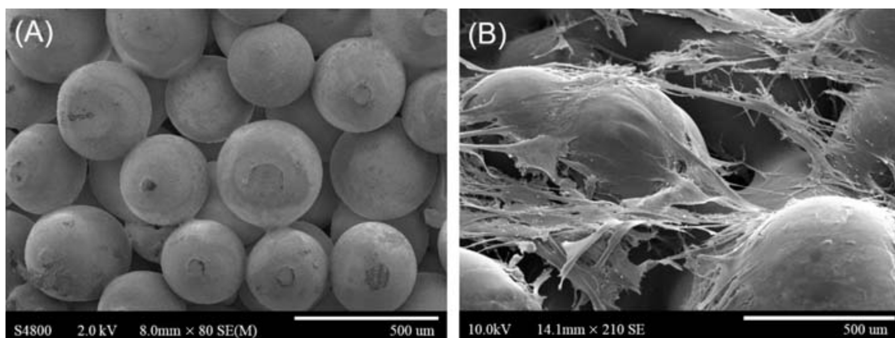


Figure 10.1 Prepared scaffold using microsphere sintering method (A) and osteoblast attachment and growth on the scaffold (B)

different characteristics of the scaffold such as pore size and stiffness at any region of interest. On the other hand, such scaffolds are capable of controlled release of growth factors or other substances that may accelerate regeneration process (Wang et al., 2010a).

Table 10.1 summarizes some of the studies in which microsphere sintering has been used to fabricate tissue engineering scaffolds.

10.2.7 Additive manufacturing

In the last decade, researchers have focused on the use of computer-aided design (CAD) for additive fabrication technology. Additive manufacturing (AM), solid freeform fabrication (SFF), RP, and 3D printing are the terms usually used to describe the technology. 3D printing has been used to produce a wide variety of scaffolds with appropriate porosity, biocompatibility, and mechanical properties for tissue engineering applications (Khoshroo et al., 2016). AM can be used where the scaffold characteristics such as overall shape, pore size, and inner structure need to be precisely controlled in microscale by means of computational topology and advanced 3D fabrication techniques or where large-scale fabrication is required in medical applications (Wong and Hernandez, 2012).

Stereolithography (SLA), Fused Deposition Modeling (FDM), the most common for tissue engineering and bioprinting applications, and Selective Laser Sintering (SLS) are the examples of AM.

SLA is a photopolymerization-based technique that facilitates the fabrication of complex designs through a layer-by-layer approach. The microstructures are produced with high accuracy according to a computer-driven model by controlling the radiation beam toward a liquid resin. During fabrication, ultraviolet (UV) light is irradiated according to a precise pattern created by CAD on the surface of a vat filled with photosensitive resin. The irradiation of UV light leads to polymerization and consequently solidification of the resin to form the first layer. The first layer is fixed on a prebuilt mobile platform used as a support for completing the structure and the next layers are formed through polymerization of liquid resin filled between the irradiative surface and the last built layer by moving the platform up to a certain height. This process is repeated layer-by-layer until completion of the desired 3D structure (Skoog et al., 2014).

FDM or fused filament fabrication (FFF) is one of the RP systems that build the 3D objects based on deposition of extruded material. Thermoplastic polymers, ceramic pastes, cements, and solution could be extruded and used for the fabrication of different constructs. The extrusion head controlled by the computer tracks the exact outline of each cross-section layer while dispensing the loaded material. The driving force for extrusion could be a pneumatic system, precision pump, etc. After extruding a layer and solidification through cooling or other approaches, the next layer will be built by moving the nozzle up or platform down a programmed distance in the z direction (Yan and Gu, 1996).

SLS, also known as SFF, is a powder-based layer-AM process in which continuous or pulsatile laser beams are used for scanning and heat-induced fusion of

Table 10.1 **Microsphere sintering studies to fabricate tissue engineering scaffolds**

Formulation	Application	Characteristics	Ref.
Poly (lactic-co-glycolide)	Bone tissue repair applications	Porosity 30%–95%	Wang et al. (2009)
Poly(lactide-co-glycolide)/Titania	Bone tissue engineering	Porosity 30%–40%	Wang et al. (2010b)
Poly(lactic acid glycolic acid)/Nano-Hydroxyapatite	Bone tissue engineering	Scaffolds sintered at 90°C for 3 h had Compressive modulus of 150–200 MPa Compressive strength of 3–3.5 MPa Porosity of ~30% Median pore size ~200 μm	Lv et al. (2009)
Poly(lactic-co-glycolic acid) (PLGA)	Cartilage tissue engineering	Porosity 41% Modulus: 100–200 kPa Pore size: 40–80 μm	Singh et al. (2010)
Chitosan/Poly(lactic acid glycolic acid)	Bone tissue engineering	Total pore volume 28%–37% Pore size 170–200 μm Compressive modulus in the range of trabecular bone	Jiang et al. (2006)
Biodegradable Polyphosphazene/Nano-Hydroxyapatite	Bone tissue engineering	Compressive moduli of 46–81 MPa Mean pore diameter 86–145 μm	Nukavarapu et al. (2008)

powder particles in predetermined outlines. The next layer is built following deposition of loose powder over the previous one and repeating the process. A wide variety of materials have been used in SLS including ceramics (e.g., Alumina, Zirconia, Silica), metal-polymer powders, different metals (e.g., Aluminum, Titanium, Copper, Iron) and their alloys (e.g., stainless steel, Ti−6Al−4V), polymers (e.g., polycarbonate, PCL), polymer-glass composites, etc. While some materials can be laser sintered without sacrificial binder, some need to be combined with a sacrificial low-melting-point polymeric binder. In both cases, the sintering is influenced by powder density, shape, size distribution, and flow rate. The limitations of SLS include but not limited to agglomeration of the powder, poor cohesion, irregular surfaces, and curling of layers. Such defects might cause shrinkage, inaccuracy in dimensions, porosity, and insufficient mechanical strength. Therefore, postprocessing operations such as thermal treatment, coating, polishing, or plating might be required to improve structural integrity, mechanical properties, and surface smoothness (Kumar, 2003).

Some of the remarkable applications of AM are the production of physical models of human anatomy, used in medicine and dentistry. AM is a useful tool for producing customized implants such as orbital floor, onlays, and cranioplasty implants (Hoang et al., 2016). Nowadays, AM is widely employed to manufacture surgical guide stents used for precise placement of dental implants. AM's application is gently being broadened for the fabrication of prosthetics, temporary crowns, bridges, and resin models for lost wax casting (Hoang et al., 2016). AM has also been used to fabricate 3D scaffolds for tissue engineering applications. Figure shows scaffolds prepared using AM. Fig. 10.2 shows the prepared tissue engineering scaffolds through AM (FDM)

10.2.8 Microfabrication approach

Improving remarkable progress in tissue engineering highly depends on successful engineering of organs including skin and cartilage. However, numerous challenges

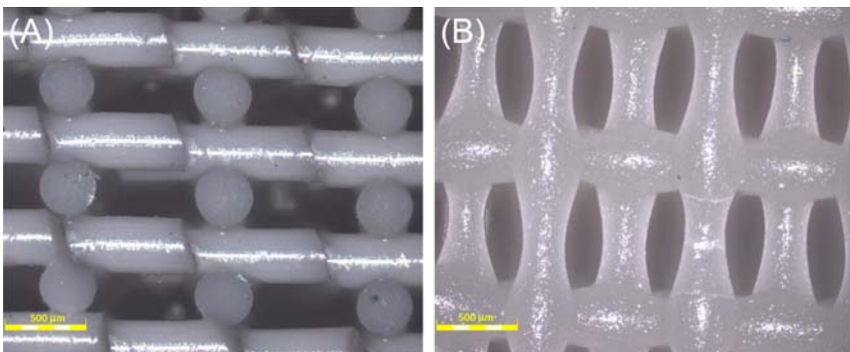


Figure 10.2 Prepared tissue engineering scaffolds through additive manufacturing (fused deposition modeling).

such as lack of a renewable sources and functional cells which are immunologically compatible with host body system may hinder successful engineering of organs (Venugopal et al., 2008). As a matter of fact, deficiency of biomaterials with favorable physicochemical, biological properties, facile merging into the host's circulatory system with the architectural elaboration of native tissues, and the inability to create huge and vascularized tissues are the most important limitations in developing tissue engineering. Microscale technologies are potentially strong tools for solving the above-mentioned challenges in tissue engineering. In fact, these technologies are compatible with cells and easily merge with biomaterials for facile fabrication of scaffolds (Smith et al., 2009). Microfabrication techniques were initially utilized in the microelectronics industry and have been developed to generate physiological-based materials and devices to emulate in vivo cells (Martins-Júnior et al., 2013). Using microfabricated devices has brought up remarkable biomedical applications such as tissue engineering, dental tissue injury, drug delivery, and biosensing. Generally, microfabricated apparatus are constructed through various steps such as photolithography, metal deposition, and dry or wet etching. Due to the importance of microfabrication techniques and devices in tissue engineering, the design and fabrication of tissue engineering scaffolds with micron- or submicron-scale features is discussed with a specific attention to the application of microfabricated scaffolds in dentistry (Chang et al., 2013; Neel et al., 2014; Porter et al., 2016; Uyar et al., 2016).

10.2.8.1 Soft lithography

Soft lithography is a general term that encompasses a wide range of lithography techniques including replica molding (RM), microcontact printing (μCP), micromolding in microcapillaries (MIMIC), microtransfer molding (μTM), and solvent-assisted micromolding (SAMIM). These techniques usually employ a patterned elastomer of polydimethylsiloxane (PDMS) as the mold or stamp to create or transfer pattern (Yang et al., 2010). The fundamental description of soft lithography, RM and micromolding in microcapillaries processes will be discussed in the following sections. Soft lithography can be used as a surface patterning or microfabrication technique for biodegradable polymers (Kim et al., 2008). RP and simple fabrication procedure with low-cost equipment are two main advantages of soft lithography method (Kim et al., 2008). The main principle in soft lithography is the replication of a microfabricated stamp or mold which has the contrary geometry of desired polymeric structure (Obregón et al., 2014). Briefly in soft lithography, an elastomer such as PDMS including a liquid silicone rubber and a curing agent is mixed. Then the as-prepared mixture shed to a master mold. At first, PDMS elastomer is heated and the liquid mixture became solid in a few hours; then, a PDMS stamp is ready to use after slowly peeling off from the master mold. The polymeric solution which is mixed with solvent is shed on the silicon wafer. The PDMS stamp is then squeezed to the silicon wafer with a little force and left at room temperature for 24 h. Finally, the fabricated polymer structure can be peeled off from the PDMS stamp whenever the solvent is evaporated. It should be mentioned that preparation

of master mold is expensive but it can be used several times to fabricate a diversity of geometric shapes (Xu et al., 2007). Also, multilayer polymer structures can be fabricated by soft lithography method. Hence, soft lithography technique can be used as an appropriate approach in tissue engineering especially for the fabrication of scaffold, bone repair and regeneration, cardiac constructs, and oriented cell growth (Peng et al., 2013; Gemberling et al., 2013; Wray et al., 2013). Considering dentistry approaches, the presence of patterned surfaces can facilitate osseointegration in dental implant by using soft lithography technique (Laranjeira et al., 2014; Shayganpour et al., 2015).

10.2.8.2 Replica molding

Generally, molding processes including solvent casting, injection molding, and hot embossing are categorized as RM. In tissue engineering, RM of biomaterials is the most uncomplicated technique for generating microfabricated structures. Decreasing the feature resolution to ~30 nm by using polymeric materials such as PDMS, facile processing, scalable and rapid fabrication of microstructures is known as the main advantage of RM. However, the 2D nature of replica molds may restrict its utilization. Compounding elastomeric materials in the fabrication of molds can greatly improve the capability and possibility of RM (Kumbar et al., 2008). For example, PDMS is poured into a solid master and the elastomeric replica is eventually used as a mold against which new replicas are generated in organic polymers. The usage of elastomers in RM makes it easier to fabricate tiny, fragile structures on the original master (Yang et al., 2010). Extraction or loss of the tooth is caused by the loss of function in the affected area. Grafting materials such as hydroxyapatite are extremely used as bone biomaterials to prevent collapse of bone walls and simplify future implant placement (Sheikh et al., 2015; Eslami et al., 2008, 2009, 2010; Tahriri and Moztarzadeh, 2014; Fatehi et al., 2008; Bizari et al., 2011; Shafiei et al., 2012). Biodegradable polymers in the form of solid and porous blocks can be used to overcome nonresorbable limitation of grafting biomaterials. For instance, biodegradable scaffolds of poly lactide-co-glycolide (PLGA) 50:50 with acidic microclimate controlling additives were prepared by solvent-casting and particulate-leaching method with few modifications to fill the extraction site (Ginjupalli and Averineni, 2014).

10.2.8.3 Micromolding in microcapillaries

Micromolding in microcapillaries (MIMIC) is a lithographic-based technique relied on microfluidic systems and is known as an appropriate method to fabricate patterned microstructures of polymers on the surface of different substrates (Lima et al., 2014). Initially, an empty mesh of channels (PDMS mold) is formed with the contact between mold and substrate. Then a polymeric solution shed to the channels and filled them through capillary forces (Beria et al., 2014). Pressure difference between two hydraulically connected areas of liquid mass causes the flow of polymeric solution in the capillaries. The PDMS mold can be removed from the

substrate after channels are fulfilled and polymer is cured. The advantages of MIMIC such as inexpensive and possibility of choosing wide range of polymers to pattern make this technique a suitable method for patterning different materials (Lima et al., 2014). There is a wide range of polymers which can be used as an inexpensive precursor for patterning by MIMIC technique. In contrast to the current patterning approaches for polymeric and biomolecules, patterning ceramic-based materials has been restricted due to their brittle and hard structures. However, it has been shown that ceramic-based materials possess numerous clinical applications. For example, Yttria-stabilized zirconia (YSZ) is widely used in dentistry due to better crack formation resistance, higher corrosion resistance, and improved esthetics (Barata et al., 2016). Also, hybridization of YSZ with silicon substrates or bioactive calcium phosphate (CaP) ceramics can be patterned by using MIMIC method to be used in dentistry (Choi et al., 2014).

10.2.9 Self-assembly approach

Self-assembly refers to a spontaneous formation of amorphous molecular units into regular supramolecular structures due to molecular interactions, such as hydrogen bonding, van der Waals and electrostatic interactions. This process is regulated by equilibration of attractive and repulsive forces between molecules. Self-assembly technique plays a pivotal role in the production of many biological nanostructures and biomolecules, such as proteins or DNA. Nowadays, peptides are known as exceptionally beneficial building blocks for generating self-assembled nanostructures in healthcare and medical applications due to their inherent biocompatibility and biodegradability (Mandal et al., 2014). Self-assembly of peptide systems into supramolecular structures requires logical interpolation of particular and elective interactions between amino acid building blocks. The created structures can be adjusted by manipulating amino acid sequences that provide proper compound of properties such as charge and residue hydrophilicity. Beta sheet structures can be generated by compounding peptides with one hydrophobic and one hydrophilic surface in an aqueous solution. The hydrophobic surfaces might be protected by contacting between the remained charge and aqueous environment (Venugopal et al., 2008). Self-assembled peptide nanostructures have represented remarkable potential as biomaterials and their application for carrier-mediated drug delivery and tissue engineering especially in dentistry (Habibi et al., 2016). Consequently, the self-assembly properties of peptides have been utilized to produce a wide range of nanoscale structures including nanofibers, nanotubes, and hydrogels (Feyzizarnagh et al., 2016). The nanofiber scaffolds support osteoblast including stem cell viability, proliferation, and migration without interfering with cell osteogenic differentiation (Kolambkar et al., 2013). For example, self-assembling peptide nanofibrous hydrogel scaffolds such as PuraMatrix can be used as a fascinating tool for therapeutic transplant of human mesenchymal stem cells into bone defects. It is known as a novel, injectable, and synthetic scaffold specially for osseoregeneration and encouraging material for dental pulp regeneration (Haidar, 2015).

10.3 Multi-tissue scaffolds for oral and dental regeneration

Regenerative dentistry aims to regenerate various oral and dental tissues by combining a triad of elements: biomaterial scaffolds, growth factors, and stem/ progenitor cells (Jazayeri et al., 2017; Khojasteh et al., 2016a, 2016b). The hierarchical structure of native oral/dental tissues requires combination of various scaffold fabrication techniques to fulfill anisotropic pore sizes while adjusting spatial physicochemical and mechanical features. In fact, combinatorial scaffold fabrication techniques should be exploited to precisely provide desired pore size, porosity, interconnectivity, mechanical properties, and geometry. Multiphasic constructs act as templates for tissue regeneration to guide the growth of new tissues. A major challenge in scaffold design for oral and dental multi-tissue engineering is to properly employ various fabrication techniques to achieve multiphasic scaffolds with desired features which may consist of different materials. The microstructural features in multiphasic scaffolds have been found to profoundly affect the cell attachment, migration, proliferation, and differentiation, and cell–cell interactions. Single or multiple scaffold fabrication techniques including porogen leaching, gas foaming, freeze drying, electrospinning, 3D printing, or other AM techniques can be employed to design multiphasic constructs depending on the biomaterial choices. There are several reports on employing combinatorial scaffold fabrication techniques for bone as well as interfacial bone/periodontal ligament (PDL) tissues regeneration. The combination of solid free form fabrication technique with freeze-drying method has been reported to design biomaterial constructs in order to facilitate cellular activities (e.g., cell adhesion and proliferation) while providing appropriate mechanical properties (see Fig. 10.3) (Hong et al., 2012).

One approach for ligamentous tissue formation is PDL cell sheet engineering. Cell sheet engineering as a cell-rich biomaterial-free method has shown successful clinical transplantations to other wound sites, like cornea reconstruction and cardiac patches. This methodology has emerged as a promising clinically applicable approach to regenerate periodontal tissues. In several studies, PDL cell sheets were transplanted for cementum and PDL tissue regenerations around the tooth root surface (Bartold et al., 2016; Iwata et al., 2015; Ishikawa et al., 2009). Multi-layered PDL cell sheets have been transplanted to periodontal defect sites (PDS) with osteoconductive biomaterials (e.g., tricalcium phosphate, TCP) for bone formation and cementum-PDL regeneration, respectively. It has been found that a three-layered PDL cell sheets transplanted onto an osteoconductive multi-compartmentalized biomaterial construct could promote periodontal regeneration (Iwata et al., 2009).

A biphasic scaffold combined with cell sheet method has been designed and evaluated for simultaneous alveolar bone/PDL complex regeneration. The biphasic scaffold was fabricated using electrospinning technique for periodontal compartment and fused deposition modeling method for alveolar bone compartment (see Fig. 10.4). In order to accomplish simultaneous alveolar bone and PDL regeneration, a cell-based strategy was carried out through combination of osteoblast culture

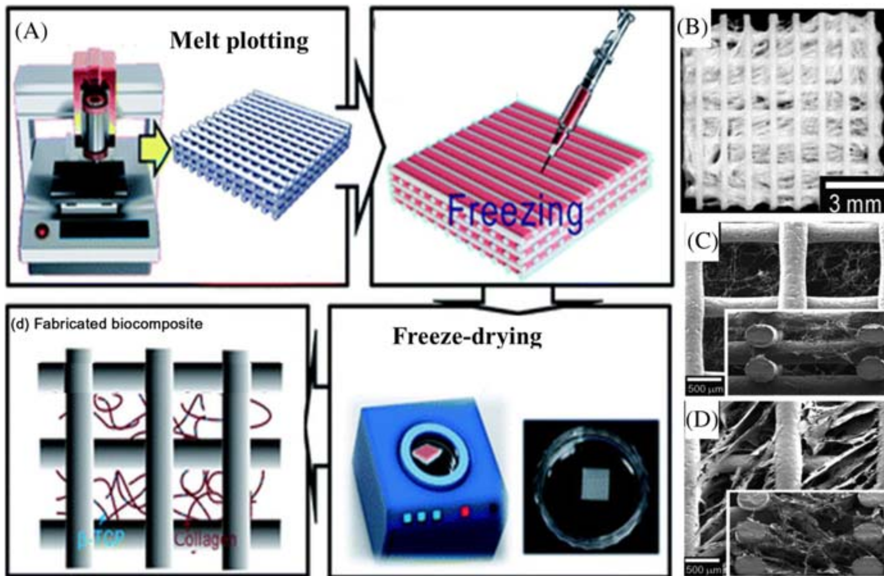


Figure 10.3 (A) Schematic representation of combined melt-plotting and freeze-drying techniques to fabricate hybrid biocomposite constructs. (B) Optical images of hybrid biocomposite construct (C, D) Scanning electron micrographs of surface and cross-section of the hybrid biocomposite construct (Hong et al., 2012).

in the bone compartment and placement of PDL cell sheets onto the electrospun membrane. In vitro results showed that the osteoblasts developed mineralized matrix in the bone compartment after 3 weeks incubation in culture medium and the PDL cell sheet did not show significant cell death. It was shown that the incorporation of multiple PDL cell sheets onto the biphasic scaffold offers the simultaneous delivery of the cells necessary for PDL, alveolar bone, and cementum regeneration in vivo (Vaquette et al., 2012).

Liao et al. (2005) designed and examined a functionally gradient three-layered polylactide-co-glycolide acid (PLGA) biomaterial construct for periodontal regeneration; the designed construct was composed of a three-layered design of a PLGA with low macro-porosity and high mechanical properties.

In another study, a semi-rigid PLGA/CaP bilayered construct was designed, which encompassed a continuous outer barrier membrane and an inner topographically complex component (Reis et al., 2011). The macroporous compartment of the composite scaffold was fabricated by porogen leaching technique. The other layer of the construct was fabricated by molding a PLGA/CaP composite suspension. The integration between layers was achieved by placing the macroporous compartment on the organic/inorganic composite suspension just prior to setting. The advantages of the developed PLGA/CaP construct were included not collapsing into the defect, and ability to retain the blood clot throughout the buccal defect (see Fig. 10.5).

A drawback of the PDL cell sheet methodology for periodontal complex regeneration is complications to localize the cell sheets on the correct defect site of the

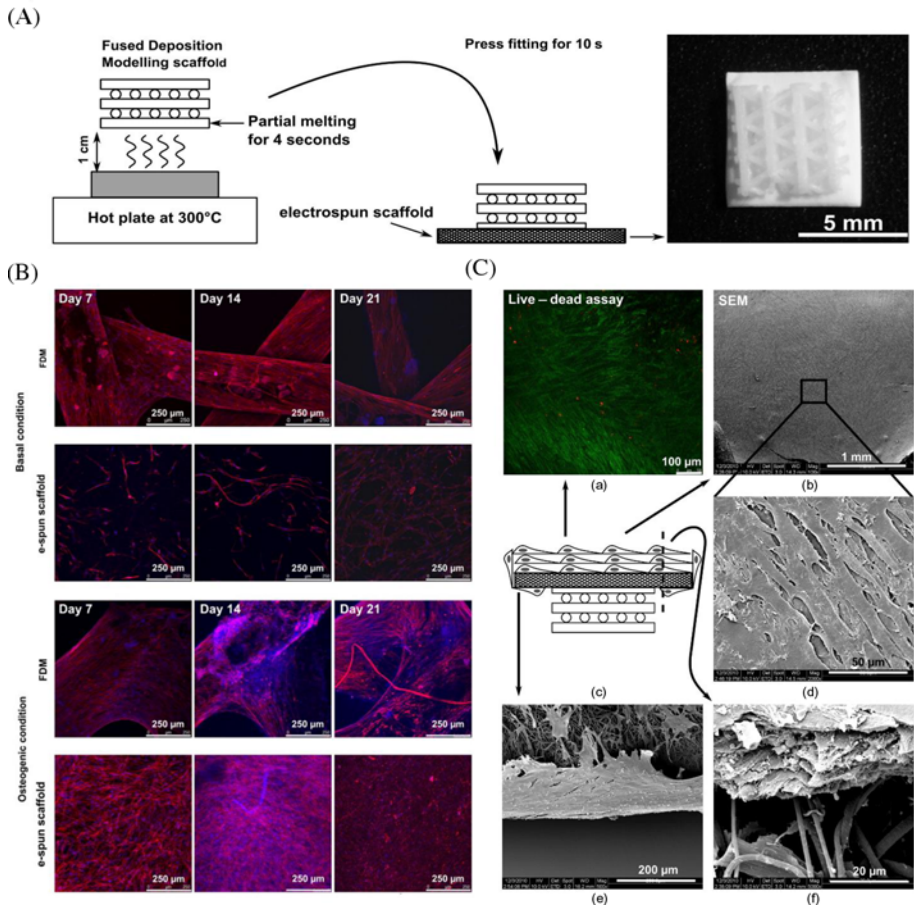


Figure 10.4 Biphasic scaffold fabricated using electrospinning (periodontal compartment) and fused deposition modeling (bone compartment) techniques. (A) Fabrication scheme, (B) confocal imaging of the osteoblast seeded biphasic scaffolds (The nuclei are stained blue and the actin filaments are in red), (C) transplantation of multiple cell sheets onto the electrospun membrane (periodontal compartment in the biphasic scaffold): (a) live−dead assay, green staining indicates live cells and red staining indicates dead cells, (b, d) scanning electron micrographs of the last cell sheet morphology, (c) schematic illustration of the biphasic scaffold, (e) anchorage points on the electrospun membrane (periodontal compartment), (f) cross-section view of the transplanted three cell sheets.
Image adapted from Vaquette, C., Fan, W., Xiao, Y., Hamlet, S., Hutmacher, D.W., Ivanovski, S., 2012. A biphasic scaffold design combined with cell sheet technology for simultaneous regeneration of alveolar bone/periodontal ligament complex. Biomaterials 33, 5560−5573 with permission.

tooth root surface. Dan et al. (2014) have studied CaP-coated PCL scaffold supported cell sheets, and investigated the influence of cellular source on periodontal regeneration. They have fabricated an electrospun PCL membrane and modified it using alkaline treatment and CaP-coating onto the surface of the scaffolds to convey

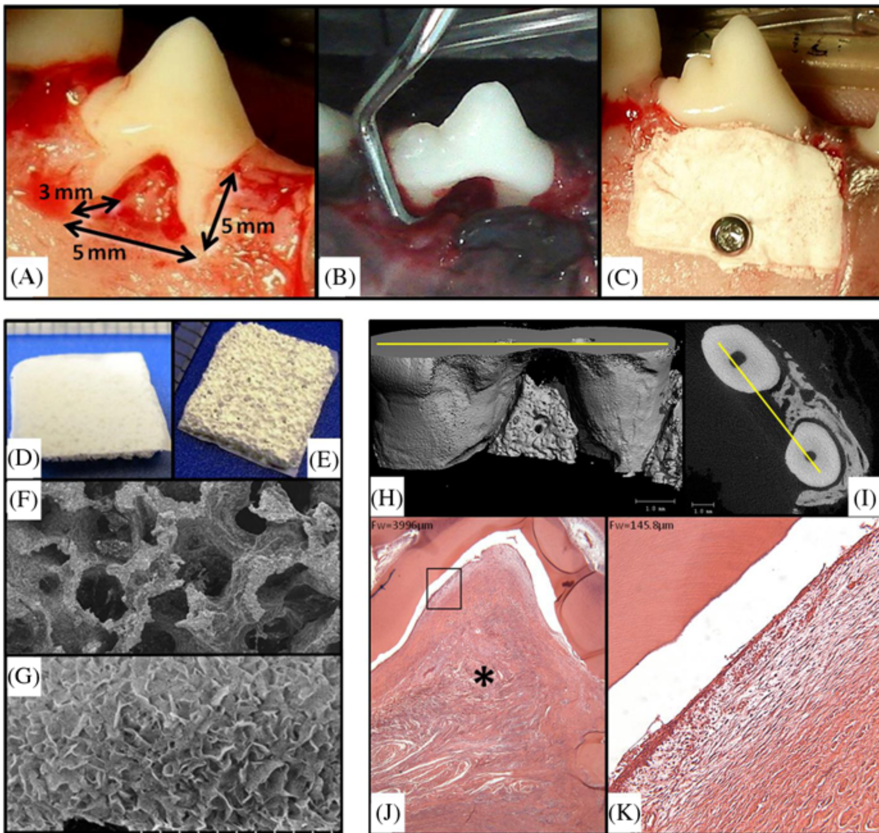


Figure 10.5 Periodontal regeneration via a multi-layered PLGA/calcium phosphate (CaP) construct fabricated by porogen leaching and solution cast/ solvent evaporation techniques. (A–C) Sequence of in vivo experiment for implantation of constructs: (A) 5 mm × 5 mm × 3 mm class II furcation defect to partially expose the root, (B) scaling was performed after 3 weeks periodontal disease induction, (C) 2 weeks later, the PLGA/CaP bilayered construct was placed to cover the defect in treated group. (D) The membrane and (E) the macroporous surfaces of the bilayered construct. (F) Macro- and (G) Nano-pores are observed by scanning electron microscopy. (H) MicroCT images of a control sample at 4 months and the corresponding optical micrographs. (H, I) Small extent of bone in the buccal and coronal parts of the furcation. (J, K) Yellow lines refer to the mesiodistal sections (J: dense connective tissue filled the defect in the central area (*), and K: loose connective tissue observed at the root interface).
Adapted from Reis, E.C.C., Borges, A.P., Araújo, M.V., Mendes, V.C., Guan, L., Davies, J. E., 2011. Periodontal regeneration using a bilayered PLGA/calcium phosphate construct. Biomaterials 32, 9244–9253 with Permission.

the PDL cell sheets. The fabricated modified PCL membranes have simplified delivering and protecting biological cell sheets to the root-exposed periodontal defect. Furthermore, the osteoconductive characteristics of PCL/CaP membranes improved osteogenic acceleration, and PDL cell sheets kept the PDL interfaces

between the cementum and alveolar bone. They reported that the PDL cell sheets significantly enhanced the cementogenesis and bone formation compared to different cell sheet groups including gingival margin-derived cells (GMC) and alveolar bone cells (ABC) (see Fig. 10.6).

The regeneration of diseased periodontal multi-tissues is a complicated process, which involves in the formation of cementum on the root surface, PDL fibers, and adjacent resorbed alveolar bone. In this view, various surgical techniques based on the principles of Guided Tissue Regeneration (GTR) have been developed to promote periodontal regeneration. In GTR-based procedures, barrier membranes are commonly used to selectively promote the repopulation of cells capable of periodontal tissues regeneration (e.g., PDL cells, osteoblasts) on the root surface and prohibit infiltration of those that do not (gingival epithelial cells).

Recently, an electrospun chitosan (CH) membrane with a low and high degree of fiber orientation was used for GTR (Qasim et al., 2017). It has been demonstrated that the GTR membrane can facilitate integration with the periodontal tissue by controlling the direction of cell growth (see Fig. 10.7). Functionally graded CH membrane construct consisted of freeze gelated core layer sandwiched between oriented and random electrospun layers. The membranes were found to be favorable for cellular attachment and proliferation. The electrospun constructs supported matrix deposition and histological sections showed cellular infiltration.

10.4 Applications of scaffolds in oral tissue engineering

A variety of natural and synthetic biomaterials such as ceramics, polymers, metals, and proteins have been used or modified with additives including particles and enzymes to be suitable for dental applications (Gupte and Ma, 2012). Polymeric nanofibers and bio-ceramic nanoparticles have been recently used in dentistry owing to high flexibility of these materials which improves dental tissue regeneration (Liu et al., 2017). Nanofibrous features such as high surface area, enhanced cellular interaction, suitable protein absorption to facilitate binding sites for cell receptors, high surface functionalization, and porosity in the range of submicron to nanoscale may highlight the role of polymeric nanofibers in dental regeneration (Liu et al., 2017; Zafar et al., 2016). Due to the fact that bio-ceramics are low flexible and brittle, biodegradable polymers such as polyethylene glycol (PEG), poly lactic acid (PLA), PGA, PCL, and their copolymers can be used as an appropriate material in dental applications. Using nanostructure implants has shown excellent mechanical stability and suitable cell responses. In fact, proper cell adhesion, differentiation, growth, and survival are the main advantages of nanostructure implants. Natural ECM has a key role in choosing appropriate matrix for periodontal tissues regeneration. For instance, collagens and noncollagenous proteins are mainly used in tooth regeneration and periodontium. In fact, biodegradable scaffolds facilitate delivery of genes, cells, and proteins during treatment. It should also be mentioned that this material must provide adequate mechanical support and enhance cells growth and proliferation to induce ECM production. Among the conventional

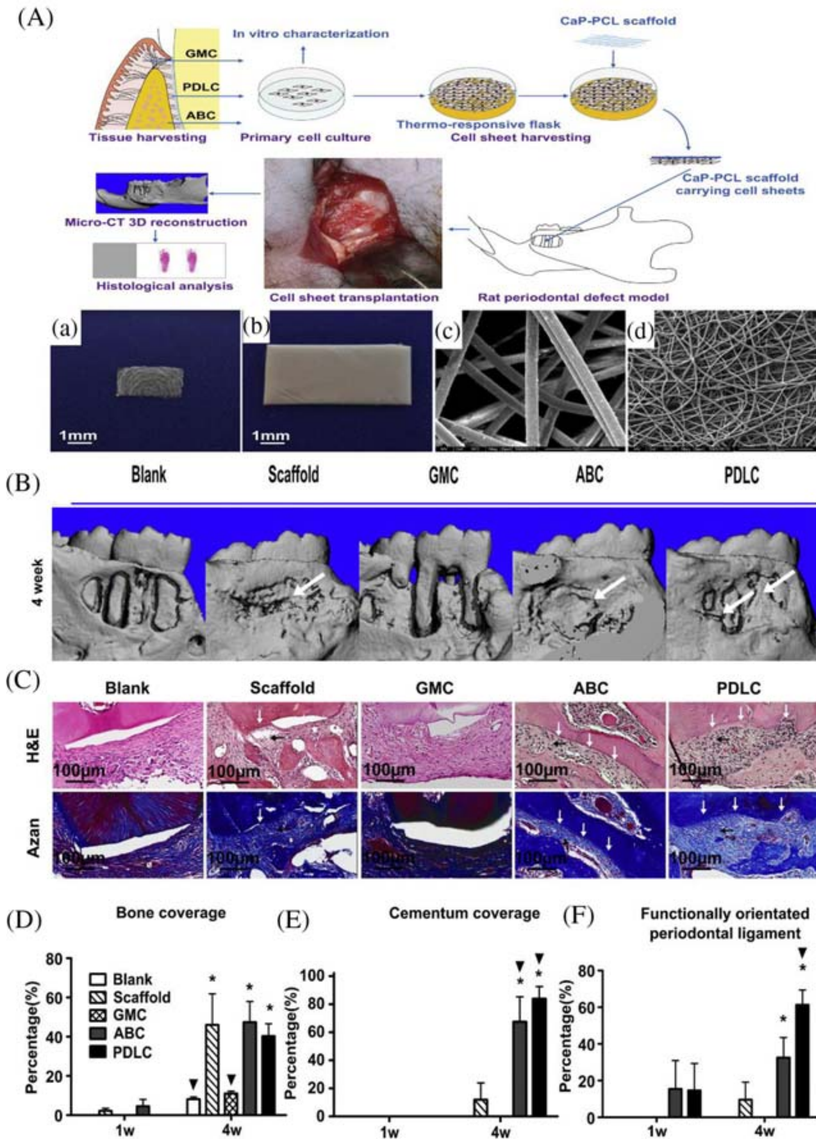


Figure 10.6 (A) Schematic illustration of in vitro and in vivo procedures, from cell sheet harvesting and transplantation, employed for periodontal multi-tissues regeneration. Macro- and microstructure of (a, c) PCL/CaP, and (b, d) electrospun membrane constructs. (B) MicroCT images showed new bone formation in different groups after 4 weeks. New bone formation (white arrows) was observed in scaffolds seeded alveolar bone cells (ABC) and PDL cells (PDLC) groups after 4 weeks. (C) Hematoxylin & eosin and Azan staining showed new cementum and periodontal ligament formation after 4 weeks. (D) Histomorphometry of bone, cementum, and periodontal ligament formation. Percentage of the defect covered by new bone at different time points.
Adapted from Dan, H., Vaquette, C., Fisher, A.G., Hamlet, S.M., Xiao, Y., Hutmacher, D. W., et al., 2014. The influence of cellular source on periodontal regeneration using calcium phosphate coated polycaprolactone scaffold supported cell sheets. Biomaterials 35, 113−122 with permission.

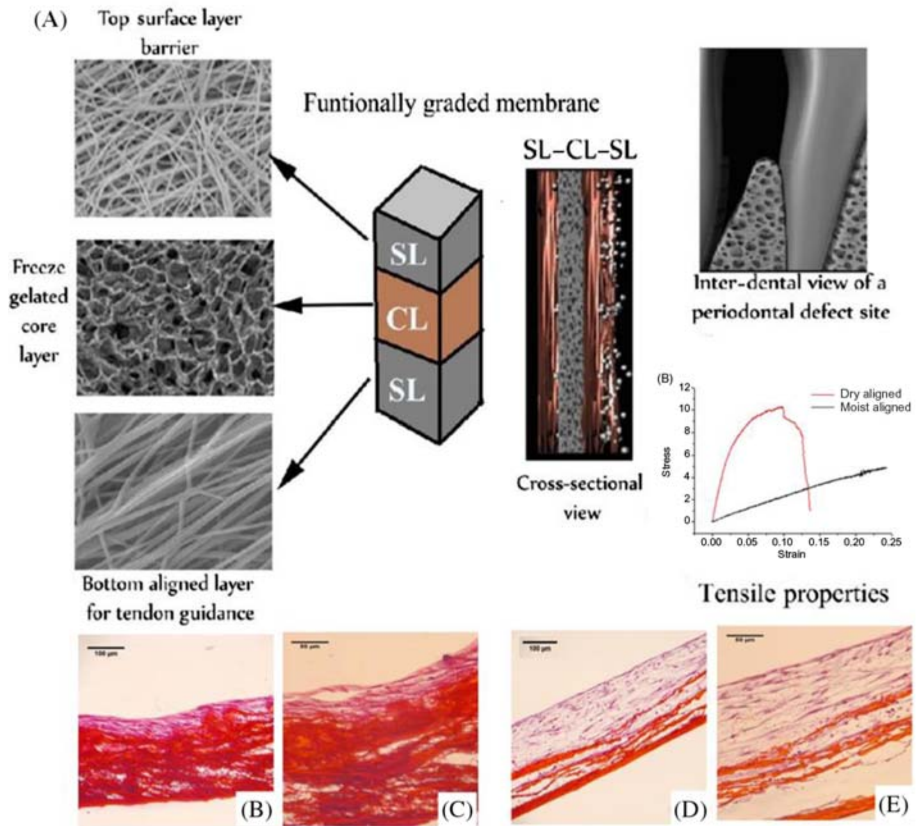


Figure 10.7 (A) Multi-layered electrospun chitosan (CH) membranes with a low and high degree of fiber orientation used for guided tissue regeneration. (B, C) Hematoxylin and eosin staining of random, and (D, E) aligned CH fibers performed after cryo-sectioning after 2 weeks culture with hES-MP cell line.
Adapted from Qasim, S.B., Najeeb, S., Delaine-Smith, R.M., Rawlinson, A., Rehman, I.U., 2017. Potential of electrospun chitosan fibers as a surface layer in functionally graded GTR membrane for periodontal regeneration. Dent. Mater. 33, 71−83 with permission.

nanostructure biomaterials, nanofibrous scaffolds are easily fabricated and possess cell-favored properties such as high surface area, controllable alignment of fibers to direct regenerative tissue, controllable shape, and high porosity (Neel et al., 2014). Furthermore, these nanofibers have great potential application in repairing and regeneration of different oral and dental tissues including dental pulp, dentin, periodontal tissues, oral mucosa, and skeletal tissues. In periodontal tissue regeneration, gelatin membrane showed decent cell attachment and proliferation over 7 days (Gupte and Ma, 2012). The effect of antibiotic-based delivery on changing the color of human dentin was also investigated. For this purpose, polydioxanone was dissolved in 10 wt% hexafluoro propanol and 25 wt% TAPMINO added. The solution

was electrospun (flow rate of 2 mL/h, 18 cm distance, and electrical voltage between 15 and 19 kV) and finally scaffolds were dried in vacuum to remove remained solvent. Antibiotic-free solution was used as control and the change of color for all groups was recorded. In particular, the change of color in all groups including control (saline), scaffold (S) based on polydioxanone/PDS, triple antibiotic paste/TAP prepared with doxycycline/DOX or minocycline/MINO, and scaffolds containing 25 or 50 wt% of DOX or MINO at different periods of storage was thoroughly investigated. The results showed that scaffolds using MINO and DOX had similar color changes for dentin. Also, DOX had the lower discoloration effect on materials compared to MINO (Porter et al., 2016). In order to investigate the effect of aligned or nonaligned fibers on mechanical properties in dental composites, polyvinylalcohol (PVA) solution was electrospun (voltage = 12 kV, 25% w/w concentration, tip to collector distance = 12 cm and syringe pump speed = 2 mL/h). The process was optimized based on minimum diameter of fibers. Then, the nanofiller mat was put in polymethylmethacrylate (PMMA) dough. Finally, the mechanical properties of PMMA-PVA nanofiber mat-PMMA composite were examined. After obtaining stress−strain graphs for composites and MTT analysis, the results showed that aligned nanofibers had the highest amount of absorbed energy, force, and flexibility. Also, the best group in the field of maximum flexural strength, elastic modulus, and toughness was reported in electrospinning system by focusing on electro-manipulated electrodes (Uyar et al., 2016). In vitro and in vivo behavior of dental pulp stem cells (DPSCs) on PCL/gelatin scaffold with or without nano-hydroxyapatite (nHA) was determined by Yang et al. (2010). PCL and gelatin were dissolved in trifluroethanol (TFE) and electrospinning was completed. DPSCs were seeded on scaffolds and major analysis such as alkaline phosphatase, real time PCR, SEM, and in vivo tests was completely investigated. The results showed that by the addition of nHA, proliferation and differentiation of DPSCs were increased (Yang et al., 2010). Using PLLA/nHA scaffolds in dental tissue engineering with normal cells MG-63 was studied by Xu et al. For this purpose, scaffolds were fabricated in optimal condition of electrospinning process and the morphology of cells on scaffolds was observed. The cell carrier showed suitable attachment to the nHA/PLLA fibers (Xu et al., 2007). In order to mimic the morphology and composition, the lowest level of bone structure, PVA and HA nanoparticles was electrospun. Morphology, crystallinity, and thermal properties of scaffold were fully analyzed by SEM, TEM, X-ray diffraction (XRD), differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA). Morphological and crystallographic investigation of scaffolds revealed that rod-like HA nanostructures can be oriented parallel to the longitudinal direction of the fibers which approximately resemble the hardness of bone tissues. These nanofibers are extensively used in many applications related to hard tissue replacement and tissue (bone and dentin) regeneration (Kim et al., 2008). Another important application of scaffolds in dental and oral is related to cranial sutures which possesses very complicated molecular pattern. In dental regeneration, releasing cytokines is a critical issue which may improve by using biodegradable PLGA microspheres. Generally,

3D printed scaffolds can be used for restoring defects through inkjet printing of biodegradable polymers. Various biomarkers such as living cells, proteins, peptides, and DNA have been printed by using 3D scaffolds. Using this technique can provide 3D models for generating ECM on scaffolds which leads to better bone healing. In addition, 3D printing scaffolds may provide an opportunity for better construction of soft tissues through direct incorporation of living cells while keeping normal cell's activity. Recently, 3D printing scaffolds have been used for building periodontium-like complex tissues which is appropriate in periodontal repairing and temporomandibular joints owing to high capacity for cell carrying (Asa'ad et al., 2016). Tissue engineering has great potential application periodontal regeneration due to degeneration and restoration of the PDL. Tissue engineering and scaffolds have been widely used for oral mucosa, nasal hypoplasia patients, congenital and scalp reconstruction. For instance, buccal fat pad (BFP) grafts have been recently used as a suitable support for bone grafting and root covering for teeth due to its high concentration of stem cells (Larsson et al., 2016). Thus, BFPs can be used in tissue engineering and clinical applications especially in surgical procedures. Allogenic acellular dermal matrix is also used in soft tissue construction for the treatment of oral mucosal defects and diseases because of high biocompatibility, supporting natural tissue architecture and descent cellular revascularization (Larsson et al., 2016). In fact, biocompatible scaffolds and matrices can provide further space for the formation of fresh periodontal tissues which is necessary for the regeneration of periodontal tissues. Synthetic alloplastic materials have been extensively utilized in soft tissue engineering specially for the treatment of oral cavities and orthopedic surgery. Natural scaffolds usually obtained from animals or cadaver have shown great potential application in soft tissue engineering. The combination of natural scaffolds with human fibroblasts can be used for the creation of keratinized gingival tissues (Moioli et al., 2007).

10.5 Conclusion

In the last decades, many developments in tissue engineering have led to the production of novel constructs for repair and regeneration especially in the field of bone and dental implants. The combined utilization of CAD with RP procedures and developing the basis of AM approaches provided an impressive progression in scaffold fabrication. Further research linked to addressing the advantages of compounding different technologies for manufacturing scaffolds for tissue engineering and optimizing the entire supply chains from personalized implant design to rapid microfabrication of implantable parts. The application of nanomaterials in the fabrication of dental scaffolds is considerably developing. Combination of different nanoparticles such as metallic nanoparticles, ceramics, and carbon nanostructures with nanofibrous biopolymers is among the most commonly used approaches to fabricate scaffolds for applications in oral and dental tissue engineering.

References

Abdel-Fattah, W.I., Jiang, T., El-Bassyouni, G.E.-T., Laurencin, C.T., 2007. Synthesis, characterization of chitosans and fabrication of sintered chitosan microsphere matrices for bone tissue engineering. Acta Biomater. 3, 503–514.

Arras, M.M., Grasl, C., Bergmeister, H., Schima, H., 2012. Electrospinning of aligned fibers with adjustable orientation using auxiliary electrodes. Sci. Technol. Adv. Mater. 13, 035008.

Asa'ad, F., Pagni, G., Pilipchuk, S.P., Giannì, A.B., Giannobile, W.V., Rasperini, G., 2016. 3D-printed scaffolds and biomaterials: review of alveolar bone augmentation and periodontal regeneration applications. Int. J. Dent. 2016.

Barata, D., Resmini, A., Pereira, D., Veldhuis, S., Van Blitterswijk, C., Ten Elshof, J., et al., 2016. Surface micropatterning with zirconia and calcium phosphate ceramics by micromoulding in capillaries. J. Mater. Chem. B. 4, 1044–1055.

Bartold, P., Gronthos, S., Ivanovski, S., Fisher, A., Hutmacher, D., 2016. Tissue engineered periodontal products. J. Periodontal Res. 51, 1–15.

Beria, L., Gevrek, T.N., Erdog, A., Sanyal, R., Pasini, D., Sanyal, A., 2014. 'Clickable' hydrogels for all: facile fabrication and functionalization. Biomater. Sci. 2, 67–75.

Bizari, D., Moztarzadeh, F., Rabiee, M., Tahriri, M., Banafatizadeh, F., Ansari, A., et al., 2011. Development of biphasic hydroxyapatite/dicalcium phosphate dihydrate (DCPD) bone graft using polyurethane foam template: in vitro and in vivo study. Adv. Appl. Ceram. 110, 417–425.

Bloch, J., Fine, E.G., Bouche, N., Zurn, A.D., Aebischer, P., 2001. Nerve growth factor-and neurotrophin-3-releasing guidance channels promote regeneration of the transected rat dorsal root. Exp. Neurol. 172, 425–432.

Borden, M., Attawia, M., Khan, Y., Laurencin, C.T., 2002. Tissue engineered microsphere-based matrices for bone repair: design and evaluation. Biomaterials. 23, 551–559.

Chang, C., Peng, N., He, M., Teramoto, Y., Nishio, Y., Zhang, L., 2013. Fabrication and properties of chitin/hydroxyapatite hybrid hydrogels as scaffold nano-materials. Carbohydr. Polym. 91, 7–13.

Choi, A.H., Ben-Nissan, B., Conway, R.C., Macha, I.J., 2014. Advances in calcium phosphate nanocoatings and nanocomposites. Advances in Calcium Phosphate Biomaterials. Springer.

Cordeiro, M.M., Dong, Z., Kaneko, T., Zhang, Z., Miyazawa, M., Shi, S., et al., 2008. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J. Endod. 34, 962–969.

Costello, B.J., Shah, G., Kumta, P., Sfeir, C.S., 2010. Regenerative medicine for craniomaxillofacial surgery. Oral Maxillofac. Surg. Clin. N. Am. 22, 33–42.

Dan, H., Vaquette, C., Fisher, A.G., Hamlet, S.M., Xiao, Y., Hutmacher, D.W., et al., 2014. The influence of cellular source on periodontal regeneration using calcium phosphate coated polycaprolactone scaffold supported cell sheets. Biomaterials. 35, 113–122.

Eslami, H., Solati-Hashjin, M., Tahriri, M., 2008. Synthesis and characterization of nanocrystalline fluorinated hydroxyapatite powder by modified wet-chemical process. J. Ceram. Process. Res. 9, 224–229.

Eslami, H., Solati-Hashjin, M., Tahriri, M., 2009. The comparison of powder characteristics and physicochemical, mechanical and biological properties between nanostructure ceramics of hydroxyapatite and fluoridated hydroxyapatite. Mater. Sci. Eng. C. 29, 1387–1398.

Eslami, H., Solati-Hashjin, M., Tahriri, M., Bakhshi, F., 2010. Synthesis and characterization of nanocrystalline hydroxyapatite obtained by the wet chemical technique. Mater. Sci. Pol. 28, 5−13.

Fatehi, K., Moztarzadeh, F., Solati-Hashjin, M., Tahriri, M., Rezvannia, M., Ravarian, R., 2008. In vitro biomimetic deposition of apatite on alkaline and heat treated Ti6A14V alloy surface. Bull. Mater. Sci. 31, 101.

Feyzizarnagh, H., Yoon, D.Y., Goltz, M., Kim, D.S., 2016. Peptide nanostructures in biomedical technology. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology.

Gemberling, M., Bailey, T.J., Hyde, D.R., Poss, K.D., 2013. The zebrafish as a model for complex tissue regeneration. Trends Genet. 29, 611−620.

Ghorani, B., Tucker, N., 2015. Fundamentals of electrospinning as a novel delivery vehicle for bioactive compounds in food nanotechnology. Food Hydrocoll. 51, 227−240.

Ginjupalli, K., Averineni, R.K., 2014. Preparation and characterization of biodegradable scaffolds of poly (lactide-co-glycolide) 50:50 and hydroxyapatite for dental applications. Compos. Interfaces. 21, 31−44.

Gupte, M., Ma, P., 2012. Nanofibrous scaffolds for dental and craniofacial applications. J. Dental Res. 91, 227−234.

Habibi, N., Kamaly, N., Memic, A., Shafiee, H., 2016. Self-assembled peptide-based nanostructures: smart nanomaterials toward targeted drug delivery. Nano Today. 11, 41−60.

Haidar, Z.S., 2015. NanoDentistry: perspectives on the role of nanobiotechnology in biomaterials, pharmaceutics and biodental tissue engineering. EC Dent. Sci. 3, 506−507.

Heidari, F., Bahrololoom, M.E., Vashaee, D., Tayebi, L., 2015. In situ preparation of iron oxide nanoparticles in natural hydroxyapatite/chitosan matrix for bone tissue engineering application. Ceram. Int. 41, 3094−3100.

Hile, D.D., Amirpour, M.L., Akgerman, A., Pishko, M.V., 2000. Active growth factor delivery from poly (D,L-lactide-co-glycolide) foams prepared in supercritical CO_2. J. Control. Release. 66, 177−185.

Hoang, D., Perrault, D., Stevanovic, M., Ghiassi, A., 2016. Surgical applications of three-dimensional printing: a review of the current literature & how to get started. Ann. Transl. Med. 4, 456.

Hong, S., Kim, M., Kim, G., 2012. Collagen−β-TCP conjugated PCL biocomposites for bone tissue regeneration: fabrication, physical properties, and cellular activities. J. Mater. Chem. 22, 22565−22574.

Ishikawa, I., Iwata, T., Washio, K., Okano, T., Nagasawa, T., Iwasaki, K., et al., 2009. Cell sheet engineering and other novel cell-based approaches to periodontal regeneration. Periodontology 2000. 51, 220−238.

Iwata, T., Yamato, M., Tsuchioka, H., Takagi, R., Mukobata, S., Washio, K., et al., 2009. Periodontal regeneration with multi-layered periodontal ligament-derived cell sheets in a canine model. Biomaterials. 30, 2716−2723.

Iwata, T., Washio, K., Yoshida, T., Ishikawa, I., Ando, T., Yamato, M., et al., 2015. Cell sheet engineering and its application for periodontal regeneration. J. Tissue Eng. Regen. Med. 9, 343−356.

Jaklenec, A., Wan, E., Murray, M.E., Mathiowitz, E., 2008. Novel scaffolds fabricated from protein-loaded microspheres for tissue engineering. Biomaterials. 29, 185−192.

Jazayeri, H.E., Tahriri, M., Razavi, M., Khoshroo, K., Fahimipour, F., Dashtimoghadam, E., et al., 2017. A current overview of materials and strategies for potential use in maxillofacial tissue regeneration. Mater. Sci. Eng. C. 70, 913−929.

Jiang, T., Abdel-Fattah, W.I., Laurencin, C.T., 2006. In vitro evaluation of chitosan/poly (lactic acid-glycolic acid) sintered microsphere scaffolds for bone tissue engineering. Biomaterials. 27, 4894–4903.

Jiang, T., Carbone, E.J., Lo, K.W.-H., Laurencin, C.T., 2015. Electrospinning of polymer nanofibers for tissue regeneration. Prog. Polym. Sci. 46, 1–24.

Khojasteh, A., Fahimipour, F., Eslaminejad, M.B., Jafarian, M., Jahangir, S., Bastami, F., et al., 2016a. Development of PLGA-coated β-TCP scaffolds containing VEGF for bone tissue engineering. Mater. Sci. Eng. C. 69, 780–788.

Khojasteh, A., Fahimipour, F., Jafarian, M., Sharifi, D., Jahangir, S., Khayyatan, F., et al., 2016b. Bone engineering in dog mandible: coculturing mesenchymal stem cells with endothelial progenitor cells in a composite scaffold containing vascular endothelial growth factor. J. Biomed. Mater. Res. Part B: Appl. Biomater..

Khoshroo, K., Almela, T., Tahriri, M., Fahimipour, F., Metalwala, Z., Moharamzadeh, K., et al., 2016. 3D-printing of porous calcium phosphate cements for bone tissue engineering. Dent. Mater. 32, e56–e57.

Kim, G.-M., Asran, A.S., Michler, G.H., Simon, P., Kim, J.-S., 2008. Electrospun PVA/HAp nanocomposite nanofibers: biomimetics of mineralized hard tissues at a lower level of complexity. Bioinspiration Biomim. 3, 046003.

King, G., King, N., Cruchley, A., Wozney, J., Hughes, F., 1997. Recombinant human bone morphogenetic protein-2 promotes wound healing in rat periodontal fenestration defects. J. Dent. Res. 76, 1460–1470.

Kolambkar, Y.M., Bajin, M., Wojtowicz, A., Hutmacher, D.W., García, A.J., Guldberg, R.E., 2013. Nanofiber orientation and surface functionalization modulate human mesenchymal stem cell behavior in vitro. Tissue Eng. Part A. 20, 398–409.

Kumar, S., 2003. Selective laser sintering: a qualitative and objective approach. J. Miner. Metals Mater. Soc. 55, 43–47.

Kumbar, S., James, R., Nukavarapu, S., Laurencin, C., 2008. Electrospun nanofiber scaffolds: engineering soft tissues. Biomed. Mater. 3, 034002.

Kumbar, S., Laurencin, C., Deng, M., 2014. Natural and Synthetic Biomedical Polymers. Newnes.

Laranjeira, M.S., Carvalho, Â., Pelaez-Vargas, A., Hansford, D., Ferraz, M.P., Coimbra, S., et al., 2014. Modulation of human dermal microvascular endothelial cell and human gingival fibroblast behavior by micropatterned silica coating surfaces for zirconia dental implant applications. Sci. Technol. Adv. Mater. 15, 025001.

Larsson, L., Decker, A., Nibali, L., Pilipchuk, S., Berglundh, T., Giannobile, W., 2016. Regenerative medicine for periodontal and peri-implant diseases. J. Dent. Res. 95, 255–266.

Li, D., Xia, Y., 2004. Electrospinning of nanofibers: reinventing the wheel? Adv. Mater. 16, 1151–1170.

Liao, S., Wang, W., Uo, M., Ohkawa, S., Akasaka, T., Tamura, K., et al., 2005. A three-layered nano-carbonated hydroxyapatite/collagen/PLGA composite membrane for guided tissue regeneration. Biomaterials. 26, 7564–7571.

Lima, M., Correlo, V., Reis, R.L., 2014. Micro/nano replication and 3D assembling techniques for scaffold fabrication. Mater. Sci. Eng. C. 42, 615–621.

Liu, J.-H., Ma, X., Xu, Y., Tang, H., Yang, S.-T., Yang, Y.-F., et al., 2017. Low toxicity and accumulation of zinc oxide nanoparticles in mice after 270-day consecutive dietary supplementation. Toxicol. Res.. Available from: http://dx.doi.org/10.1039/C6TX00370B.

Liu, X., Won, Y., Ma, P.X., 2005. Surface modification of interconnected porous scaffolds. J. Biomed. Mater. Res. Part A. 74, 84–91.

Lo, H., Ponticiello, M., Leong, K., 1995. Fabrication of controlled release biodegradable foams by phase separation. Tissue Eng. 1, 15–28.
Luciani, A., Coccoli, V., Orsi, S., Ambrosio, L., Netti, P.A., 2008. PCL microspheres based functional scaffolds by bottom-up approach with predefined microstructural properties and release profiles. Biomaterials. 29, 4800–4807.
Lv, Q., Nair, L., Laurencin, C.T., 2009. Fabrication, characterization, and in vitro evaluation of poly (lactic acid glycolic acid)/nano-hydroxyapatite composite microsphere-based scaffolds for bone tissue engineering in rotating bioreactors. J. Biomed. Mater. Res. Part A. 91, 679–691.
Ma, P.X., 2004. Scaffolds for tissue fabrication. Mater. Today. 7, 30–40.
Mandal, D., Shirazi, A.N., Parang, K., 2014. Self-assembly of peptides to nanostructures. Org. Biomol. Chem. 12, 3544–3561.
Martins-Júnior, P., Alcântara, C., Resende, R., Ferreira, A., 2013. Carbon nanotubes: directions and perspectives in oral regenerative medicine. J. Dent. Res. 92, 575–583.
Moioli, E.K., Clark, P.A., Xin, X., Lal, S., Mao, J.J., 2007. Matrices and scaffolds for drug delivery in dental, oral and craniofacial tissue engineering. Adv. Drug Deliv. Rev. 59, 308–324.
Mooney, D.J., Cima, L., Langer, R., Johnson, L., Hansen, L.K., Ingber, D.E., et al., 1991. Principles of tissue engineering and reconstruction using polymer-cell constructs. In: MRS Proceedings. Cambridge University Press, p. 345.
Mozafari, M., Mehraien, M., Vashaee, D., Tayebi, L., 2012. Electroconductive nanocomposite scaffolds: a new strategy into tissue engineering and regenerative medicine. Nanocomposites—New Trends and Developments. InTech.
Neel, E.A.A., Chrzanowski, W., Salih, V.M., Kim, H.-W., Knowles, J.C., 2014. Tissue engineering in dentistry. J. Dent. 42, 915–928.
Nukavarapu, S.P., Kumbar, S.G., Brown, J.L., Krogman, N.R., Weikel, A.L., Hindenlang, M.D., et al., 2008. Polyphosphazene/nano-hydroxyapatite composite microsphere scaffolds for bone tissue engineering. Biomacromolecules. 9, 1818–1825.
Obregón, R., Ramón-Azcón, J., Ahadian, S., Shiku, H., Bae, H., Ramalingam, M., et al., 2014. The use of microtechnology and nanotechnology in fabricating vascularized tissues. J. Nanosci. Nanotechnol. 14, 487–500.
Patrick JR, C.W., Mikos, A.G., Mcintire, L., 1998. Prospectus of tissue engineering. Front. Tissue Eng.3–14.
Peng, L., Deng, Y., Yi, P., Lai, X., 2013. Micro hot embossing of thermoplastic polymers: a review. J. Micromech. Microeng. 24, 013001.
Peterson, L., Brittberg, M., Kiviranta, I., Åkerlund, E.L., Lindahl, A., 2002. Autologous chondrocyte transplantation biomechanics and long-term durability. Am. J. Sports Med. 30, 2–12.
Porter, M.L.A., Münchow, E.A., Albuquerque, M.T., Spolnik, K.J., Hara, A.T., Bottino, M.C., 2016. Effects of novel 3-dimensional antibiotic-containing electrospun scaffolds on dentin discoloration. J. Endod. 42, 106–112.
Qasim, S.B., Najeeb, S., Delaine-Smith, R.M., Rawlinson, A., Rehman, I.U., 2017. Potential of electrospun chitosan fibers as a surface layer in functionally graded GTR membrane for periodontal regeneration. Dent. Mater. 33, 71–83.
Rasoulianboroujeni, M., Yazdimamaghani, M., Khoshkenar, P., Pothineni, V.R., Kim, K.M., Murray, T.A., et al., 2016. From solvent-free microspheres to bioactive gradient scaffolds. Nanomedicine: Nanotechnol. Biol. Med..
Reis, E.C.C., Borges, A.P., Araújo, M.V., Mendes, V.C., Guan, L., Davies, J.E., 2011. Periodontal regeneration using a bilayered PLGA/calcium phosphate construct. Biomaterials. 32, 9244–9253.

Shabafrooz, V., Mozafari, M., Köhler, G.A., Assefa, S., Vashaee, D., Tayebi, L., 2014. The effect of hyaluronic acid on biofunctionality of gelatin–collagen intestine tissue engineering scaffolds. J. Biomed. Mater. Res. Part A. 102, 3130–3139.

Shafiei, F., Behroozibakhsh, M., Moztarzadeh, F., Haghbin-Nazarpak, M., Tahriri, M., 2012. Nanocrystalline fluorine-substituted hydroxyapatite [$Ca_5(PO_4)_3(OH)_{1-x}F_x$ ($0 \leqslant x \leqslant 1$)] for biomedical applications: preparation and characterisation. IET Micro Nano Lett. 7, 109–114.

Shayganpour, A., Rebaudi, A., Cortella, P., Diaspro, A., Salerno, M., 2015. Electrochemical coating of dental implants with anodic porous titania for enhanced osteointegration. Beilstein J. Nanotechnol. 6, 2183–2192.

Sheikh, Z., Sima, C., Glogauer, M., 2015. Bone replacement materials and techniques used for achieving vertical alveolar bone augmentation. Materials. 8, 2953–2993.

Singh, M., Sandhu, B., Scurto, A., Berkland, C., Detamore, M.S., 2010. Microsphere-based scaffolds for cartilage tissue engineering: using subcritical CO_2 as a sintering agent. Acta Biomater. 6, 137–143.

Skoog, S.A., Goering, P.L., Narayan, R.J., 2014. Stereolithography in tissue engineering. J. Mater. Sci.: Mater. Med. 25, 845–856.

Smith, I., Liu, X., Smith, L., Ma, P., 2009. Nanostructured polymer scaffolds for tissue engineering and regenerative medicine, Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, vol. 1. pp. 226–236.

Tahmasbi Rad, A., Ali, N., Kotturi, H.S.R., Yazdimamaghani, M., Smay, J., Vashaee, D., et al., 2014. Conducting scaffolds for liver tissue engineering. J. Biomed. Mater. Res. Part A. 102, 4169–4181.

Tahriri, M., Moztarzadeh, F., 2014. Preparation, characterization, and in vitro biological evaluation of PLGA/nano-fluorohydroxyapatite (FHA) microsphere-sintered scaffolds for biomedical applications. Appl. Biochem. Biotechnol. 172, 2465–2479.

Uyar, T., Çökeliler, D., Doğan, M., Koçum, I.C., Karatay, O., Denkbaş, E.B., 2016. Electrospun nanofiber reinforcement of dental composites with electromagnetic alignment approach. Mater. Sci. Eng. C. 62, 762–770.

Vacanti, J.P., Langer, R., 1999. Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation. The Lancet. 354, S32–S34.

Vaquette, C., Fan, W., Xiao, Y., Hamlet, S., Hutmacher, D.W., Ivanovski, S., 2012. A biphasic scaffold design combined with cell sheet technology for simultaneous regeneration of alveolar bone/periodontal ligament complex. Biomaterials. 33, 5560–5573.

Venugopal, J., Low, S., Choon, A.T., Ramakrishna, S., 2008. Interaction of cells and nanofiber scaffolds in tissue engineering. J. Biomed. Mater. Res. Part B: Appl. Biomater. 84, 34–48.

Villar, C.C., Cochran, D.L., 2010. Regeneration of periodontal tissues: guided tissue regeneration. Dent. Clin. N. Am. 54, 73–92.

Wang, Y., Shi, X., Ren, L., Wang, C., Wang, D.-A., 2009. Porous poly (lactic-co-glycolide) microsphere sintered scaffolds for tissue repair applications. Mater. Sci. Eng. C. 29, 2502–2507.

Wang, Y., Shi, X., Ren, L., Yao, Y., Wang, D.-A., 2010a. In vitro osteogenesis of synovium mesenchymal cells induced by controlled release of alendronate and dexamethasone from a sintered microspherical scaffold. J. Biomater. Sci. Polym. Ed. 21, 1227–1238.

Wang, Y., Shi, X., Ren, L., Yao, Y., Zhang, F., Wang, D.A., 2010b. Poly (lactide-co-glycolide)/titania composite microsphere-sintered scaffolds for bone tissue engineering applications. J. Biomed. Mater. Res. Part B: Appl. Biomater. 93, 84–92.

Wendorff, J.H., Agarwal, S., Greiner, A., 2012. Electrospinning: Materials, Processing, and Applications. John Wiley & Sons.
Whang, K., Thomas, C., Healy, K., Nuber, G., 1995. A novel method to fabricate bioabsorbable scaffolds. Polymer. 36, 837–842.
Wong, K.V., Hernandez, A., 2012. A review of additive manufacturing. ISRN Mech. Eng.2012.
Wray, L.S., Tsioris, K., Gil, E.S., Omenetto, F.G., Kaplan, D.L., 2013. Microfabricated porous silk scaffolds for vascularizing engineered tissues. Adv. Funct. Mater. 23, 3404–3412.
Xu, M., Mei, F., Li, D., Yang, X.P., Sui, G., Deng, X.L., et al., 2007. Electrospun poly (L-lacticacid)/nano-hydroxyapatite hybrid nanofibers and their potential in dental tissue engineering. In: Key Engineering Materials. Trans Tech Publications, pp. 377–380.
Yan, X., Gu, P., 1996. A review of rapid prototyping technologies and systems. Comput.Aid. Des. 28, 307–318.
Yang, X., Yang, F., Walboomers, X.F., Bian, Z., Fan, M., Jansen, J.A., 2010. The performance of dental pulp stem cells on nanofibrous PCL/gelatin/nHA scaffolds. J. Biomed. Mater. Res. Part A. 93, 247–257.
Yao, J., Radin, S., Leboy, P.S., Ducheyne, P., 2005. The effect of bioactive glass content on synthesis and bioactivity of composite poly (lactic-co-glycolic acid)/bioactive glass substrate for tissue engineering. Biomaterials. 26, 1935–1943.
Yazdimamaghani, M., Pourvala, T., Motamedi, E., Fathi, B., Vashaee, D., Tayebi, L., 2013. Synthesis and characterization of encapsulated nanosilica particles with an acrylic copolymer by in situ emulsion polymerization using thermoresponsive nonionic surfactant. Materials. 6, 3727–3741.
Yazdimamaghani, M., Razavi, M., Vashaee, D., Pothineni, V.R., Rajadas, J., Tayebi, L., 2015. Significant degradability enhancement in multilayer coating of polycaprolactone-bioactive glass/gelatin-bioactive glass on magnesium scaffold for tissue engineering applications. Appl. Surf. Sci. 338, 137–145.
Yazdimamaghani, M., Razavi, M., Vashaee, D., Tayebi, L., 2014a. Development and degradation behavior of magnesium scaffolds coated with polycaprolactone for bone tissue engineering. Mater. Lett. 132, 106–110.
Yazdimamaghani, M., Razavi, M., Vashaee, D., Tayebi, L., 2014b. Microstructural and mechanical study of PCL coated Mg scaffolds. Surf. Eng. 30, 920–926.
Yazdimamaghani, M., Vashaee, D., Assefa, S., Shabrangharehdasht, M., Rad, A.T., Eastman, M.A., et al., 2014c. Green synthesis of a new gelatin-based antimicrobial scaffold for tissue engineering. Mater. Sci. Eng. C. 39, 235–244.
Zafar, M., Najeeb, S., Khurshid, Z., Vazirzadeh, M., Zohaib, S., Najeeb, B., et al., 2016. Potential of electrospun nanofibers for biomedical and dental applications. Materials. 9, 73.
Zhang, C.-L., Yu, S.-H., 2014. Nanoparticles meet electrospinning: recent advances and future prospects. Chem. Soc. Rev. 43, 4423–4448.

This page intentionally left blank

Stem cells from oral and maxillofacial tissues

11

Mohammadreza Tahriri, Rizwan Bader, Kimia Khoshroo, Morteza Rasoulianboroujeni and Lobat Tayebi
Marquette University School of Dentistry, Milwaukee, WI, United States

11.1 Introduction

Complex or multitissue dental, oral, and maxillofacial defects and disorders present surgeons with various reconstructive challenges. For example, conditions such as cleft palate, oncology defects, jaw cysts, osteomyelitis, gunshot wounds, osteoradionecrosis, and medication-related osteonecrosis of the jaw and even small defects such as those seen in the alveolar ridges resulting from dentoalveolar trauma, periodontal disease, and hypodontia, necessitate puzzling surgical reconstruction approaches. Researchers are investigating different regenerative techniques to facilitate handling such challenges (Jazayeri et al., 2017; Razavi et al., 2014, 2015; Amrollahi et al., 2016; Tayebi, 2016; Fahmy et al., 2016; Hasani-Sadrabadi et al., 2015; Yazdimamaghani et al., 2014).

In this context, stem cell therapies have significant promise to revolutionize human healthcare and can be useful to overcome the difficulties in oral and maxillofacial surgery. Oral and maxillofacial clinics are an important source of tissues from which various stem cells can be extracted.

Stem cells have two fundamental characteristics: (1) self-renewal, which allows cells to be multipotent and divide without any differentiation, and (2) potency, which allows them the capacity to differentiate into a plethora of cell types (Shakoori et al., 2017). In these capacities stem cells will potentially facilitate the regeneration of certain tissues needed in various defects. The focus of this chapter is the study of stem cells, which are undifferentiated during division and are able to differentiate later allowing tissues for self-cell repair (Caplan, 1991). They can support growth of key cell types in oral and maxillofacial region including osteoblasts, chondrocytes, and adipocytes (Shakoori et al., 2017). Obtaining mesenchymal stem cells from bone tissue is more challenging than obtaining them from dental pulp-derived stem cells (DPSC), apical papilla, human exfoliated deciduous teeth, periodontal ligaments, and dental follicle precursor cells (Asutay et al., 2015; Estrela et al., 2011). This chapter introduces different stem cells that can be extracted from oral and maxillofacial tissues and potentially used for treatment of the defects in this area.

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00011-6
© 2017 Elsevier Ltd. All rights reserved.

11.2 Types of stem cells extracted from oral and maxillofacial tissues dental pulp stem cells

DPSCs are mesenchymal stem cells found inside the dental pulp. Gronthos et al. (2000) extracted these cells as the first tooth-derived stem cells in 2000. DPSCs have the capability to differentiate into various types of cells and tissues, such as osteoblasts, adipocyte-like cells, smooth muscle cells, neurons, dentin, and a dentin-pulp-like complex (Saito et al., 2015). In addition, some studies have revealed the chondrogenic potential of DPSCs in vitro (Alge et al., 2010; Zhang et al., 2006).

DPSCs have been found to have a shorter doubling time (higher proliferation rate) and a higher percentage of stem/progenitor cells in the population. They have presented a higher alkaline phosphatase activity than BMMSCs in osteogenic medium suggesting the potential application for mineralized tissue regeneration (Alge et al., 2010).

11.2.1 Stem cells from human exfoliated deciduous teeth

SHEDs are immature multipotent clonogenic cells isolated from the pulp remnant of exfoliated deciduous teeth (Shakoori et al., 2017; Sunil et al., 2012; Alge et al., 2010). SHEDs, also classified as progenitor cells, have a higher proliferation rate as well as larger population doublings compared to other stem cells like DPSCs and bone marrow mesenchymal stem cells (BMMSCs) (Shakoori et al., 2017; Sunil et al., 2012; Alge et al., 2010). Osteoblasts, odontoblasts, adipocytes, and neural cells are some of the cell types that can be obtained through differentiation of SHEDs (Saito et al., 2015).

11.2.2 Periodontal ligament stem cells

Multipotent periodontal ligament stem cells (PDLSCs), successfully isolated from human impacted third molars by Seo et al. (2004), reside in the perivascular wall of periodontal ligaments. Despite their embryologic neural crest origin, PDLSCs are very similar to mesenchymal stem cells (MSCs) in characteristics such as phenotype, morphology, and differentiation potential (Saito et al., 2015; Gronthos et al., 2000; Wada et al., 2009).

Furthermore, PDLSCs have a immunomodulatory ability which allows them to share a slight resemble to BMMSCs to some degree (Wada et al., 2009). PDLSCs have the capability to differentiate into osteoblasts, cementoblasts, adipocytes, and chondrocytes. These cells have been differentiated into periodontal ligament, alveolar bone, cementum, peripheral nerves, and blood vessels (Park et al., 2011; Liu et al., 2008; Huang et al., 2009) and have been reported to form periodontal ligament and cementum-like tissue in vivo (Park et al., 2015).

11.2.3 Stem cells from apical papilla

Stem cells from apical papilla (SCAPs) are stems cells isolated from apical papilla, the soft tissue at the apices of developing permanent teeth, and as the source of

primary odontoblasts are thought to be influential in the formation of root dentin (Guo et al., 2013). They can undergo adipogenic, dentinogenic, and neurogenic differentiation under appropriate stimuli/conditions (Sonoyama et al., 2006, 2008) and represent features of MSCs (Sedgley and Botero, 2012).

11.2.4 Dental follicle progenitor cells

Dental follicle progenitor cells (DFPCs) are stem cells obtained from dental follicles, an ectomesenchymal tissue around the enamel organ and the dental papilla harboring of heterogeneous population of cells such as progenitor cells that develop the periodontium. Dental follicle encompasses a tooth germ in early tooth formation phases (Annibali et al., 2014). DFPCs also carry the potential to differentiate into different cell types such as osteoblasts, chondrocytes, adipocytes, and neural cells (Mangano et al., 2010).

11.3 Application of clinically relevant stem cells in oral and maxillofacial surgery

Among the various types of stem cells introduced in the previous section, DPSCs can be considered as the most clinically relevant cells.

In a clinical study, application of biocomplexes of DPSCs-collagen sponges for human mandible tissue repair, demonstrated excellent results (Sunil et al., 2012). Osteogenic differentiation capability of DPSCs when used in conjunction with biodegradable scaffolds plays an important role in clinical regeneration (Akkouch et al., 2014; Annibali et al., 2014; Mangano et al., 2010).

Liu et al. indicated that when DPSCs and recombinant human bone morphogenetic protein 2 (rhBMP-2) are incorporated together in a tissue-engineered bone complex, an earlier mineralization and more alveolar bone formation in a rabbit model could be achieved compared to DPSC-free constructs and even autologous bone (Liu et al., 2011). Preceding this perception, DPSCs had been reported to show remarkable Osseo induction and mineralized tissue formation when treated with BMP-2 (Ikeda et al., 2011; Yang et al., 2009, 2008). Moreover, examination of platelet rich plasma/DPSCs used as a graft material in canine models revealed the capability of the graft to conduct mature bone formation and neovascularization (Park et al., 2015; Yamada et al., 2011, 2010).

The application of DPSCs complexes for bone regeneration around the dental implants has been also studied (Yamada et al., 2010; Ito et al., 2011). The obtained results suggest that DPSCs have a high osteogenic potential and may be used as a cell source for the regeneration of the bone tissue surrounding the dental implants (Ito et al., 2011).

It seems that the osteogenic differentiation of DPSCs can be also influenced by the substrate used. Mangano et al. showed that DPSCs can differentiate into osteoblasts when cultured in vitro on various titanium surfaces. Their results show that

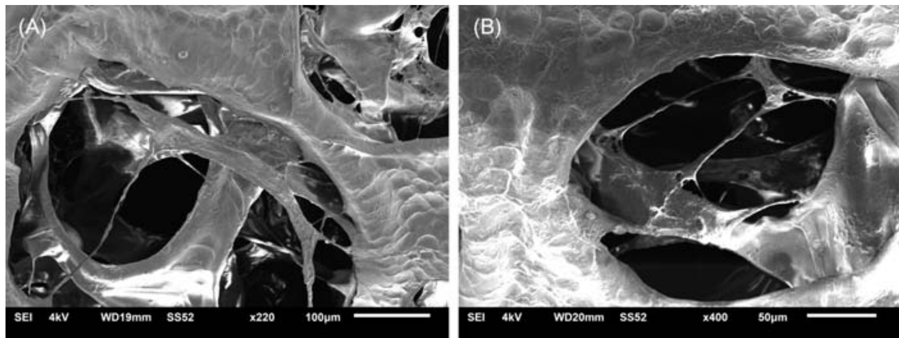


Figure 11.1 DPSC attachment to the gelatin-based scaffolds.

the application of human DPSCs leads to complete osteointegration through quick differentiation into osteoblasts and endotheliocytes and, then, bone tissue production along the implant surfaces (Mangano et al., 2010). Kanafi et al. examined the possibility of immobilizing DPSCs within alginate microspheres and assessed the in vitro osteogenic differentiation potential of the cells. They concluded that immobilization can result in enhanced mineralization and improved secretion of bone related proteins and an upregulated osteo-related gene profile. Interestingly, osteogenic differentiation of DPSCs has been triggered only by immobilization and without the utilization of common induction elements in the media (Kanafi et al., 2014).

DPSCs can also be predifferentiated and used. Akkouch et al. described the predifferentiation of DPSCs into osteoblast-like cells. They seeded these cells onto collagen-hydroxyapatite-poly(L-lactide-co-ε-caprolactone) composite scaffolds and found notable adhesion, proliferation, and differentiation of the osteoblast-like cells along with ECM mineralization throughout the scaffold (Akkouch et al., 2014). Cell attachment plays a critical role in preparation of cell-scaffold complexes for clinical applications. Fig. 11.1 demonstrates DPSC attachment to gelatin-based scaffolds prepared by freeze-drying in our laboratory. DPSCs grew within the pores of the scaffold while attempting to anchor their cytoskeletal projections onto the prewalls.

Other types of stem cells have also been studied as promising cell sources for bone regeneration. As an example, Chadipiralla et al. examined the influence of retinoic acid and dexamethasone treatment on the in vitro proliferation and calcium deposition of PDLSCs and SHEDs. The results demonstrating significantly higher cell proliferation of PDLSCs and consequently greater calcium deposition after a certain period of culture, suggest that PDLSC is a better osteogenic stem cell source (Chadipiralla et al., 2010).

11.4 Future trends

It is worth mentioning that regardless of the clinical success achieved to date, the utilization of stem cells for clinical applications is still in its early stages and pose

several limitations and challenges that yet to be overcome (Almela et al., 2016). Physicians still have much to discover about how to use stem cells adequately and safely in their practice. Such knowledge requires not only to identify the most appropriate source for collecting the stem cells, but also to improve the methods for encouraging stem cell migration and differentiation where needed.

References

Akkouch, A., Zhang, Z., Rouabhia, M., 2014. Engineering bone tissue using human dental pulp stem cells and an osteogenic collagen-hydroxyapatite-poly (l-lactide-co-ε-caprolactone) scaffold. J. Biomater. Appl. 28, 922–936.

Alge, D.L., Zhou, D., Adams, L.L., Wyss, B.K., Shadday, M.D., Woods, E.J., et al., 2010. Donor-matched comparison of dental pulp stem cells and bone marrow-derived mesenchymal stem cells in a rat model. J. Tissue Eng. Regenerative Med. 4, 73–81.

Almela, T., Brook, I.M., Moharamzadeh, K., 2016. The Significance of cell-related challenges in the clinical application of tissue engineering. J. Biomed. Mater. Res. A. 104 (12), 3157–3163.

Amrollahi, P., Shah, B., Seifi, A., Tayebi, L., 2016. Recent advancements in regenerative dentistry: a review. Mater. Sci. Eng. C. 69, 1383–1390.

Annibali, S., Bellavia, D., Ottolenghi, L., Cicconetti, A., Cristalli, M.P., Quaranta, R., et al., 2014. Micro-CT and PET analysis of bone regeneration induced by biodegradable scaffolds as carriers for dental pulp stem cells in a rat model of calvarial "critical size" defect: preliminary data. J. Biomed. Mater. Res. B: Appl. Biomater. 102, 815–825.

Asutay, F., Polat, S., Gül, M., Subaşı, C., Kahraman, S.A., Karaöz, E., 2015. The effects of dental pulp stem cells on bone regeneration in rat calvarial defect model: micro-computed tomography and histomorphometric analysis. Arch. Oral Biol. 60, 1729–1735.

Caplan, A.I., 1991. Mesenchymal stem cells. J. Orthopaed. Res. 9, 641–650.

Chadipiralla, K., Yochim, J.M., Bahuleyan, B., Huang, C.-Y.C., Garcia-Godoy, F., Murray, P.E., et al., 2010. Osteogenic differentiation of stem cells derived from human periodontal ligaments and pulp of human exfoliated deciduous teeth. Cell Tissue Res. 340, 323–333.

Estrela, C., Alencar, A.H.G.D., Kitten, G.T., Vencio, E.F., Gava, E., 2011. Mesenchymal stem cells in the dental tissues: perspectives for tissue regeneration. Braz. Dental J. 22, 91–98.

Fahmy, M.D., Jazayeri, H.E., Razavi, M., Masri, R., Tayebi, L., 2016. Three-dimensional bioprinting materials with potential application in preprosthetic surgery. J. Prosthodont. 25 (4), 310–318.

Gronthos, S., Mankani, M., Brahim, J., Robey, P.G., Shi, S., 2000. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc. Natl. Acad. Sci. 97, 13625–13630.

Guo, L., Li, J., Qiao, X., Yu, M., Tang, W., Wang, H., et al., 2013. Comparison of odontogenic differentiation of human dental follicle cells and human dental papilla cells. PLoS One. 8, e62332.

Hasani-Sadrabadi, M.M., Hajrezaei, S.P., Emami, S.H., Bahlakeh, G., Daneshmandi, L., Dashtimoghadam, E., et al., 2015. Enhanced osteogenic differentiation of stem cells via microfluidics synthesized nanoparticles. Nanomed. Nanotechnol. Biol. Med. 11, 1809–1819.

Huang, C.C., Pelaez, D., Bendala, J.D., Garcia-Godoy, F., Cheung, H.S., 2009. Plasticity of stem cells derived from adult periodontal ligament. Regenerative Med. 4, 809−821.

Ikeda, H., Sumita, Y., Ikeda, M., Ikeda, H., Okumura, T., Sakai, E., et al., 2011. Engineering bone formation from human dental pulp-and periodontal ligament-derived cells. Annal. Biomed. Eng. 39, 26−34.

Ito, K., Yamada, Y., Nakamura, S., Ueda, M., 2011. Osteogenic potential of effective bone engineering using dental pulp stem cells, bone marrow stem cells, and periosteal cells for osseointegration of dental implants. Int. J. Oral Maxillofac. Implants. 26 (5), 947−954.

Jazayeri, H.E., Tahriri, M., Razavi, M., Khoshroo, K., Fahimipour, F., Dashtimoghadam, E., et al., 2017. A current overview of materials and strategies for potential use in maxillofacial tissue regeneration. Mater. Sci. Eng. C. 70, 913−929.

Kanafi, M., Ramesh, A., Gupta, P., Bhonde, R., 2014. Dental pulp stem cells immobilized in alginate microspheres for applications in bone tissue engineering. Int. Endodont. J. 47, 687−697.

Liu, H.-C., Wang, D.-S., Su, F., Wu, X., Shi, Z.-P., LV, Y., et al., 2011. Reconstruction of alveolar bone defects using bone morphogenetic protein 2 mediated rabbit dental pulp stem cells seeded on nano-hydroxyapatite/collagen/poly (L-lactide). Tissue Eng. A. 17, 2417−2433.

Liu, Y., Zheng, Y., Ding, G., Fang, D., Zhang, C., Bartold, P.M., et al., 2008. Periodontal ligament stem cell-mediated treatment for periodontitis in miniature swine. Stem Cells. 26, 1065−1073.

Mangano, C., de Rosa, A., Desiderio, V., D'aquino, R., Piattelli, A., de Francesco, F., et al., 2010. The osteoblastic differentiation of dental pulp stem cells and bone formation on different titanium surface textures. Biomaterials. 31, 3543−3551.

Park, J.-Y., Jeon, S.H., Choung, P.-H., 2011. Efficacy of periodontal stem cell transplantation in the treatment of advanced periodontitis. Cell Transplant. 20, 271−285.

Park, Y.-J., Cha, S., Park, Y.-S., 2015. Regenerative applications using tooth derived stem cells in other than tooth regeneration: a literature review. Stem Cells Int. 2016, 9305986.

Razavi, M., Fathi, M., Savabi, O., Razavi, S.M., Heidari, F., Manshaei, M., et al., 2014. In vivo study of nanostructured diopside ($CaMgSi_2O_6$) coating on magnesium alloy as biodegradable orthopedic implants. Appl. Surf. Sci. 313, 60−66.

Razavi, M., Fathi, M., Savabi, O., Vashaee, D., Tayebi, L., 2015. In vivo assessments of bioabsorbable AZ91 magnesium implants coated with nanostructured fluoridated hydroxyapatite by MAO/EPD technique for biomedical applications. Mater. Sci. Eng. C. 48, 21−27.

Saito, M.T., Silvério, K.G., Casati, M.Z., Sallum, E.A., Nociti Jr, F.H., 2015. Tooth-derived stem cells: update and perspectives. World J. Stem Cells. 7, 399−407.

Sedgley, C.M., Botero, T.M., 2012. Dental stem cells and their sources. Dental Clin. N. Am. 56, 549−561.

Seo, B.-M., Miura, M., Gronthos, S., Bartold, P.M., Batouli, S., Brahim, J., et al., 2004. Investigation of multipotent postnatal stem cells from human periodontal ligament. The Lancet. 364, 149−155.

Shakoori, P., Zhang, Q., Le, A.D., 2017. Applications of mesenchymal stem cells in oral and craniofacial regeneration. Oral Maxillofac. Surg. Clin. N. Am. 29, 19−25.

Sonoyama, W., Liu, Y., Fang, D., Yamaza, T., Seo, B.-M., Zhang, C., et al., 2006. Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS One. 1, e79.

Sonoyama, W., Liu, Y., Yamaza, T., Tuan, R.S., Wang, S., Shi, S., et al., 2008. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J. Endodont. 34, 166−171.

Sunil, P., Manikandhan, R., Muthu, M., Abraham, S., 2012. Stem cell therapy in oral and maxillofacial region: an overview. J. Oral Maxillofac. Pathol. 16, 58.

Tayebi, L., 2016. 3D printing of customized implants for treatment of orofacial deformities and defects. FASEB J. 30, 13.3-13.3.

Wada, N., Menicanin, D., Shi, S., Bartold, P.M., Gronthos, S., 2009. Immunomodulatory properties of human periodontal ligament stem cells. J. Cell. Physiol. 219, 667−676.

Yamada, Y., Ito, K., Nakamura, S., Ueda, M., Nagasaka, T., 2011. Promising cell-based therapy for bone regeneration using stem cells from deciduous teeth, dental pulp, and bone marrow. Cell Transplant. 20, 1003−1013.

Yamada, Y., Nakamura, S., Ito, K., Sugito, T., Yoshimi, R., Nagasaka, T., et al., 2010. A feasibility of useful cell-based therapy by bone regeneration with deciduous tooth stem cells, dental pulp stem cells, or bone-marrow-derived mesenchymal stem cells for clinical study using tissue engineering technology. Tissue Eng. A. 16, 1891−1900.

Yang, X., van Der Kraan, P., Bian, Z., Fan, M., Walboomers, X., Jansen, J., 2009. Mineralized tissue formation by BMP2-transfected pulp stem cells. J. Dental Res. 88, 1020−1025.

Yang, X., Walboomers, X.F., van Den Beucken, J.J., Bian, Z., Fan, M., Jansen, J.A., 2008. Hard tissue formation of STRO-1−selected rat dental pulp stem cells in vivo, Tissue Eng. A, 15. pp. 367−375.

Yazdimamaghani, M., Vashaee, D., Assefa, S., Shabrangharehdasht, M., Rad, A.T., Eastman, M.A., et al., 2014. Green synthesis of a new gelatin-based antimicrobial scaffold for tissue engineering. Mater. Sci. Eng. C. 39, 235−244.

Zhang, W., Walboomers, X.F., Shi, S., Fan, M., Jansen, J.A., 2006. Multilineage differentiation potential of stem cells derived from human dental pulp after cryopreservation, Tissue Eng., 12. pp. 2813−2823.

This page intentionally left blank

Bioreactor design for oral and dental tissue engineering

12

Pouya Amrollahi[1], *Farzaneh Moghadam*[1] *and Lobat Tayebi*[2]
[1]Arizona State University, Tempe, AZ, United States,
[2]Marquette University School of Dentistry, Milwaukee, WI, United States

12.1 Introduction

Tissue engineering (TE) is the science of recreating and restoring the function of body parts and organs. It was in the mid-1980s that TE was recognized as an interdisciplinary field at the intersection of cellular and molecular biology, materials science, and stem cell engineering. Since then the researchers from various scientific disciplines are building the pillars of a biologic renaissance (Vacanti and Vacanti, 2007). TE aims to surmount the shortage of transplanting organs by designing more patient-particular tissues and organs, and to cover the needs of patients suffering from slight injuries to end-stage failure of different tissues and organs. Although human dental and orofacial tissues possess high abrasion resistance and lifelong architectural durability, they are highly vulnerable to chemical, physical, and microbial attacks due to their permanent responsibilities including phonation, mastication, esthetics, respiration, and emotional and facial expressions. Hard and soft tissue defects, diseases, and traumas account for almost half of the medical costs in the United States (Kaigler and Mooney, 2001). This makes oral and dental tissues one of the main targets for TE to tackle.

In TE the best way to encourage neo-tissue growth and formation is to establish the appropriate environmental conditions (for example, oxygen supply, temperature, pH, etc.) for cells embedded in biologically inspired three-dimensional (3D) structures. This provides the cells with the prerequisites for remodeling, interacting, and functioning toward tissue development and regeneration. The fact that the living cells can survive, function, and proliferate in a suitable environment outside the body, which was first demonstrated by Rose G. Harrison et al. on nerve fiber in 1907 (Harrison et al., 1907), has given rise to cell culturing. Cell culture provides optimized, quantifiable, and tunable microenvironmental conditions for cell proliferation into both two-dimensional (2D) and 3D structures. As a crucial component of TE, the most important application of cell culturing is in stem cell engineering. Stem cells have major capabilities such as self-renewal, programmability, and the potential to produce various cell types; therefore they are highly attractive to be used in every field of medicine. It is essential to understand the native physiological conditions of the tissues and try to imitate and experimentally express those factors. Given the appropriate microenvironmental conditions, stem cells can differentiate into lineages of all three germ layers: endoderm (such as stomach, lungs, colon, and

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00012-8
© 2017 Elsevier Ltd. All rights reserved.

liver), mesoderm (such as muscle, bone, fat, and circulatory system), and ectoderm (such as hair, nails, cornea, tooth enamel, and neural crest). In order to fulfill this requirement, bioreactors are the tools that utilize different physical manipulations, mechanical means, and chemical stimulations to mimic the actual physiological situations to orchestrate the tissue formation (Hansmann et al., 2013). It is believed that introducing multiple physical, chemical, and molecular factors can change the differentiation rate of the cells and hence the neo-tissue formation rate (Vunjak-Novakovic and Freshney, 2006). In this chapter, after a brief introduction of principles of bioreactors design which are used as efficient instruments for promoting tissue formation, the literature of their application in dental and orofacial tissue generation have been widely investigated.

12.2 Fundamentals of bioreactors and bioreactor design

Since the 1980s, bioreactors have wide applications in food and drug industries by providing completely controllable and cautiously monitored environments. The main difference between these industrial bioreactors and TE bioreactors is the crucial need for intensive controllability and accurate manipulation of stimuli and parameters in the latter. Moreover, there is widely known, thoroughly accurate correlation between the process parameters and the quality of the product in industrial bioreactors; whereas, the characteristics of the neo-tissue stem from more complex interactions of the culture microenvironment and are hence not easily predictable. Initially, biomedical scientists and engineers started using bioreactors to study the biomaterials; however, they began to grow interest in some of the bioreactors that were designed in order to enable extracorporal tissue growth (Meyer et al., 1993). TE bioreactors are relatively newer devices that can (1) optimize cell growth, (2) improve homogeneity of cell stimulation and distribution at large scale, (3) accelerate extracellular matrix production with enhanced mechanical stability, and (4) ensure nutrient delivery to and waste removal from the scaffolds, while mimicking the native milieu of the tissues by applying regulatory signals and growth factors (Amrollahi and Tayebi, 2015).

The approaches through which bioreactors function can be broadly classified into two main categories. One category of TE bioreactors expedites cell viability and uniformity through regulating the cellular microenvironment and introducing physiological, mechanical, and electrical stimuli. They provide quantifiable means to increase cell seeding, enhance mas/gas transfer rate, and control pH and temperature. On the other hand, another category of TE bioreactors improves monitoring the physiological parameters and optimizes process variables. These bioreactors are useful in investigating the effect of different parameters (such as oxygen flow, temperature change, etc.) on cell proliferation. In general, TE bioreactors have remarkable impact on the cell biology of the cells and impose the conditions for cell or tissue functionalization and formation. The results of these bioreactors are tissues mature enough to be implanted in the patient's body. Primarily the most efficient

TE bioreactor design must support uniform cell distribution, feed the construct with enough amounts of oxygen and nutrients, remove the waste products of the tissue formation effectively, and persuade the extracellular matrix formation by applying external stimuli.

Uniform tissue growth in a TE bioreactor entails an efficient mass transport system for feeding in the essential nutrients, cells, and oxygen and remove wastes and carbon dioxide. Oxygen transport is primarily of great importance, because it acts as metabolic and signaling molecule with low aqueous solubility and low diffusional penetration depth (Martin et al., 2004). It is important to consider both the external (governed by the hydrodynamic conditions) and internal (depending on diffusion and convection processes) mass transfer in a TE bioreactor. Inside a scaffold, mass transfer is only through diffusion. 3D neo-tissue formation dictates more stringent mass transport considerations compared to 2D constructs. The development of larger 3D tissues needs enlarged devices and systems for cell placement (Griffith and Naughton, 2002). Therefore when the size of the scaffold gets bigger and bigger, it is going to be more complicated and more difficult for the nutrients and wastes to transfer in an out of the scaffold. This can lead to heterogeneity in cell distribution and hence the tissue growth. Thus several external forces such as vacuum pressure (Van Wachem et al., 1990), electromagnetic forces (Ito et al., 2005), acoustic waves (Li et al., 2007), and cell suspensions (Shirota et al., 2004) should be used in order to achieve optimal cell seeding and distribution. Although most tissues can be stimulated via applying mechanical forces, they do not react very similarly towards the other parameters of microenvironment (Meyer et al., 2006).

Innovative TE bioreactors should also be able to support the means to provide complex biophysical signals through various sources; such as, applying static and dynamic stresses (stretch, shear, compression, and torsion), enable fluid flow, and applying magnetic or electrical signals. Therefore an efficient TE bioreactor must provide the necessary tools for delicate and accurate generation of mechanical forces. To mimic the in vivo environment effectively, TE bioreactors should be equipped with several stimulating sources, considering the complex nature of the forces, chemicals and stimuli in the human body. Moreover, it is essential for the operator to be able to monitor the tissue formation process; hence, making it favorable to use transparent walls in the design process. Not only all the materials used in the structure of a TE bioreactor must be biocompatible or at least bio-inert and avoid stimulating adverse reactions during culturing in a humid environment at body temperature, but also should be sterilizable and flexible. For this purpose, biodegradable polymers with appropriate degradation rate that matches the regeneration rate of the neo-tissue, such as polylactic acid, polyglycolic acid, and poly (lactic-co-glycolic acid) (YazdiMamaghani et al., 2016) have been the essential ingredient of TE scaffolds and bioreactors for years and their interactions with cells have been widely investigated. However, materials and devices with biomedical applications can be synthesized and fabricated through various chemical and mechanical routes (Amrollahi et al., 2015, 2014, 2016a). Being easy to assemble and disassemble is an important property for commercializing a bioreactor. One other critical concern is the possibility to scale up the neo-tissue generation to fill

the need for extensively defective cases. Another requirement is the means for real-time monitoring of process parameters (like oxygen/nutrition concentration, temperature, and pH). Providing these factors can ensure controllability, operational monitoring, and easy assembly (Brown, 2013).

With all that said, it can be concluded that the design of bioreactors is extremely dependent on the function, geometry, and characteristics of the intended tissue. From a technical point of view, although in some cases it might be possible to use basic bioreactors for empirical purposes, developing a bioreactor system with sufficient operational gadgets is exceedingly complicated due to the complex nature of the body and its organs. So far, scientists have come up with various bioreactor designs and made several major technical advances. In the next section the typical TE bioreactors are discussed.

12.3 Different types of bioreactors

The necessity of culturing various cell types and tissues has impelled scientists to design several different types of bioreactors (schematically shown in Fig. 12.1) that can accommodate the specific requirements of each individual cell/tissue type. To the contrary of the idea of having one design for all cells and tissues, diversity in bioreactor design can help with making cell/tissue-specific optimizations; therefore increasing the efficiency and effectivity of bioreactors. So far, the common bioreactor designs can be classified by their main mode of operation. However, each class of bioreactors has its unique way of applying the stimulus, distinct volumetric capacity to hold the culture, and different process for maintaining the culture. Spinner flasks, rocker platforms, rotating wall vessels, compression, and perfusion are the typical bioreactor designs in use (Amrollahi and Tayebi, 2015).

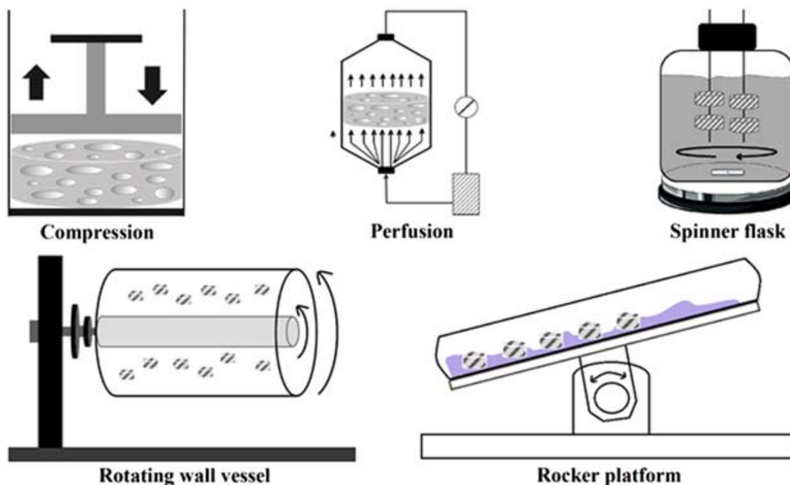


Figure 12.1 Schematic depiction of typical bioreactor designs.

The spinner-flask bioreactor, which provides a dynamic flow via magnetic stirring of the media, is simply made from a cylindrical glass container and multiple needles that hold the tissue constructs. Located at the bottom of the glass container, the stirring bar is in charge of generating the flow of nutrients. In this design, all of the major parameters, such as mass-transfer rate, mixing rate, etc., and hence the morphology and structure of the resulting tissue are controlled and governed by the stirring bar, which generates a turbulent and unsteady flow. In spinner-flask bioreactors the intensity of mixing cannot go above a certain limit, as very high generated shear can disturb tissue formation. These bioreactors are very popular for dynamic seeding of scaffolds and have applications in in vitro production of articular cartilage (Sucosky et al., 2004).

The rocking platform bioreactors generate the mixing process through a side-to-side wavelike motion. It is very important to set the rocking speed and the tilt angle to provide a uniform suspension. Compared to the spinner-flask bioreactors the rocking platform bioreactors have lower risks of contamination, improved applicability, and cheaper costs. These bioreactors have a great potential for cultivation of bacteria and recombinant protein production (Glazyrina et al., 2010; Eibl et al., 2009).

The rotating wall vessels, which have been first developed by National Aeronautics and Space Administration (NASA) to protect 3D culturing from the turbulence caused by takeoff and landings of space shuttles (Granet et al., 1998), exploit gravitational and rotational forces in order to create laminar flow and reduce the mechanical stress, fluid shear, and turbulence on the cell constructs. It has been suggested that for optimal suspension culture conditions, a solid-body rotation Couette-flow is required (Hammond and Hammond, 2001).

The compression bioreactors work with both static and dynamic compressive force modes to mimic the in vivo mechanical stresses that tissues undergo. Although, this will help improve the mechanical properties of the neo-tissue, the optimum loading regimen must be determined in each case (Chen and Hu, 2006). It has also been shown that these forces can regulate and modify the metabolism of articular cartilage (Davisson et al., 2002).

The perfusion bioreactors that provide the means to drive and force the medium through the scaffold have shown to be very effective in improving the cell seeding efficiency, allowing more than one perfusion chambers, and developing more uniform tissues usually over a prolonged period; while being simple to operate, and having a great potential for scaling up. Compared to the other types of bioreactors, perfusion systems provide higher final cell densities (Zhao and Ma, 2005).

Due to the physiological and pathophysiological conditions and the complex nature of the tissues, usually a customized combination of the aforementioned bioreactors must be utilized to achieve improved neo-tissue structures.

12.4 Application of bioreactors for tissue engineering of oral tissue

The size of an oral or maxillofacial defect can determine the approach needed to be taken for the reconstruction procedure. When dealing with relatively large

(critically sized) defects, autologous bone grafts, allografts, osteodistraction, and microvascular procedures show several disadvantages that can remarkably limit their utilization (Torroni, 2009). Thus the quest for coming up with innovative techniques that can fundamentally tackle these limitations and provide patient/case-specific solutions is ongoing. In addition, considering the tremendous role of the oral and maxillofacial tissue in aesthetics, requiring a second surgical procedure (which is common in the aforementioned techniques) bears a risk that is very important to avoid. Large mandibular defects not only disturb mastication, but also can leave shocking psychosocial damage (Hickey and Salter, 2006). Moreover, when it comes to oral or maxillofacial defects, any biomaterial or device that is intended to be utilized in the oral cavity need to satisfy extra requirements other than being biocompatible; it should efficiently initiate cell interactions, induce signaling pathways, and tolerate complex mechanical forces of the wound healing process (due to suturing, wound contractions, etc.) (Mathes et al., 2010), muscular contractions, mastication, speech, and body movements (Depprich et al., 2008). Also the use of animal models in studying the effectiveness of the designed dental biomaterials is very complicated, expensive, and not that straightforward in applying to human beings in nature. Therefore a device that can simulate these mechanical forces in vitro, while minimizing the probability of further surgeries and mimicking the conditions of critically sized defects can add invaluable insight into the material selection and design process for each patient.

In an attempt to evaluate the potential substitutes for connective tissue grafts, Mathes et al. (2010) designed and constructed an in vitro bioreactor with six stainless steel reaction chambers (diameter: 16 mm, height: 5.5 mm) to study the effect of mechanical stimulation on "tissue-specific human primary gingival fibroblasts attached to a collagen sponge" and the viability of gingiva-specified markers. All six chambers were kept under continuous flow of culture medium, CO_2-incubation, and adjustable pressure (through a silicone membrane) for 14 days. A 0.1 bar pressure was applied to compress the collagen sponge in every other 30 min, while shear forces were introduced to the construct for 30 min with 60 min intervals. Methyl-thiazolyl-tetrazolium assay was done to determine the cell number, while enzyme-linked immunosorbent assay (ELISA) kits were used to assess cell viability and expression of the markers (collagen type I, fibronectin, and tenascin-c). To evaluate the possibility of using collagen sponge as a suitable substitute, human primary fibroblasts were cultured for 24 days on each specimen. Because of the compressive mechanical forces the stiffness of the collagen sponges was increased, which works in favor of volume augmentation in a soft tissue wound. Also, mechanical strain improved the fibroblast proliferation and increased the matrix protein. The perfusion system led to the induction of signaling pathways and enhanced the expression of markers. Therefore it can be concluded that the static conditions do not promote natural cell interactions.

Loss of tooth will change the normal compression loading profile applied to the alveolar bone and it would alter its morphology, function, and the outcome for a successful implant treatment (Tolstunov, 2006). Ji et al. (2014) studied how applying in vitro pulsatile compressive forces that can simulate chewing would help with

the osteogenesis of the alveolar bone. The sample was agarose gel seeded with human dental pulp stromal cells, along with fetal bovine serum, penicillin, and streptomycin. They used a loading component to apply symmetrical cyclic compressive force with 1 Hz frequency (average chewing frequency; Buschang et al., 2000) for 30 min, three times a day; while the vertical displacement was fixed to be at 0.5 mm. Studies have shown that 5−10% strain can promote the proliferation of the human dental pulp stromal cells (Yu et al., 2009). The results indicated that the applied compressive force has overall enhanced the viability of the cells; however, this enhancement is influenced by how deep the cells are seeded in the gel, how far they are from the loading component, and how long it has been since the cyclic compression has been applied. Another group of researchers, Woloszyk et al. (2014) have used spinner-flask bioreactors to promote the differentiation of human dental pulp stem cells. After culturing the cells under both static and dynamic conditions for 47 days, they observed that the level of calcium had a twofold increase in the dynamic culture compared to the static condition. In another investigation, Han et al. targeted the osteogenic potential of human periodontal dental pulp stem cells under a 0.2 Hz frequency cyclic stretch (imposing a 10% strain) (Han et al., 2010). Their results indicated that the appropriate amount of cyclic tensile mechanical force can increase the differentiation of human periodontal dental pulp stem cells while decreasing the α-smooth muscle actin. Kanzaki et al. (2006) studied the effect of applying cyclic tensile force to induce 15% elongation on samples containing human periodontal ligament cells and peripheral blood mononuclear cells that had osteoclast precursors. They applied the cyclic force over various periods of time and observed significant up-regulation of osteoprotegerin expression.

Usually bioreactors are considered to be instruments that work ex vivo (Salehi-Nik et al., 2013); however, a novel emerging approach to generate oral neo-tissue is to use in vivo bioreactors, schematically demonstrated in Fig. 12.2. The concept of in vivo bioreactors was introduced to take advantage of the regenerative capacity of the body to cover the shortcoming of ex vivo systems in fully mimicking the physiological conditions of body. Although a lot of efforts have been put in designing ex vivo bioreactors that can simulate the in vivo interactions with more precision and accuracy, imitating vascularization and all the architectural aspects of large organs are still very challenging. In vivo bioreactors were first used for new bone

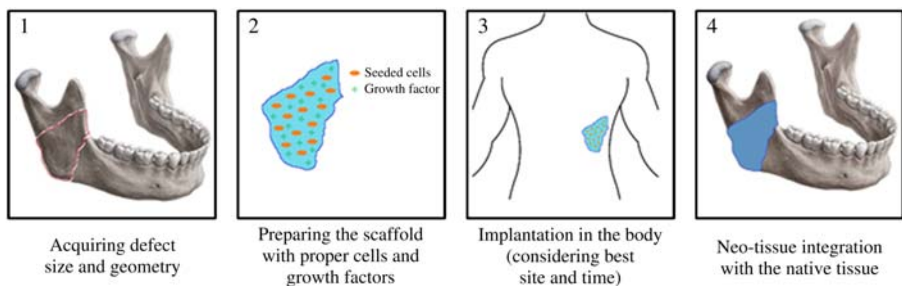


Figure 12.2 Schematic depiction of the steps used in the in vivo bioreactor approach.

growth in a rabbit model to repair musculoskeletal defects (Stevens et al., 2005). When it comes to the facial bone defects, the higher precision and more geometrical control are required. Therefore many preclinical investigations have been done on the regeneration of mandibular tissue. Although using small animal models (mice, rats, and rabbits) in the preclinical stage is more attractive due to being relatively inexpensive (Çelik et al., 2000), these animals cannot mimic all of the diffusional challenges of human body and therefore have limited application (Tatara et al., 2014). On the other hand, large animal models such as sheep (Kokemüller et al., 2014), miniature pigs (Terheyden et al., 1999), and nonhuman primates (Zhou et al., 2010) have widespread application in preclinical studies on mandibular reconstruction. Overall, primates are the best approximations for the human body, but their high cost and ethical controversies make miniature pigs the most viable option. The preclinical studies on mandibular reconstruction have helped scientists find optimal fabrication time (with the highest quality and quantity between 6 and 9 weeks) and implantation site (against the periosteum) (Cheng et al., 2005; Brey et al., 2007). The fabrication time governs the quality of the neo-tissue, while the implantation site can determine the use of various physiological factors and minimize inflammation and pain. Two decades after the first reported case of using in vivo bioreactors for mandibular reconstruction in human patients (Orringer et al., 1999), Kokemueller et al. (2010) have reported the successful and durable implantation of four β-tricalcium phosphate cylinders loaded with iliac crest cells in the defect location of a patient with chronic mandibular osteomyelitis.

Better understanding of the molecular and cellular biology of morphogenesis can make remarkable contributions to the architecture of the scaffolds, and hence, bioreactors (Reddi, 1998). Teeth development, described in details in our previous work (Amrollahi et al., 2016b), initiates from surface epithelium (originated from ectoderm) and underlying mesenchyme (derived from the cranial neural crest cells) as ectodermal appendages, sharing the same core regulatory network with other ectodermal organs. Epithelial-mesenchymal interactions that are mediated by signaling pathways, namely hedgehog (Hh), Wnt, fibroblast growth factor (FGF), transforming growth factor β (Tgfβ), bone morphogenic protein (BMP), and ectodysplasin (Eda), regulate teeth development by expressing growth factors at the placodes, the primary enamel knots, and the secondary enamel knots (Jussila and Thesleff, 2012). Placode formation is regulated by transcription factor *p63*, which plays crucial role in the expression of important signaling pathways (such as BMP, FGF, and Eda) (Laurikkala et al., 2006). Eda signaling pathway is expressed in the placode and any anomaly, such as mutation and overexpression, would manifest as missing teeth (Mikkola, 2009) and developing an extra tooth (Mustonen et al., 2003), respectively. Wnt signaling is important in enamel knot regulation and plays a crucial role in the tooth morphogenesis; initiating the bud to cap stage transition and the primary enamel knot formation. Manipulating the activity of the aforementioned signaling pathways can lead to changes in species-specific cusp patterns (Salazar-Ciudad and Jernvall, 2002), size and number of teeth (Munne et al., 2010). It is noteworthy that although all of these genes are also expressed in other organs, the odontogenic competence is confined to the oral tissue (Wang et al., 2009).

Providing the possibility of real-time monitoring of gene expressions (for example through setting off chain reactions) during the in vitro culturing of the dental tissue, can open new directions in further perfecting the design of dental and oral bioreactors.

12.5 Conclusion

To date, many efforts have been made in the design and utilization of both ex vivo and in vivo bioreactors for oral and dental tissue regeneration and defect reconstruction and the results have been to some extent promising. However, due to the complex nature of these tissues and human musculoskeletal structure, there is still significant room for more investigations, both on the design part as well as the preclinical and clinical studies. Design, development, and the perfection of tissue-specific bioreactors for each oral and dental tissue, based on their contents, geometry, and role, could take a huge step toward whole-tooth generation. Further investment in designing a bioreactor that can provide a more accurate approximation of human oral and dental tissue, with high controllability on the TE factors and proliferation stimuli could change the future of regenerative dentistry.

References

Amrollahi, P., Ataie, A., Nozari, A., Sheibani, S., 2014. Synthesis and characterization of CuNi magnetic nanoparticles by mechano-thermal route. J. Superconduct. Novel Magnet. 27, 481−485.

Amrollahi, P., Ataie, A., Nozari, A., Seyedjafari, E., Shafiee, A., 2015. Cytotoxicity evaluation and magnetic characteristics of mechano-thermally synthesized CuNi nanoparticles for hyperthermia. J. Mater. Eng. Performance. 24, 1220−1225.

Amrollahi, P., Krasinski, J.S., Vaidyanathan, R., Tayebi, L., Vashaee, D., 2016a. Electrophoretic deposition (EPD): fundamentals and applications from nano-to micro-scale structures. Handbook of Nanoelectrochemistry: Electrochemical Synthesis Methods, Properties, and Characterization Techniques. Springer, Berlin, Heidelberg, pp. 561−591.

Amrollahi, P., Shah, B., Seifi, A., Tayebi, L., 2016b. Recent advancements in regenerative dentistry: a review. Mater. Sci. Eng. C. 69, 1383−1390.

Amrollahi, P., Tayebi, L., 2015. Bioreactors for heart valve tissue engineering: a review. J. Chem. Technol. Biotechnol. 91 (4), 847−856.

Brey, E.M., Cheng, M.-H., Allori, A., Satterfield, W., Chang, D.W., Patrick, J.R., et al., 2007. Comparison of guided bone formation from periosteum and muscle fascia. Plastic Reconstruct. Surg. 119, 1216−1222.

Brown, R.A., 2013. Extreme Tissue Engineering: Concepts and Strategies for Tissue Fabrication. John Wiley & Sons, Ltd, West Sussex, United Kingdom.

Buschang, P., Hayasaki, H., Throckmorton, G., 2000. Quantification of human chewing-cycle kinematics. Arch. Oral Biol. 45, 461−474.

Çelik, M., Tuncer, S., Emekli, U., Kesim, S.N., 2000. Histologic analysis of prefabricated, vascularized bone grafts: an experimental study in rabbits. J. Oral Maxillofac. Surg. 58, 292−295.

Chen, H.-C., Hu, Y.-C., 2006. Bioreactors for tissue engineering. Biotechnol. Lett. 28, 1415–1423.

Cheng, M.-H., Brey, E.M., Allori, A., Satterfield, W.C., Chang, D.W., Patrick, J.R., et al., 2005. Ovine model for engineering bone segments. Tissue Eng. 11, 214–225.

Davisson, T., Kunig, S., Chen, A., Sah, R., Ratcliffe, A., 2002. Static and dynamic compression modulate matrix metabolism in tissue engineered cartilage. J. Orthopaed. Res. 20, 842–848.

Depprich, R., Handschel, J., Wiesmann, H.-P., Jäsche-Meyer, J., Meyer, U., 2008. Use of bioreactors in maxillofacial tissue engineering. Br. J. Oral Maxillofac. Surg. 46, 349–354.

Eibl, R., Werner, S., Eibl, D., 2009. Bag bioreactor based on wave-induced motion: characteristics and applications. Disposable Bioreactors. Springer, Berlin, Heidelberg.

Glazyrina, J., Materne, E.-M., Dreher, T., Storm, D., Junne, S., Adams, T., et al., 2010. High cell density cultivation and recombinant protein production with *Escherichia coli* in a rocking-motion-type bioreactor. Microb. Cell Fact. 9, 1–11.

Granet, C., Laroche, N., Vico, L., Alexandre, C., Lafage-Proust, M., 1998. Rotating-wall vessels, promising bioreactors for osteoblastic cell culture: comparison with other 3D conditions. Med. Biol. Eng. Comput. 36, 513–519.

Griffith, L.G., Naughton, G., 2002. Tissue engineering-current challenges and expanding opportunities. Science. 295, 1009–1014.

Hammond, T.G., Hammond, J.M., 2001. Optimized suspension culture: the rotating-wall vessel. Am. J. Physiol. Renal Physiol. 281, F12–F25.

Han, M.-J., Seo, Y.-K., Yoon, H.-H., Song, K.-Y., Park, J.-K., 2010. Upregulation of bone-like extracellular matrix expression in human dental pulp stem cells by mechanical strain. Biotechnol. Bioproc. Eng. 15, 572–579.

Hansmann, J., Groeber, F., Kahlig, A., Kleinhans, C., Walles, H., 2013. Bioreactors in tissue engineering—principles, applications and commercial constraints. Biotechnol. J. 8, 298–307.

Harrison, R.G., Greenman, M.J., Mall, F.P., Jackson, C.M., 1907. Observations of the living developing nerve fiber. Anat. Rec. 1, 116–128.

Hickey, A.J., Salter, M., 2006. Prosthodontic and psychological factors in treating patients with congenital and craniofacial defects. J. Prosthetic Dent. 95, 392–396.

Ito, A., Ino, K., Hayashida, M., Kobayashi, T., Matsunuma, H., Kagami, H., et al., 2005. Novel methodology for fabrication of tissue-engineered tubular constructs using magnetite nanoparticles and magnetic force. Tissue Eng. 11, 1553–1561.

Ji, J., Sun, W., Wang, W., Munyombwe, T., Yang, X.B., 2014. The effect of mechanical loading on osteogenesis of human dental pulp stromal cells in a novel in vitro model. Cell Tissue Res. 358, 123–133.

Jussila, M., Thesleff, I., 2012. Signaling networks regulating tooth organogenesis and regeneration, and the specification of dental mesenchymal and epithelial cell lineages. Cold Spring Harbor Perspect. Biol. 4, a008425.

Kaigler, D., Mooney, D., 2001. Tissue engineering's impact on dentistry. J. Dent. Educ. 65, 456–462.

Kanzaki, H., Chiba, M., Sato, A., Miyagawa, A., Arai, K., Nukatsuka, S., et al., 2006. Cyclical tensile force on periodontal ligament cells inhibits osteoclastogenesis through OPG induction. J. Dent. Res. 85, 457–462.

Kokemueller, H., Spalthoff, S., Nolff, M., Tavassol, F., Essig, H., Stuehmer, C., et al., 2010. Prefabrication of vascularized bioartificial bone grafts in vivo for segmental mandibular reconstruction: experimental pilot study in sheep and first clinical application. Int. J. Oral Maxillofac. Surg. 39, 379–387.

Kokemüller, H., Jehn, P., Spalthoff, S., Essig, H., Tavassol, F., Schumann, P., et al., 2014. En bloc prefabrication of vascularized bioartificial bone grafts in sheep and complete workflow for custom-made transplants. Int. J. Oral Maxillofac. Surg. 43, 163−172.

Laurikkala, J., Mikkola, M.L., James, M., Tummers, M., Mills, A.A., Thesleff, I., 2006. p63 regulates multiple signalling pathways required for ectodermal organogenesis and differentiation. Development. 133, 1553−1563.

Li, H., Friend, J.R., Yeo, L.Y., 2007. A scaffold cell seeding method driven by surface acoustic waves. Biomaterials. 28, 4098−4104.

Martin, I., Wendt, D., Heberer, M., 2004. The role of bioreactors in tissue engineering. Trends Biotechnol. 22, 80−86.

Mathes, S.H., Wohlwend, L., Uebersax, L., von Mentlen, R., Thoma, D.S., Jung, R.E., et al., 2010. A bioreactor test system to mimic the biological and mechanical environment of oral soft tissues and to evaluate substitutes for connective tissue grafts. Biotechnol. Bioeng. 107, 1029−1039.

Meyer, U., Büchter, A., Nazer, N., Wiesmann, H.P., 2006. Design and performance of a bioreactor system for mechanically promoted three-dimensional tissue engineering. Br. J. Oral Maxillofac. Surg. 44, 134−140.

Meyer, U., Szulczewski, H.D., Moller, K., Heide, H., Jones, D.B., 1993. Attachment kinetics and differentiation of osteoblasts on different biomaterials. Cells Mater. 3, 129−140.

Mikkola, M.L., 2009. Molecular aspects of hypohidrotic ectodermal dysplasia. Am. J. Med. Genet. A. 149, 2031−2036.

Munne, P.M., Felszeghy, S., Jussila, M., Suomalainen, M., Thesleff, I., Jernvall, J., 2010. Splitting placodes: effects of bone morphogenetic protein and Activin on the patterning and identity of mouse incisors. Evol. Dev. 12, 383−392.

Mustonen, T., Pispa, J., Mikkola, M.L., Pummila, M., Kangas, A.T., Pakkasjärvi, L., et al., 2003. Stimulation of ectodermal organ development by Ectodysplasin-A1. Dev. Biol. 259, 123−136.

Orringer, J.S., Shaw, W.W., Borud, L.J., Freymiller, E.G., Wang, S.A., Markowitz, B.L., 1999. Total mandibular and lower lip reconstruction with a prefabricated osteocutaneous free flap. Plastic Reconstruct. Surg. 104, 793−797.

Reddi, A.H., 1998. Role of morphogenetic proteins in skeletal tissue engineering and regeneration. Nat. Biotechnol. 16, 247−252.

Salazar-Ciudad, I., Jernvall, J., 2002. A gene network model accounting for development and evolution of mammalian teeth. Proc. Natl. Acad. Sci. U.S.A. 8116−8120.

Salehi-Nik, N., Amoabediny, G., Pouran, B., Tabesh, H., Shokrgozar, M.A., Haghighipour, N., et al., 2013. Engineering parameters in bioreactor's design: a critical aspect in tissue engineering. BioMed Res. Int. 2013.

Shirota, T., He, H., Yasui, H., Matsuda, T., 2004. Human endothelial progenitor cell-seeded hybrid graft: proliferative and antithrombogenic potentials in vitro and fabrication processing. Tissue Eng. 9, 127−136.

Stevens, M.M., Marini, R.P., Schaefer, D., Aronson, J., Langer, R., Shastri, V.P., 2005. In vivo engineering of organs: the bone bioreactor. Proc. Natl. Acad. Sci. U. S. A. 102, 11450−11455.

Sucosky, P., Osorio, D.F., Brown, J.B., Neitzel, G.P., 2004. Fluid mechanics of a spinner-flask bioreactor. Biotechnol. Bioeng. 85, 34−46.

Tatara, A., Wong, M., Mikos, A., 2014. In vivo bioreactors for mandibular reconstruction. J. Dental Res. 93, 1196−1202.

Terheyden, H., Jepsen, S., Rueger, D.R., 1999. Mandibular reconstruction in miniature pigs with prefabricated vascularized bone grafts using recmombinant human osteogenic protein-1: a preliminary study. Int. J. Oral Maxillofac. Surg. 28, 461−463.

Tolstunov, L., 2006. Dental implant success-failure analysis: a concept of implant vulnerability. Implant Dent. 15, 341–346.

Torroni, A., 2009. Engineered bone grafts and bone flaps for maxillofacial defects: state of the art. J. Oral Maxillofac. Surg. 67, 1121–1127.

Vacanti, J., Vacanti, C.A., 2007. The history and scope of tissue engineering. Principles of Tissue Engineering. Elsevier Academic Press, Massachusetts, United States.

Van Wachem, P.B., Stronck, J.W.S., Koers-Zuideveld, R., Dijk, F., Wildevuur, C.R.H., 1990. Vacuum cell seeding: a new method for the fast application of an evenly distributed cell layer on porous vascular grafts. Biomaterials. 11, 602–606.

Vunjak-Novakovic, G., Freshney, R.I., 2006. Culture of Cells for Tissue Engineering. John Wiley & Sons, Inc, New Jersey, United States.

Wang, X.-P., O'connell, D.J., Lund, J.J., Saadi, I., Kuraguchi, M., Turbe-Doan, A., et al., 2009. Apc inhibition of Wnt signaling regulates supernumerary tooth formation during embryogenesis and throughout adulthood. Development. 136, 1939–1949.

Woloszyk, A., Dircksen, S.H., Bostanci, N., Müller, R., Hofmann, S., Mitsiadis, T.A., 2014. Influence of the mechanical environment on the engineering of mineralised tissues using human dental pulp stem cells and silk fibroin scaffolds. PloS One. 9, e111010.

YazdiMamaghani, M., Davachi, S.M., Amrollahi, P., Vashaee, D., Tayebi, L., 2016. Conducting polymers: developments. Encyclopedia of Biomedical Polymers and Polymeric Biomaterials. Taylor & Francis, United States.

Yu, J., Xie, Y.-J., Xu, D., Zhao, S.-L., 2009. Effect of cyclic strain on cell morphology, viability and proliferation of human dental pulp cells in vitro. Shang. J. Stomatol.18.

Zhao, F., Ma, T., 2005. Perfusion bioreactor system for human mesenchymal stem cell tissue engineering: dynamic cell seeding and construct development. Biotechnol. Bioeng. 91, 482–493.

Zhou, M., Peng, X., Mao, C., Xu, F., Hu, M., Yu, G.-Y., 2010. Primate mandibular reconstruction with prefabricated, vascularized tissue-engineered bone flaps and recombinant human bone morphogenetic protein-2 implanted in situ. Biomaterials. 31, 4935–4943.

Growth factors for oral and maxillofacial regeneration applications

13

Mohammadreza Tahriri[1], *Morteza Rasoulianboroujeni*[1], *Rizwan Bader*[1], *Daryoosh Vashaee*[2] *and Lobat Tayebi*[1]
[1]Marquette University School of Dentistry, Milwaukee, WI, United States,
[2]North Carolina State University, Raleigh, NC, United States

13.1 Introduction

Growth factors (GFs) are essential substances found in humans that enhance the cellular growth, proliferation, and cellular differentiation. These factors play also crucial roles in tissue regeneration and engineering. GFs may have variety of functions, e.g., angiogenesis (Lee, 2013), bone morphogenic proteins (BMPs) are capable of empowering bone cell differentiation, and fibroblast GF and vascular endothelial GFs help to trigger vessel differentiation. Different GFs can regenerate defective tissues with oral and maxillofacial surgery to improve therapeutic treatment. The GFs, BMP-2, and BMP-7 have been also used in combination with collagen composite scaffolds (Payne et al., 2014). A concept existed that when BMP-2 and BMP-7 was combined with a collagen sponge, it could act as a carrier for the reconstruction of mandibular defect measuring 4−8 cm, maxillary sinus augmentation, and cleft alveolus (Spagnoli and Marx, 2011). Refractory diseases such as bisphosphonate-related osteonecrosis of the jaw have also been tried to be treated using BMPs (Janssen et al., 2014). Moreover, platelet-derived growth factor (PDGF) has been considered a "starter" for the wound healing process in tissue regeneration, offering great results on both soft and hard tissue regeneration, a prime example being alveolar bone defects. In addition, insulin-like growth factors (IGFs) and transforming growth factor beta (TGF-β) were also found to be potent GFs for a variety of cells and could be possible candidates for commercially accessible products such as BMPs and PDGF (Payne et al., 2014). It has been found that IGF and TGF-β both play key roles in cartilage regeneration and tempomandibular joint (Payne et al., 2014). The following sections will discuss the effects of PDGF, IGFs, TGF-β, BMP, platelet-rich plasma (PRP), and vascular endothelial growth factor (VEGF).

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00013-X
© 2017 Elsevier Ltd. All rights reserved.

13.2 Platelet-derived growth factor

PDGF is a basic dimeric glycoprotein of 30 kDa consisted of two disulfide bonded polypeptides that are encoded by various genes (Schliephake, 2002). There are three isoforms of PDGF: the combination of A- and B-chains, two homodimeric (PDGF-AA and PDGF-BB), and one heterodimeric isoform (PDGF-AB). The use of PDGF-BB and PDGF-AB allows them to be contained in alpha granules of platelets that, when released, attach to the platelets of harmed locales of vessel walls, while the PDGF-AA is secreted by the osteoblastic linage (Rydziel et al., 1994).

PDGF is also involved in embryo development, in the creation of the neural crest and patterning of limbs and myotomes in early embryogenesis, alongside mesoderm induction and mesenchymal-epithelial interactions amidst organ development (Shinbrot et al., 1994). PDGF is involved in the growth and creation of the central nervous system (Hutchins and Jefferson, 1992), myoblasts (Yablonka-Reuveni, 1995), oligodendrocytes (Pringle and Richardson, 1993), blood vessels (Soriano, 1997), mesangial cells of kidney glomeruli (Budde et al., 1997), and alveolar muscle cells of the lung (Betsholtz and Raines, 1997). The ligands of PDGF are produced in epithelial and endothelial cells, but the receptors are located in the mesenchymal cells across the embryo (Cicciù et al., 2012). A disruption to this system, demonstrated by two studies, can cause perinatal lethality of more than 50% (Betsholtz and Raines, 1997).

The cell surface receptors contain tyrosine kinase, which facilitates the activity of PDGF such as PDGF-BB. PDGF-BB binds to the PDGF-receptor beta and activates the extracellular signal-regulated kinase 1 and 2 (ERK1 + ERK2) (Chaudhary and Avioli, 1997). Thus the cellular proliferation is induced by transforming inactive cells into the proliferation portion of the cell cycle (Yang et al., 2000). The protein kinase B (PKB/Akt), a serine-threonine protein kinase, activated by phosphatidylinositol 3-kinase (PI 3-k) acts as a negative feedback system to control normal cell growth and cell cycle progression (Chaudhary and Hruska, 2001). Moreover, TGF-β1 neutralizes the mitogenic effect of PDGF, which interferes with receptor autophosphorylation and somewhat blocks PDGF-R tyrosine kinase. This concludes with PDGF becoming internalized and degraded (Roberts and Sporn, 1990).

In vitro, PDGF-AA, and PDGF-BB allow an increase in the various types of bone cells, such as osteoblast and osteoclast lineages (Hock and Canalis, 1994), but the proliferation of PDGF depends on the donor's age (Tanaka and Liang, 1995), which allows cells to differentiate (Abdennagy et al., 1992). PDGF can stimulate the chondrocyte proliferation; however, not the endochondral maturation (Kieswetter et al., 1997). PDGF can also enhance the creation of osteopontin (Saygin et al., 2000), which, therefore, limits the creation of bone sialoprotein and osteocalcin in bone cells (Yu et al., 1997). In osteosarcoma tissues a positive feedback system allows PDGF-AA and its receptor uncontrolled growth, which is due to PDGF-AA being in an autocrine loop in osteoblast cultures (Scharf et al., 2001). PDGF also has capabilities of decreasing alkaline phosphatase (ALP) activity and mineralization after long exposure (Centrella et al., 1991).

PDGF use in skeletal reconstruction has contradictory results in a variety of experimental settings. PDGF is shown to enhance bone mineral density and compressive strength in the vertebrae of ovariectomized rats (Mitlak et al., 1996); however, it lacked bone ingrowth in HA carriers in rat femora (Arm et al., 1996). PDGF-BB treatment of tibial osteotomies in the rat sample had little bone regeneration (Nash et al., 1994). It has been shown that osteogenin had a repressing factor of bone regeneration in rat calvarial defects (Marden et al., 1993). However, when rats were given a restorable membrane as a carrier, PDGF was shown to enhance the bone formation in the rat calvarial defects (Marden et al., 1993).

PDGF showed great results in the periodontal lesions of monkeys with an increase in height of alveolar bone. Combined with barrier membranes, there was also great periodontal ligament regeneration in beagle dogs (Rutherford et al., 1993). The use of the IGF-I showed similar growth by filling the bone defects in monkeys (Giannobile et al., 1996). Results indicated that PDGF along with IGF had significant bone regeneration after periodontal treatment in stage I/II clinical trials (Howell et al., 1997). The use of these GFs allowed the enhancement of the coronal peri-implant bone formation with ePTFE membranes in dogs (Becker et al., 1992), and, without membranes, allowed the enhancement of apical bone regeneration around the implants (Lynch et al., 1991).

13.3 Insulin-like growth factors

IGFs are single chain peptides that have 40−50% homology with insulin, and coexist in two isoforms of IGF-I (70 amino acids) and IGF-II (67 amino acids). Despite the homology between the three, they all have unique binding sites to their receptors (Schliephake, 2002).

IGFs contain metabolic and growth promoting activity in numerous cells and tissue sorts. One of the isoforms, IGF-I, has been evaluated as a circulating mediator (somatomedin C) of growth hormone, suggesting a functional relationship from the somatomedin hypothesis (Yakar et al., 1999). However, studies indicated that GH-IGF-I might no longer be legitimate due to an experiment that tested liver expression and found no growth or development, despite a 75−80% decrease in circulating IGF-I (Yakar et al., 1999). IGF-I, hence, showed signs of autocrine or paracrine function adding to the idea of a purely endocrine activity that completes general growth and tissue specific development (Butler and LeRoith, 2001).

IGFs have shown developments in fetal and postnatal growth function (Kimble et al., 1999). IGF-I has shown progress in prenatal and postnatal development factors, while IGF-II has shown progress in prenatal stages (Maitre et al., 1995) explaining the increasing ratio of IGF-I/IGF-II seen with aging in numerous amounts of tissue (Bikle et al., 1994). IGFs are also significant factors for muscle creation (Richardson et al., 1991), skeletal growth, and skeletal maintenance (Tirapegui, 1999). Studies showed that IGF-I correlated highly with bone mass in

young women more so than in postmenopausal women with osteoporosis (Ravn et al., 1995). These factors of IGF-activity in postnatal development and growth were organ-specific and influenced by factors such as nutrition. A nutritional deficiency showed signs of liver reduction, a decreased level of circulating IGF-I (Tirapegui, 1999), and a stable supply in the brain (Calikoglu et al., 2001). IGFs are mediated through transmembrane receptors, which compromise the IGF-I receptor, the IGF-II/mannose phosphate receptor (Ataliotis and Mercola, 1997), and the insulin receptor (O'Connor, 1998). IGF-I receptor is a tyrosine kinase that has a high affinity for IGF-I, a 2−50 fold lower affinity for IGF-II, and a 100−500 fold lower affinity for insulin (Morgan et al., 1987).

IGF-I has had minimal effects on isolated application and more towards reconstructive surgery such as the systemic utilization of IGF-I of bone formation in calvarial defects in irradiated rats (Thaller et al., 1998). Investigators have looked at the combined utilization of IGF-I with PDGF, demonstrating bone ingrowth into Ti implants and bone contacts located in the extraction sockets with methycellulose gel acting as the carrier (Lynch et al., 1991). IGF-I/PDGF with expanded polytetrafluoroethylene (ePTFE) membranes were engaged in fresh extraction pockets, where it finished a twofold enhancement in the bone implant contact promoting the guided bone regeneration (Becker et al., 1992). IGF-I showed little function in the development of new attachments for the periodontal regeneration while PDGF-BB showed exceptionally better results (Giannobile et al., 1996).

13.4 Transforming growth factor beta

TGF-β is a multifunctional homodimeric proteins of 25 kDa with a unique receptor that regulates proliferation, differentiation, and other functions in several cell types (Schliephake, 2002). TFG-β can form connective tissues in vivo proposing various therapeutic applications (Sporn et al., 1986). Moreover, it controls the actions of other peptide GFs and decides whether the action will have a positive or negative effect. TFG-β, one of the 30 proteins in its superfamily, has been considered one of the most versatile out of all the cytokines and plays a crucial role in the development of multiple cells (Gao et al., 1998).

TFG-β has an essential role in nervous system creation and in the regulation of neuronal precursors and neuronal differentiation. Any damage or loss of TFG-β or its receptors can lead to perinatal lethality (Dünker and Krieglstein, 2000). Amid embryogenesis, many different morphogenic impacts lead to various genes being activated allowing TFG-β to establish a concentration gradient to position information for the patterning of the embryo. This concentration gradient is accomplished with the help of intracellular trafficking via receptor-mediated endocytosis followed by endocytotic sorting (Entchev et al., 2000). TFG-β plays a prominent role as a morphogen in early embryogenesis such as cranial suture fusion (Roth et al., 1997b) and regulation of the cranial bone growth by patterning the forming dura mater underneath the suture area (Roth et al., 1997a). TFG-β are first secreted as inactive precursors that are activated by slicing the N-terminal of the molecule.

This allows the secreted ligand to bind to the transmembraneous heterodimeric receptor of the cell surface. This receptor is made out of two sections and activates a group of intracellular proteins (SMAD proteins) by serin-threonine kinase and tyrosine kinase activity (Centrella et al., 1987). Phosphorylated SMAD proteins act as transcription complexes to achieve intracellular signal transduction. Receptor SMADs, which come in the complexes of SMAD2 and 3, bind to Co-SMAD (SMAD4) to relocate the nucleus and activate a specific set of target genes (Hanai et al., 1999). TFG-β also has a transcription factor, RUNx3, that helps form a complex with TFG-β activated SMAD3 (Hanai et al., 1999). Runx3 and BMP-initiated SMAD proteins allow for osteoblastic differentiation (Lee et al., 2000).

Use of TFG-β in skeletal reconstructive surgery has been examined in multiple studies. The results showed significant improvement of the bone regeneration. However, TFG-β1 on calcium carbonate carriers in supra-alveolar jaw defects in dogs did not show any bone formation (Tatakis et al., 2000), while utilization of a membrane and the mixture of BMP and PDGF in an absorbable GF cement created significantly larger bone fill (Meraw et al., 2000). In contrast, single dose application of TFG-β in rat calvarial defects showed less bone healing (Bosch et al., 1996). Analysis shows that bone regeneration is dependent of the employed carrier type and its degradation kinetics. The use of free TFG-β in rabbit calvarial defects found no influence, but gelatin capsules were concluded to lead an increase in one's regeneration (Hong et al., 2000). TFG-β1 with hydrogel carriers with 90−95% water content was also found to have beneficial impacts on rat calvarial regeneration, but carriers with too quick or too slow degradation had no impact (Yamamoto et al., 2000). TFG-β, with DMB use, in a segmental mandibular defect in dogs brought remarkably stronger bridging than unfilled control; however, it achieved a breaking strength of 9.4% of the unoperated control side (Sherris et al., 1998). Studies indicate that TFG-β bone regeneration is dependent upon the presence of committed cells, as the prevention of the periosteum brought failure to the TFG-β when trying to restore bone regeneration in rat calvarial defects (Zellin et al., 1998). Moreover, bone inductive activity is proposed since TFG-β lacked bone formation in the calvarial defects of baboons (Duneas et al., 1998). However, it did induce ectopic bone formation when implanted in abdominal muscles of the site (Duneas et al., 1998).

TFG-β that was tested in conjunction with the implant placement in extraction sockets proved to fail the rate of the osteointegration (Smith, 1995). In another study, when TFG-β was used in periodontal repair in conjunction with barrier membranes, it was found that it lacked osteointegration and restricted the improvement of the alveolar bone regeneration (Wikesjö et al., 2004).

13.5 Bone morphogenic proteins

BMPs are homodimeric proteins of 30 kDa consisting of 20 variations and modifications that have two indistinguishable strands linked by a cysteine binding group (Jessell et al., 2009). BMP-2−BMP9 belong to the TFG-β superfamily and have shown a high degree of homology with TFG-β. In comparison, BMP and TFG-β

both have a typical scaffold with a cysteine knot motif and two double stranded beta sheets (Smith, 1995). BMP-2, BMP4, and BMP-7 have been used in clinical and experimental situations.

BMPs are members of the TFG-β family of growth and differentiation factors and play various roles in embryonic and postembryonic creation as signaling molecules in a variety of tissues (Azari et al., 2002). BMPs have been involved in skeletogenesis and bone formation (Reddi, 1998) along with mesoderm patterning, neurogenesis, and organogenesis (Mehler et al., 1997). The genetic and cellular approaches have brought much insight into the BMP signaling mechanisms, most of which coming from the research on model organisms including the mouse, nematode, frog, and fruit fly. The extracellular BMPs attach and activate the multimeric transmembrane receptor complex. This ligand-activated receptor, serine/threonine kinase, phosphorylates members of the SMAD family of the signal transducers. This causes the direct translocation of SMADs to the nucleus allowing regulation of the expression of target genes (Wrana, 2000). To allow BMPs to serve as powerful therapeutic agents (Sakou, 1998), their action must be modified through their signaling, the mechanism as how they are regulated intracellularly or extracellularly, and the potential for BMPs agonistic and antagonistic activities (Sakou, 1998). Studies indicate that the BMP signaling pathway, GFs, and hormonal signaling pathways must be further inspected to prevent undesirable side effects from therapies (Kretzschmar et al., 1997). The role of BMPs can be determined by the expression from a plethora of tissues (Helder et al., 1995). Studies conducted on mice have shown BMPs roles in skeletogenesis and other developmental aspects. A dysfunction in the role of BMP genes has been shown to cause multiple mutations such as short ear and brachypod. Short ear has been the result of the abnormal growth of skeletal structures, which has also shown to cause reduced repair of bone fractures in the adults by the lesion in the BMP-5 gene (Marker et al., 1997). When testing the mice, it was found that a mutation of the GDF-5 (a member of the BMP family) resulted in brachypod and autosomal latent syndromes Hunter-Thompson and Grebe-type chondrodysplasias in the adults (EE, 1994). Syndromes such as these have been characterized by the shortening of the attached skeletal and the abnormal or unusual development of the joints showing minimal impact of the axial skeleton; however, BMPs are yet to be tested in clinical cases (EE, 1994). During the eighties, purified extracts of bovine noncollagenous proteins were employed in the effort to clone BMPs and test human recombinant proteins. The research utilized a single-shot protocol because the injection of aqueous solution was impossible due to the quick dissolution. The use of proteolytic degradation, collagen, poly-alpha-hydroxy acids, calcium phosphates, deproteinized bovine bone, titanium, and deactivated de-mineralized bone autogenous blood clots have been used as carriers to sustain BMP activity levels (Cook et al., 1994). Loading carriers were achieved by BMPs achieving surface absorption along with the quick dilution to washout the GF in the first 24 h (Cook et al., 1994). Many scientists have used collagen and gelatin solution mixtures with BMP to infiltrate porous carriers (Kawai et al., 1993). Few researchers have also used long-term controlled release of BMPs from resorbable polymer carriers (Lu et al., 2000). Higher dosages (300 ng/mL) were utilized in the experiments to achieve osteogenic differentiation in vivo (Yamaguchi et al., 1996).

The use of rhBMP2, rhBMP4, and rhBMP7 induced ectopic and orthotopic bone formation at the site of use. It is critical that a high washout rate be accounted for in the carriers to achieve endochondral bone formation. A quick declining BMP signal was adequate to establish the bone regeneration in the model defects. Spinal fusions and segmental defects of femur, tibiae, ulnae, and mandibles have also been mended in animal models such as rats, rabbits, dogs, and sheep.

13.6 Platelet rich plasma

Platelets contain a number of different GFs and cytokines that may accelerate tissue regeneration process (Dugrillon et al., 2002). PDGF, transforming growth factors β1 and β2 (TGF-β1 and TGF-β2), IGF, epidermal growth factor (EGF), epithelial cell growth factor, platelet-derived angiogenesis factor, platelet factor 4, and hepatocyte growth factor are the GFs found in the platelets (Kiuru et al., 1991). Normal platelet counts in blood range from 150,000/μL to 350,000/μL with an average of 200,000/μL. Platelet-rich plasma (PRP) is defined as a 5 mL volume of plasma with a platelet concentration of at least 1,000,000 platelets/μL (Marx, 2001). Therefore PRP contains a 3- to 5-fold increase in the GF concentration. These factors induce the local mesenchymal and epithelial cells to migrate, divide, and increase matrix synthesis (Sánchez et al., 2003). As a result, PRP-related products, also known as PRP, platelet-rich concentrate, platelet gel, preparation rich ingrowth factors, and platelet releasate have been suggested to enhance bone and soft tissue healing (Marx, 2001). It is worth mentioning that PRP may modulate cell proliferation in a cell type-specific manner. While osteoblastic DNA synthesis and cell division as well as DNA synthesis in gingival fibroblasts and periodontal ligament cells are stimulated by PRP, down-regulation of ALP and suppression of epithelial cell division occurs when PRP is present (Okuda et al., 2003).

PRP-related products have been studied in vitro and in vivo in the fields of maxillofacial surgery and general surgery and are currently used for various orthopedic applications (Mehta and Watson, 2008). PRP has been recommended to be used prior to or in conjunction with dental implant placement to increase the rate and quality of the bone deposition regeneration (Sánchez et al., 2003).

The effect PRP concentrate addition as a source of autologous GFs to autogenous bone grafts and bone formation is still controversial. While some authors suggest that the addition of PRP does not seem to enhance the new bone formation in the autogenous bone grafts used in the mandible of a canine model (Choi et al., 2004) or the noncritical sized defects in the rabbit cranial model (Aghaloo et al., 2002), others have shown that the use of PRP concentrate leads to an increase of the bone formation and the density after autologous bone grafting in patients (Marx et al., 1998). Such controversy may arise from the fact that the platelet concentration necessary for a positive PRP effect on bone regeneration seems to embrace a very limited range (Weibrich et al., 2004). Delivering an appropriate amount of GFs through topically applied PRP may up-regulate cellular activities and subsequently promote periodontal regeneration (Okuda et al., 2003).

13.7 Vascular endothelial growth factor

For appropriate healing to happen the defect should have proper vascularization. Much of the creation and maintenance of the angiogenesis is modulated by VEGF. It has been well known that VEGF, as a part of the cascade, controls the bone formation, especially during the bone healing, through the encouragement of the vascular structures by operating on the osteoblasts (Herford et al., 2017). Just as blood vessel creation does not take place at a specific time, a single deposition of a specific type of VEGF at a high concentration within a defect is not likely to form a vasculature system. The release of VEGF over time and other dumbfounding factors develop a distinctive challenge in the development of the vasculature that could support a regenerative tissue construct. A temporal creation of VEGF, locally applied at the site of the bone destruction, may demonstrate to be a powerful therapy to encourage human bone repair (Barati et al., 2016). Zhang et al. (2011) evaluated the influence of VEGF alone and in association to BMP-2. They ascertained that the application of a single angiogenic agent was not enough for bone formation. However, the influence of VEGF simultaneously applied with BMP-2 increased the bone generation in terms of both density and volume. Additionally, VEGF was remarkably effective in enhancing the resorption speed of the carrier utilized in the research.

Table 13.1 summarizes the functions of the main GFs in relation to the bone regeneration.

Table 13.1 Functions of the main growth factors in relation to the bone regeneration (Kaigler et al., 2006)

Growth factors	Cell of origin	Functions
BMPs 2-4	Osteoblasts	Stimulates mesenchymal progenitor cell migration
BMP-7	Osteoblasts	Stimulates osteoblast and chondroblast differentiation
IGF-2	Macrophages, fibroblasts	Stimulates the osteoblast proliferation and bone matrix synthesis
PDGF	Macrophages	Stimulates the differentiation of fibroblasts into myofibroblasts Stimulates the proliferation of mesenchymal progenitor cells
TGF-β	Fibroblasts, osteoblasts	Induces the endothelial cell and fibroblast apoptosis Induces the differentiation of fibroblasts into myofibroblasts Stimulates chemotaxis and survival of osteoblasts
VEGF	Macrophages	Chemotaxis of mesenchymal stem cells, antiapoptotic effecton the bone-forming cells, and angiogenesis promotion

13.8 Future trends

Using GFs in oral and maxillofacial tissue regeneration has created great interest and focused research efforts. However, many of the efforts, especially the ones involved clinical trials, were not successful due to problems regarding GF delivery. The bolus injection of many GFs is harmful and using a vehicle, such as micro and nanoparticles with the GF loading capability seems to be necessary (Richardson et al., 2001; Sheridan et al., 2000; Chen and Mooney, 2003; Lee et al., 2002, 2011; Simmons et al., 2004; Holland et al., 2005; Babensee et al., 2000; Yu et al., 2014; Kolambkar et al., 2011; Thomopoulos et al., 2014; Lu et al., 2014).

However, imprecise use (such as having polydisperse particles), off targeting, or unsatisfactory delivery without precise sustained release can increase the threat of risky side effects. The side effects are more severe in delivery of sensitive GFs such as VEGF, which should be localized and transported to a specific target tissue with prolonged and sustained exposure to a low dosage of VEGF, otherwise it may lead to tumorigenesis. This is a serious obstacle preventing such GFs to be approved for clinical use. Currently, delivery systems are imperfect and limited knowledge exists for precise encapsulation and sustained/controlled release of many GFs (Khojasteh et al., 2016).

Thus the authors believe that one of the most important future trend related to the application of GFs in oral and maxillofacial tissue engineering will be focused on developing technologies for precise delivery of GFs to the area of defects. Some new methods such as microfluidic approaches show promising results in terms of production of precise customized encapsulated GF particles with well-regulated release rates.

In general, utilization of GFs are among the most promising therapies, especially for treatment of critically sized and/or segmental maxillofacial defects, with encouraging progress. The GF therapies have the potential to stand among the most effective and safe modalities for oral and maxillofacial tissue regeneration in near future.

References

Abdennagy, B., Hott, M., Marie, P.J., 1992. Effects of platelet-derived growth factor on human and mouse osteoblastic cells isolated from the trabecular bone surface. Cell Biol. Int. Rep. 16, 235–247.

Aghaloo, T.L., Moy, P.K., Freymiller, E.G., 2002. Investigation of platelet-rich plasma in rabbit cranial defects: a pilot study. J. Oral Maxillofac. Surg. 60, 1176–1181.

Arm, D., Tencer, A.F., Bain, S.D., Celino, D., 1996. Effect of controlled release of platelet-derived growth factor from a porous hydroxyapatite implant on bone ingrowth. Biomaterials. 17, 703–709.

Ataliotis, P., Mercola, M., 1997. Distribution and functions of platelet-derived growth factors and their receptors during embryogenesis. Int. Rev. Cytol. 172, 95–127.

Azari, K., Doctor, J.S., Doll, B.A., Hollinger, J.O., 2002. Bone morphogenetic proteins: a review for cranial and maxillofacial surgery. Oral Maxillofac. Surg. Clin. N. Am. 14, 1–14.

Babensee, J.E., Mcintire, L.V., Mikos, A.G., 2000. Growth factor delivery for tissue engineering. Pharmaceut. Res. 17, 497–504.

Barati, D., Shariati, S.R.P., Moeinzadeh, S., Melero-Martin, J.M., Khademhosseini, A., Jabbari, E., 2016. Spatiotemporal release of BMP-2 and VEGF enhances osteogenic and vasculogenic differentiation of human mesenchymal stem cells and endothelial colony-forming cells co-encapsulated in a patterned hydrogel. J. Control. Release. 223, 126–136.

Becker, W., Lynch, S.E., Lekholm, U., Becker, B.E., Caffesse, R., Donath, K., et al., 1992. A comparison of ePTFE membranes alone or in combination with platelet-derived growth factors and insulin-like growth factor-I or demineralized freeze-dried bone in promoting bone formation around immediate extraction socket implants. J. Periodontol. 63, 929–940.

Betsholtz, C., Raines, E.W., 1997. Platelet-derived growth factor: a key regulator of connective tissue cells in embryogenesis and pathogenesis. Kid. Int. 51, 1361–1369.

Bikle, D.D., Harris, J., Halloran, B.P., Roberts, C.T., Leroith, D., Morey-Holton, E., 1994. Expression of the genes for insulin-like growth factors and their receptors in bone during skeletal growth. Am. J. Physiol.-Endocrinol. Metabol. 267, E278–E286.

Bosch, C., Melsen, B., Gibbons, R., Vargervik, K., 1996. Human recombinant transforming growth factor-[beta] in healing of calvarial bone defects. J. Craniofac. Surg. 7, 300–310.

Budde, K., Neumayer, H.-H., Salant, D.J., Cybulsky, A.V., Coleman, D.L., Sterzel, R.B., 1997. Glomerular epithelial cell products stimulate mesangial cell proliferation in culture. Kid. Int. 52, 733–741.

Butler, A.A., LeRoith, D., 2001. Minireview: tissue-specific versus generalized gene targeting of the igf1 and igf1r genes and their roles in insulin-like growth factor physiology. Endocrinology. 142, 1685–1688.

Calikoglu, A.S., Karayal, A.F., D'ercole, A.J., 2001. Nutritional regulation of IGF-I expression during brain development in mice. Pediatr. Res. 49, 197–202.

Centrella, M., Mccarthy, T.L., Canalis, E., 1987. Transforming growth factor beta is a bifunctional regulator of replication and collagen synthesis in osteoblast-enriched cell cultures from fetal rat bone. J. Biol. Chem. 262, 2869–2874.

Centrella, M., Mccarthy, T.L., Kusmik, W.F., Canalis, E., 1991. Relative binding and biochemical effects of heterodimeric and homodimeric isoforms of platelet-derived growth factor in osteoblast-enriched cultures from fetal rat bone. J. Cell. Physiol. 147, 420–426.

Chaudhary, L.R., Avioli, L.V., 1997. Activation of extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2) by FGF-2 and PDGF-BB in normal human osteoblastic and bone marrow stromal cells: differences in mobility and in-gel renaturation of ERK1 in human, rat, and mouse osteoblastic cells. Biochem. Biophys. Res. Commun. 238, 134–139.

Chaudhary, L.R., Hruska, K.A., 2001. The cell survival signal Akt is differentially activated by PDGF-BB, EGF, and FGF-2 in osteoblastic cells. J. Cell. Biochem. 81, 304–311.

Chen, R.R., Mooney, D.J., 2003. Polymeric growth factor delivery strategies for tissue engineering. Pharmaceut. Res. 20, 1103–1112.

Choi, B.-H., Im, C.-J., Huh, J.-Y., Suh, J.-J., Lee, S.-H., 2004. Effect of platelet-rich plasma on bone regeneration in autogenous bone graft. Int. J. Oral Maxillofac. Surg. 33, 56–59.

Cicciù, M., Herford, A.S., Juodžbalys, G., Stoffella, E., 2012. Recombinant human bone morphogenetic protein type 2 application for a possible treatment of bisphosphonates-related osteonecrosis of the jaw. J. Craniofac. Surg. 23, 784–788.

Cook, S.D., Baffes, G.C., Wolfe, M.W., Sampath, T.K., Rueger, D.C., 1994. Recombinant human bone morphogenetic protein-7 induces healing in a canine long-bone segmental defect model. Clin. Orthopaed. Related Res. 301, 302–312.

Dugrillon, A., Eichler, H., Kern, S., Klüter, H., 2002. Autologous concentrated platelet-rich plasma (cPRP) for local application in bone regeneration. Int. J. Oral Maxillofac. Surg. 31, 615–619.

Duneas, N., Crooks, J., Ripamonti, U., 1998. Transforming growth factor-β 1: induction of bone morphogenetic protein genes expression during endochondral bone formation in the baboon, and synergistic interaction with osteogenic protein-1 (BMP-7). Growth Fact. 15, 259–277.

Dünker, N., Krieglstein, K., 2000. Targeted mutations of transforming growth factor-β genes reveal important roles in mouse development and adult homeostasis. FEBS J. 267, 6982–6988.

EE, S., Huynh, T.V., Copeland, N.G., Jenkins, N.A., Kingsley, D.M., Lee, S.J., 1994. Limb alterations in brachypodism mice due to mutations in a new member of the TGF beta-superfamily. Nature. 368, 639–643.

Entchev, E.V., Schwabedissen, A., González-Gaitán, M., 2000. Gradient formation of the TGF-β homolog Dpp. Cell. 103, 981–992.

Gao, J., Symons, A., Bartold, P., 1998. Expression of transforming growth factor-beta 1 (TGF-β1) in the developing periodontium of rats. J. Dent. Res. 77, 1708–1716.

Giannobile, W.V., Hernandez, R.A., Finkelman, R.D., Ryarr, S., Kiritsy, C.P., D'andrea, M., et al., 1996. Comparative effects of plateletderived growth factor-BB and insulin-like growth factor-I, individually and in combination, on periodontal regeneration in *Macaca fascicularis*. J. Periodont. Res. 31, 301–312.

Hanai, J.-I., Chen, L.F., Kanno, T., Ohtani-Fujita, N., Kim, W.Y., Guo, W.-H., et al., 1999. Interaction and functional cooperation of PEBP2/CBF with smads synergistic induction of the immunoglobulin germline Cα promoter. J. Biol. Chem. 274, 31577–31582.

Helder, M.N., Ozkaynak, E., Sampath, K.T., Luyten, F., Latin, V., Oppermann, H., et al., 1995. Expression pattern of osteogenic protein-1 (bone morphogenetic protein-7) in human and mouse development. J. Histochem. Cytochem. 43, 1035–1044.

Herford, A.S., Miller, M., Signorino, F., 2017. Maxillofacial defects and the use of growth factors. Oral Maxillofac. Surg. Clin. N. Am. 29, 75–88.

Hock, J.M., Canalis, E., 1994. Platelet-derived growth factor enhances bone cell replication, but not differentiated function of osteoblasts. Endocrinology. 134, 1423–1428.

Holland, T.A., Tabata, Y., Mikos, A.G., 2005. Dual growth factor delivery from degradable oligo (poly (ethylene glycol) fumarate) hydrogel scaffolds for cartilage tissue engineering. J. Controll. Release. 101, 111–125.

Hong, L., Tabata, Y., Miyamoto, S., Yamada, K., Aoyama, I., Tamura, M., et al., 2000. Promoted bone healing at a rabbit skull gap between autologous bone fragment and the surrounding intact bone with biodegradable microspheres containing transforming growth factor-β1. Tissue Eng. 6, 331–340.

Howell, T.H., Fiorellini, J.P., Paquette, D.W., Offenbacher, S., Giannobile, W.V., Lynch, S. E., 1997. A phase I/II clinical trial to evaluate a combination of recombinant human platelet-derived growth factor-BB and recombinant human insulin-like growth factor-I in patients with periodontal disease. J. Periodontol. 68, 1186–1193.

Hutchins, J.B., Jefferson, V.E., 1992. Developmental distribution of platelet-derived growth factor in the mouse central nervous system. Dev. Brain Res. 67, 121–135.

Janssen, N.G., Weijs, W.L., Koole, R., Rosenberg, A.J., Meijer, G.J., 2014. Tissue engineering strategies for alveolar cleft reconstruction: a systematic review of the literature. Clin. Oral Invest. 18, 219–226.

Jessell, T., Wichterle, H., Wilson, S., 2009. Systems and methods for screening for modulators of neural differentiation. Google Patents.

Kaigler, D., Cirelli, J.A., Giannobile, W.V., 2006. Growth factor delivery for oral and periodontal tissue engineering. Expert Opin. Drug Deliv. 3, 647–662.

Kawai, T., Mieki, A., Ohno, Y., Umemura, M., Kataoka, H., Kurita, S., et al., 1993. Osteoinductive activity of composites of bone morphogenetic protein and pure titanium. Clin. Orthopaed. Related Res. 290, 296–305.

Khojasteh, A., Fahimipour, F., Eslaminejad, M.B., Jafarian, M., Jahangir, S., Bastami, F., et al., 2016. Development of PLGA-coated β-TCP scaffolds containing VEGF for bone tissue engineering. Mater. Sci. Eng. C. 69, 780–788.

Kieswetter, K., Schwartz, Z., Alderete, M., Dean, D.D., Boyan, B.D., 1997. Platelet derived growth factor stimulates chondrocyte proliferation but prevents endochondral maturation. Endocrine. 6, 257–264.

Kimble, R., Breier, B., Gluckman, P., Harding, J., 1999. Enteral IGF-I enhances fetal growth and gastrointestinal development in oesophageal ligated fetal sheep. J. Endocrinol. 162, 227–235.

Kiuru, J., Viinikka, L., Myllylä, G., Pesonen, K., Perheentupa, J., 1991. Cytoskeleton-dependent release of human platelet epidermal growth factor. Life Sci. 49, 1997–2003.

Kolambkar, Y.M., Dupont, K.M., Boerckel, J.D., Huebsch, N., Mooney, D.J., Hutmacher, D.W., et al., 2011. An alginate-based hybrid system for growth factor delivery in the functional repair of large bone defects. Biomaterials. 32, 65–74.

Kretzschmar, M., Doody, J., Massagu, J., 1997. Opposing BMP and EGF signalling pathways converge on the TGF-β family mediator Smad1. Nature. 389, 618–622.

Lee, B.-K., 2013. Growth factors in oral and maxillofacial surgery: potentials and challenges. J. Korean Assoc. Oral Maxillofac. Surg. 39, 255–256.

Lee, J.-Y., Nam, S.-H., Im, S.-Y., Park, Y.-J., Lee, Y.-M., Seol, Y.-J., et al., 2002. Enhanced bone formation by controlled growth factor delivery from chitosan-based biomaterials. J. Controll. Release. 78, 187–197.

Lee, K.-S., Kim, H.-J., Li, Q.-L., Chi, X.-Z., Ueta, C., Komori, T., et al., 2000. Runx2 is a common target of transforming growth factor β1 and bone morphogenetic protein 2, and cooperation between Runx2 and Smad5 induces osteoblast-specific gene expression in the pluripotent mesenchymal precursor cell line C2C12. Mol. Cell. Biol. 20, 8783–8792.

Lee, K., Silva, E.A., Mooney, D.J., 2011. Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. J. R. Soc. Interface. 8, 153–170.

Lu, L., Stamatas, G.N., Mikos, A.G., 2000. Controlled release of transforming growth factor β1 from biodegradable polymer microparticles. J. Biomed. Mater. Res. 50, 440–451.

Lu, S., Lam, J., Trachtenberg, J.E., Lee, E.J., Seyednejad, H., Van Den Beucken, J.J., et al., 2014. Dual growth factor delivery from bilayered, biodegradable hydrogel composites for spatially-guided osteochondral tissue repair. Biomaterials. 35, 8829–8839.

Lynch, S.E., Buser, D., Hernandez, R.A., Weber, H., Stich, H., Fox, C.H., et al., 1991. Effects of the platelet-derived growth factor/insulin-like growth factor-I combination on bone regeneration around titanium dental implants. Results of a pilot study in beagle dogs. J. Periodontol. 62, 710–716.

Maitre, B., Clement, A., Williams, M.C., Brody, J.S., 1995. Expression of insulin-like growth factor receptors 1 and 2 in the developing lung and their relation to epithelial cell differentiation. Am. J. Respir. Cell Mol. Biol. 13, 262–270.

Marden, L.J., Fan, R., Pierce, G.F., Reddi, A.H., Hollinger, J.O., 1993. Platelet-derived growth factor inhibits bone regeneration induced by osteogenin, a bone morphogenetic protein, in rat craniotomy defects. J. Clin. Invest. 92, 2897.

Marker, P.C., Seung, K., Bland, A.E., Russell, L.B., Kingsley, D.M., 1997. Spectrum of Bmp5 mutations from germline mutagenesis experiments in mice. Genetics. 145, 435−443.

Marx, R.E., 2001. Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dentist. 10, 225−228.

Marx, R.E., Carlson, E.R., Eichstaedt, R.M., Schimmele, S.R., Strauss, J.E., Georgeff, K.R., 1998. Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol. 85, 638−646.

Mehler, M.F., Mabie, P.C., Zhang, D., Kessler, J.A., 1997. Bone morphogenetic proteins in the nervous system. Trends Neurosci. 20, 309−317.

Mehta, S., Watson, J.T., 2008. Platelet rich concentrate: basic science and current clinical applications. J. Orthopaed. Trauma. 22, 432−438.

Meraw, S.J., Reeve, C.M., Lohse, C.M., Sioussat, T.M., 2000. Treatment of peri-implant defects with combination growth factor cement. J. Periodontol. 71, 8−13.

Mitlak, B.H., Finkelman, R.D., Hill, E.L., Li, J., Martin, B., Smith, T., et al., 1996. The effect of systemically administered PDGF-BB on the rodent skeleton. J. Bone Miner. Res. 11, 238−247.

Morgan, D.O., Edman, J.C., Standring, D.N., Fried, V.A., Smith, M.C., Roth, R.A., et al., 1987. Insulin-like growth factor II receptor as a multifunctional binding protein. Nature. 329, 301.

Nash, T., Howlett, C., Martin, C., Steele, J., Johnson, K., Hicklin, D., 1994. Effect of platelet-derived growth factor on tibial osteotomies in rabbits. Bone. 15, 203−208.

O'Connor, R., 1998. Survival factors and apoptosis. Apoptosis. Springer, Berlin Heidelberg.

Okuda, K., Kawase, T., Momose, M., Murata, M., Saito, Y., Suzuki, H., et al., 2003. Platelet-rich plasma contains high levels of platelet-derived growth factor and transforming growth factor-β and modulates the proliferation of periodontally related cells in vitro. J. Periodontol. 74, 849−857.

Payne, K.F., Balasundaram, I., Deb, S., Di Silvio, L., Fan, K.F., 2014. Tissue engineering technology and its possible applications in oral and maxillofacial surgery, Br. J. Oral Maxillofac. Surg., 52. pp. 7−15.

Pringle, N.P., Richardson, W.D., 1993. A singularity of PDGF alpha-receptor expression in the dorsoventral axis of the neural tube may define the origin of the oligodendrocyte lineage. Development. 117, 525−533.

Ravn, P., Overgaard, K., Spencer, E., Christiansen, C., 1995. Insulin-like growth factors I and II in healthy women with and without established osteoporosis. Eur. J. Endocrinol. 132, 313−319.

Reddi, A.H., 1998. Role of morphogenetic proteins in skeletal tissue engineering and regeneration. Nat. Biotechnol. 16, 247−252.

Richardson, R., Wright, J., Kim, J., Hausman, G., 1991. Expression of transforming growth factor-beta (TGF-beta 1) and insulin-like growth factor II (IGF-II) messenger RNA in the developing subcutaneous tissue (SQ) of the fetal pig. Growth Dev. Aging. 56, 149−157.

Richardson, T.P., Peters, M.C., Ennett, A.B., Mooney, D.J., 2001. Polymeric system for dual growth factor delivery. Nature Biotechnol. 19, 1029−1034.

Roberts, A., Sporn, M., 1990. The Transforming Growth Factor-βs. Peptide Growth Factors and Their Receptors I. Springer, Berlin Heidelberg.

Roth, D.A., Gold, L.I., Han, V.K., Mccarthy, J.G., Sung, J.J., Wisoff, J.H., et al., 1997a. Immunolocalization of Transforming Growth Factor [beta] 1,[beta] 2, and [beta] 3 and Insulin-Like Growth Factor I in Premature Cranial Suture Fusion. Plastic Reconstruct. Surg. 99, 300−309.

Roth, D.A., Longaker, M.T., Mccarthy, J.G., Rosen, D.M., Mcmullen, H.F., Levine, J.P., et al., 1997b. Studies in cranial suture biology: part I. Increased immunoreactivity for TGF-β isoforms (β1, β2, and β3) during rat cranial suture fusion. J. Bone Miner. Res. 12, 311–321.

Rutherford, R.B., Ryan, M.E., Kennedy, J.E., Tucker, M.M., Charette, M.F., 1993. Platelet-derived growth factor and dexamethasone combined with a collagen matrix induce regeneration of the periodontium in monkeys. J. Clin. Periodontol. 20, 537–544.

Rydziel, S., Shaikh, S., Canalis, E., 1994. Platelet-derived growth factor-AA and-BB (PDGF-AA and-BB) enhance the synthesis of PDGF-AA in bone cell cultures. Endocrinology. 134, 2541–2546.

Sakou, T., 1998. Bone morphogenetic proteins: from basic studies to clinical approaches. Bone. 22, 591–603.

Sánchez, A.R., Sheridan, P.J., Kupp, L.I., 2003. Is platelet-rich plasma the perfect enhancement factor? A current review. Int. J. Oral Maxillofac. Implants18.

Saygin, N.E., Tokiyasu, Y., Giannobile, W.V., Somerman, M.J., 2000. Growth factors regulate expression of mineral associated genes in cementoblasts. J. Periodontol. 71, 1591–1600.

Scharf, J., Dombrowski, F., Ramadori, G., 2001. The IGF axis and hepatocarcinogenesis. J. Clin. Pathol. 54, 138.

Schliephake, H., 2002. Bone growth factors in maxillofacial skeletal reconstruction. Int. J. Oral Maxillofac. Surg. 31, 469–484.

Sheridan, M., Shea, L., Peters, M., Mooney, D., 2000. Bioabsorbable polymer scaffolds for tissue engineering capable of sustained growth factor delivery. J. Controll. Release. 64, 91–102.

Sherris, D.A., Murakami, C.S., Larrabee, W.F., Bruce, A.G., 1998. Mandibular reconstruction with transforming growth factor-β1. Laryngoscope. 108, 368–372.

Shinbrot, E., Peters, K., Williams, L., 1994. Expression of the platelet-derived growth factor β receptor during organogenesis and tissue differentiation in the mouse embryo. Dev. Dyn. 199, 169–175.

Simmons, C.A., Alsberg, E., Hsiong, S., Kim, W.J., Mooney, D.J., 2004. Dual growth factor delivery and controlled scaffold degradation enhance in vivo bone formation by transplanted bone marrow stromal cells. Bone. 35, 562–569.

Smith, R., 1995. The effect on TGF-beta 1 on osseointegration. J. Calif. Dental Assoc. 23, 49–53.

Soriano, P., 1997. The PDGF alpha receptor is required for neural crest cell development and for normal patterning of the somites. Development. 124, 2691–2700.

Spagnoli, D.B., Marx, R.E., 2011. Dental implants and the use of rhBMP-2. Dental Clin. N. Am. 55, 883–907.

Sporn, M.B., Roberts, A.B., Wakefield, L.M., Assoian, R.K., 1986. Transforming growth factor-beta: biological function and chemical structure. Science. 233, 532–535.

Tanaka, H., Liang, C.T., 1995. Effect of platelet-derived growth factor on DNA synthesis and gene expression in bone marrow stromal cells derived from adult and old rats. J. Cell. Physiol. 164, 367–375.

Tatakis, D.N., Wikesjö, U.M., Razi, S.S., Sigurdsson, T.J., Lee, M.B., Nguyen, T., et al., 2000. Periodontal repair in dogs: effect of transforming growth factor-β1 on alveolar bone and cementum regeneration. J. Clin. Periodontol. 27, 698–704.

Thaller, S.R., Salzhauer, M.A., Rubinstein, A.J., Thion, A., Tesluk, H., 1998. Effect of insulin-like growth factor type I on critical size calvarial bone defects in irradiated rats. J. Craniofac. Surg. 9, 138–141.

Thomopoulos, S., Sakiyama-Elbert, S., Silva, M., Gelberman, R., Xia, Y., Schwartz, A., et al. 2014. Polymer nanofiber scaffold for a heparin/fibrin based growth factor delivery system. Google Patents.

Tirapegui, J., 1999. Effect of insulin-like growth factor-1 (IGF-1) on muscle and bone growth in experimental models. Int. J. Food Sci. Nutr. 50, 231−236.

Weibrich, G., Hansen, T., Kleis, W., Buch, R., Hitzler, W., 2004. Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. Bone. 34, 665−671.

Wikesjö, U.M., Sorensen, R.G., Kinoshita, A., Jian Li, X., Wozney, J.M., 2004. Periodontal repair in dogs: effect of recombinant human bone morphogenetic protein-12 (rhBMP-12) on regeneration of alveolar bone and periodontal attachment. J. Clin. Periodontol. 31, 662−670.

Wrana, J.L., 2000. Regulation of Smad activity. Cell. 100, 189−192.

Yablonka-Reuveni, Z., 1995. Development and postnatal regulation of adult myoblasts. Microsc. Res. Techniq. 30, 366−380.

Yakar, S., Liu, J.-L., Stannard, B., Butler, A., Accili, D., Sauer, B., et al., 1999. Normal growth and development in the absence of hepatic insulin-like growth factor I. Proc. Natl. Acad. Sci. 96, 7324−7329.

Yamaguchi, A., Ishizuya, T., Kintou, N., Wada, Y., Katagiri, T., Wozney, J.M., et al., 1996. Effects of BMP-2, BMP-4, and BMP-6 on osteoblastic differentiation of bone marrow-derived stromal cell lines, ST2 and MC3T3-G2/PA6. Biochem. Biophys. Res. Commun. 220, 366−371.

Yamamoto, M., Tabata, Y., Hong, L., Miyamoto, S., Hashimoto, N., Ikada, Y., 2000. Bone regeneration by transforming growth factor β1 released from a biodegradable hydrogel. J. Controll. Release. 64, 133−142.

Yang, D., Chen, J., Jing, Z., Jin, D., 2000. Platelet-derived growth factor (PDGF)-AA: a self-imposed cytokine in the proliferation of human fetal osteoblasts. Cytokine. 12, 1271−1274.

Yu, X., Hsieh, S.-C., Bao, W., Graves, D.T., 1997. Temporal expression of PDGF receptors and PDGF regulatory effects on osteoblastic cells in mineralizing cultures. Am. J. Physiol.-Cell Physiol. 272, C1709−C1716.

Yu, X., Khalil, A., Dang, P.N., Alsberg, E., Murphy, W.L., 2014. Multilayered inorganic microparticles for tunable dual growth factor delivery. Adv. Funct. Mater. 24, 3082−3093.

Zellin, G., Beck, S., Hardwick, R., Linde, A., 1998. Opposite effects of recombinant human transforming growth factor-β1 on bone regeneration in vivo: effects of exclusion of periosteal cells by microporous membrane. Bone. 22, 613−620.

Zhang, W., Wang, X., Wang, S., Zhao, J., Xu, L., Zhu, C., et al., 2011. The use of injectable sonication-induced silk hydrogel for VEGF 165 and BMP-2 delivery for elevation of the maxillary sinus floor. Biomaterials. 32, 9415−9424.

This page intentionally left blank

Part III

Oral and Dental Soft Tissue Engineering

This page intentionally left blank

Oral mucosa tissue engineering

14

Keyvan Moharamzadeh
University of Sheffield, Sheffield, United Kingdom

14.1 Introduction

Tissue-engineered human oral mucosal models have been developed since the early 1980s for various in vitro and in vivo applications. The author has previously published two comprehensive review articles (Moharamzadeh et al., 2007, 2012) outlining the strategies used for the production of three-dimensional (3D) human oral mucosal models, advances in tissue engineering of oral mucosa and the applications of the oral mucosal models developed up to 2012. This chapter contains an updated combined version of the previously published review articles on tissue-engineered human oral mucosa.

14.2 Normal human oral mucosa

Oral mucosa consists of two distinct layers; the surface epithelium is supported by a fibrous connective tissue layer, the lamina propria. In many places, the oral mucosa is attached to underlying structures by a loose connective tissue component, the submucosa (Fig. 14.1). These three layers are analogous to the epidermal, dermal, and hypodermal layers of the skin.

14.2.1 Oral epithelium

The epithelial layer of the oral mucosa is stratified squamous epithelium, which may be keratinized or nonkeratinized according to the area in the mouth. The epithelium exhibits four layers of cells, the basal layer, spinous layer, granular layer, and the superficial layer, which is known as the cornified layer in the skin and keratinized oral mucosa. Keratinization involves the transformation of viable keratinocytes in the granular layer into dead surface cells devoid of organelles and packed with dense masses of cytokeratin filaments. In nonkeratinized oral epithelium the granular layer is replaced by the intermediate layer whose cells lack keratohyaline granules. Basal layer keratinocytes are stem cells that undergo terminal differentiation as they migrate to the surface. As well as keratinocytes, oral epithelium contains intraepithelial clear cells; melanocytes, Langerhans cells, and Merkel

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00014-1
© 2017 Elsevier Ltd. All rights reserved.

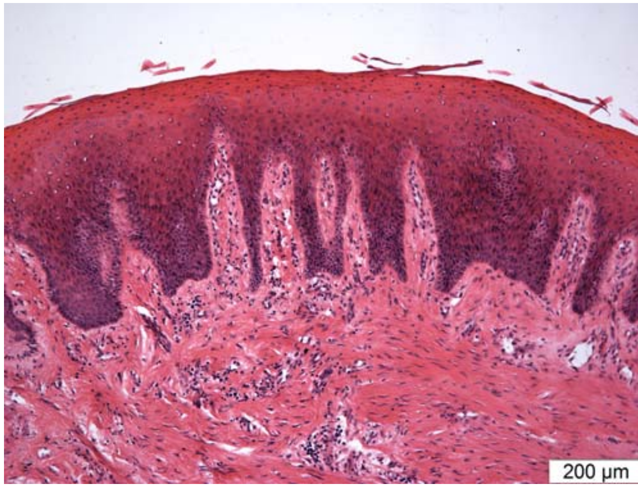


Figure 14.1 Normal human oral mucosa.

cells. Adhesion between epithelial cells is achieved by desmosomes. The basal layers are attached to the underlying lamina propria through hemidesmosomes and the basement membrane, which contains collagen type IV, laminin, and fibronectin.

Cytokeratins are intermediate filaments found in all types of epithelia and are the most fundamental markers of epithelial differentiation. Cytokeratin profile reflects both cell type and differentiation status in different types and different layers of epithelia.

14.2.2 Connective tissue

The lamina propria consists of an abundant network of type I collagen fibers and the deeper layers contain collagen type III fibers and elastic fibers in variable amounts depending upon the site. Many fibroblasts are present but only very occasional macrophages, plasma cells, mast cells, and lymphocytes are found. The lamina propria also contains vascular components, which form extensive capillary loops in the papillae between the epithelial ridges. Lymphatic vessels, nerves, and nerve endings are also present, as well as the ducts of salivary glands, whose acini are usually found in the deeper submucosa. A variable number of sebaceous glands are found in the oral cavity but are not associated with hair follicles (Atkinson et al., 2000).

14.3 Split-thickness oral mucosa engineering

In 1975 Rheinwald and Green introduced a method to grow human keratinocytes in serial culture in vitro, using a feeder layer composed of irradiated 3T3 mouse

fibroblasts and a specific culture medium called Green's medium. This method is frequently used for the culture of keratinocytes and production of single layer epithelial sheets. Several investigators have been successful in culturing sheets of oral keratinocytes without an irradiated feeder layer (Arenholt-Bindslev et al., 1987; Lauer, 1994). As already mentioned these epithelial sheets are fragile, difficult to handle, and are apt to contract. Monolayer cultures have been extremely helpful for studying the basic biology, and responses to stimuli, of both oral and skin keratinocytes and many studies have used them. However, the oral epithelium and epidermis are complex multilayer structures with cells undergoing terminal differentiation and monolayer cultures may not be a good model of what is happening in vivo. Thus the development of 3D multilayer culture system was a major breakthrough in epithelial tissue engineering and epithelial biology research.

Culturing keratinocytes on permeable cell culture membranes at the air/liquid interface permitted the construction of multilayer sheets of epithelium, which resemble native epithelium and show signs of differentiation such as basement membrane formation, different cytokeratin expression, and keratinization if the origin of the keratinocytes is keratinized mucosa (Rosdy et al., 1993; Rosdy and Clauss, 1990). A commercially available in vitro model of oral epithelium, developed by Skinethic laboratories (Nice, France), consisted of a 3D, multilayer culture of the human TR146 keratinocyte cell line on polycarbonate cell culture inserts. Since the cells are derived from an oral squamous cell carcinoma cell line, this tissue model does not fully differentiate but does form a nonkeratinizing oral epithelial model that has been extensively used for biocompatibility and other studies. SkinEthic's other product, gingival epithelium, which was produced by air−liquid interface culture of normal gingival keratinocytes, produced a keratinized stratified squamous epithelium similar to normal gingival epithelium. EpiOral and EpiGingival were developed by MatTek Corp. (NJ, Uniterd States). These are 3D reconstructs of human oral (buccal) and gingival epithelium that form multilayer, stratified nonkeratinized and keratinized oral epithelium, respectively, that exhibit in vivo-like morphological and growth characteristics. Both tissues express cytokeratin K13 and weakly express cytokeratin K14. The tissues also produce naturally occurring antimicrobial peptides including human beta defensins.

14.4 Full-thickness oral mucosa engineering

An ideal full-thickness engineered oral mucosa that resembles normal oral mucosa consists of:

1. A *lamina propria*, which consists of a 3D scaffold infiltrated by fibroblasts producing extracellular matrix (ECM). This structure can be achieved by seeding oral fibroblasts into a porous biocompatible scaffold and long-term culturing in a fibroblast differentiation medium (Berthod et al., 1993; Black et al., 2005). Possible difficulties at this stage include: poor fibroblast infiltration due to the lack of porosity, the shrinkage of the scaffold if a large number of fibroblasts are seeded, and fast biodegradation of the scaffold.

It has been shown that fibroblasts cultured in 3D porous scaffolds produce significantly higher levels of ECM than fibroblasts in monolayers (Berthod et al., 1993). Newly synthesized collagen in 3D cultures of fibroblasts can be characterized by transmission electron microscopy. Fibroblasts play an important role in epithelial morphogenesis, keratinocyte adhesion, and the formation of the complex dermal-epithelial junction (Saintigny et al., 1993). The epithelial phenotype and keratin expression is extrinsically influenced by the nature and origin of the underlying fibroblasts (Okazaki et al., 2003) and the mesenchymal substrate (Merne and Syrjanen, 2003). It has been shown that without fibroblasts in the matrix, the epithelium ceases to proliferate (Fusenig, 1994) while differentiation continues (Smola et al., 1998). The significance of fibroblasts has also been shown by an experiment in which degenerative vacuolization was seen in cocultures grown in the absence of fibroblasts. The use of oral buccal and vaginal fibroblasts led to a nonkeratinized epithelium, in contrast to cultures with skin fibroblasts, which showed slight parakeratinization (Atula et al., 1997). Thus fibroblasts may influence the differentiation potential of the epithelium towards that found at the site of origin of the fibroblasts.

2. A continuous basement membrane separating the lamina propria and the epithelium. The basement membrane can be characterized by transmission electron microscopy showing lamina lucida, lamina densa, and anchoring fibers. Immuno-staining for basement membrane antigens such as collagen type IV, laminin, fibronectin, integrins, and bullous pemphigoid antigen is also a useful characterization method (Black et al., 2005).
3. A stratified squamous epithelium on the basement membrane, including densely packed keratinocytes that undergo differentiation as they migrate to the surface. This is achieved by growing oral keratinocytes at the air−liquid interface in a chemically defined medium, which contains keratinocyte growth factors such as epidermal growth factor (EGF) (Ophof et al., 2002; Bhargava et al., 2004; Izumi et al., 1999; Rouabhia and Deslauriers, 2002; Moriyama et al., 2001). Significant issues that need to be addressed when growing multilayer epithelial constructs on connective tissue substrates include keratinocyte invasion into the connective tissue layer and poor differentiation of the epithelium.

To tackle these problems and optimize the construction of full-thickness oral mucosa many factors have to be considered. These include the choice of (A) scaffold, (B) the cell source, and (C) the culture environment.

14.4.1 Scaffolds

The most important element in oral mucosa and skin reconstructs is the scaffold that supports the cells. Choosing the right scaffold with ideal biocompatibility, porosity, biostability, and mechanical properties is a crucial step in tissue engineering.

Scaffolds used in oral mucosa and skin reconstruction fall into several different categories: (1) naturally derived scaffolds such as acellular dermis and amniotic membrane, (2) fibroblast-populated skin substitutes, (3) collagen-based scaffolds, (4) gelatin-based scaffolds, (5) fibrin-based materials, (6) synthetic scaffolds such as polymers, and (7) hybrid scaffolds, which are combination of natural and synthetic matrices.

14.4.1.1 Naturally derived scaffolds

14.4.1.1.1 Acellular dermis

Acellular cadaveric dermis (AlloDerm) was used by Izumi et al. (1999) as a scaffold for tissue engineering of oral mucosa. AlloDerm is an acellular, nonimmunogenic cadaveric human dermis (Rennekampff et al., 1997) that has a polarity by which one side has a basal lamina suitable for epithelial cells and the other side has intact vessel channels suitable for fibroblasts (Livesey et al., 1995).

Deepidermalized dermis (DED) has been extensively utilized for the preparation of human epidermal-dermal composites (Ghosh et al., 1997; Herson et al., 2001; Chakrabarty et al., 1999; Lee et al., 2000; Ralston et al., 1999) and also for in vitro reconstruction of human hard palate mucosal epithelium (Cho et al., 2000). Deepithelialized bovine tongue mucosa has also been used as a substrate for keratinocyte culture in vitro (Hildebrand et al., 2002). DED is prepared from split-thickness skin by removing the epidermis and dermal fibroblasts from the dermis. The advantages that have made the DED a popular scaffold are: good durability and reduced antigenicity, ability to retain its structural properties, even after freezing, lyophilization, and preservation in glycerol (Heck et al., 1985; Krejci et al., 1991; McKay et al., 1994; Ghosh et al., 1997). It has been shown that oral mucosal substitutes composed of oral keratinocytes cultured on skin-derived substrates (DED or AlloDerm) show histological and immunohistochemical characteristics very close to normal oral mucosa (Ophof et al., 2002). Ex vivo-produced oral mucosa equivalent has been produced with autologous oral keratinocytes seeded on acellular dermis such as AlloDerm without incorporation of fibroblasts. This may be a potential way for tissue engineering or oral mucosa with the advantages of growing keratinocytes in a chemically defined culture precluding serum or any animal-derived constituents which makes this method consistent with FDA's regulatory guidelines for clinical trial (Izumi et al., 2003a; Izumi et al., 2013). However, this technique may be considered suboptimal for many reasons. First, from a development viewpoint, the epithelial−mesenchymal interaction is crucial in oral mucosa morphogenesis and complete maturation and differentiation of the epidermal layer (El Ghalbzouri et al., 2002; Liu et al., 2011). Second, there is evidence indicates that the unique scarless healing of oral mucosa may be attributed to the oral fibroblasts phenotype and their role in collagen remodeling and growth factors secretion as well as to other mediators that aid in rapid resolution of inflammatory phase (Enoch et al., 2008; Glim et al., 2013; Mak et al., 2009). Third, even when fibroblasts were incorporated in different synthetic or natural matrix, poor fibroblast infiltration has been observed due to lack of matrix porosity which is essential for cells migration (Moharamzadeh et al., 2008b).

14.4.1.1.2 Amniotic membrane

The possibility of using human amniotic membrane as a substrate for culturing oral epithelial cells and its suitability for ocular surface reconstruction in rabbit has been

examined by Nakamura et al. (2003). They developed and characterized an engineered oral epithelium with numerous desmosomes and attached to a basement membrane with hemidesmosomes that was able to express keratins 3, 4, and 13.

14.4.1.2 Fibroblast-populated skin substitutes

Fibroblast-populated scaffolds include several commercially available living skin equivalents. Dermagraft, developed by Advanced Tissue Sciences Inc., is a dermal substitute composed of a biodegradable polymer mesh populated with dermal fibroblasts (Purdue, 1997). Another product, Apligraf(Graft skin), developed by organogenesis, is a composite graft composed of allogenic keratinocytes grown on a fibroblast-populated bovine collagen gel (Gentzkow et al., 1996; Eaglstein et al., 1995). Other living skin substitutes include Orcel (Ortec International Inc.), Polyactive (HC Implants), and Hyalograf 3D™ (Fidia Advanced Biopolymers). The fibroblasts within these scaffolds proliferate and produce ECM and growth factors within 2−3 weeks and create a dermis like matrix (Gentzkow et al., 1996).

14.4.1.3 Collagen-based scaffolds

14.4.1.3.1 Pure collagen scaffolds

In 1996, Masuda developed the first in vitro full-thickness oral mucosal model by seeding cultured normal gingival keratinocytes on contracted bovine skin collagen gels (CCG) containing fibroblasts and coculturing in a reconstruction medium at an air−liquid interface for 10 days. They obtained a well-differentiated mucosal model, which was histologically similar to native tissues. Moriyama et al. (2001) modified this method and developed a composite cultured oral mucosa utilizing an atelopeptide type I collagen sponge matrix with CCG. Their mucosal model was composed of (1) a lamina propria in which fibroblasts were embedded in CCG and a honeycomb structured collagen sponge and (2) stratified epithelial cell layers on the surface of the cultured lamina propria. The benefit of this model is that the collagen gel supports fibroblasts, which provides a suitable membrane for keratinocyte multilayer formation, and prevents epithelial cell invasion and island formation in the connective tissue layer (MacCallum and Lillie, 1990). Laminin expression was detected between the epithelium and lamina propria in Moriyama's model. However, type IV collagen expression and hemidesmosome-like structure was not recognizable. Furthermore, the fibroblasts embedded in collagen gel synthesized little ECM compared to 3D porous scaffolds (Berthod et al., 1993). Rouabhia and Deslauriers (2002) produced and characterized an in vitro engineered human oral mucosa using bovine skin collagen. Their method consisted of mixing bovine skin collagen with normal human oral fibroblasts to produce engineered lamina propria and then seeding oral epithelial cells on this matrix and growing them at an air−liquid interface. In their mucosal model, epithelial cells expressed the proliferation marker Ki-67 as well as cytokeratins K14, K19, and the differentiation marker

cytokeratin K10. Keratinocytes interacted with fibroblasts by secreting basement membrane proteins (laminins) and by expressing integrins (β1 and α2β1). They also showed that the engineered oral mucosa was able to secrete interlukins (IL-1β and IL-8), tumor necrosis factor alpha (TNF-α), and different metalloproteinases such as gelatinase-A and gelatinase-B. As a scaffold, collagen matrix is very biocompatible, but it biodegrades rapidly and has poor mechanical properties. Cross-linking of the collagen-based scaffolds is an effective method to improve biostability and mechanical properties (Ma et al., 2003). However, cross-linking of collagen-based tissues enhances the tendency to calcify, which is not desirable in clinical situations (Nimni, 1995).

Fibroblast-populated collagen gel has also been recently used for tissue engineering of 3D combined bone-oral mucosal models with promising results (Almela et al., 2016; Bae et al., 2014). Although histological appearance of the engineered connective tissue based on fibroblast-populated collagen gel highly resembles the native tissue, poor mechanical properties and the lack of suturability of the collagen gel limits its use for clinical transplantation.

14.4.1.3.2 Compound collagen scaffolds

Several compound collagen-based matrices have been introduced in order to improve the function of the scaffolds for tissue engineering. These include: collagen-chitosan scaffold (Ma et al., 2003), collagen-elastin membrane (Hafemann et al., 1999), collagen-glucosaminoglycan (C-GAG) matrix (Ojeh et al., 2001), and collagen-GAG-chitosan (CGC) scaffolds (Vaissiere et al., 2000; Black et al., 2005; Moharamzadeh et al., 2008b). Chitosan is a naturally occurring substance that is chemically similar to cellulose and is derived from chitin, a polysaccharide found in the exoskeleton of shellfish like shrimp or crabs. Chitosan functions as a bridge to increase the cross-linking efficiency of glutaraldehyde due to the longer chain of amino groups. Glycosaminoglycans are essential components of the ECM, composed of long nonbranched polymers of repeating disaccharide units, one of which is an amino sugar. The sources of GAGs include shark cartilage, bovine trachea, and pork cartilage. GAGs such as chondroitin sulfate and hyaluronic acid are hydrophilic, attracting large amounts of water and form hydrated gels, enabling the rapid diffusion of water-soluble molecules (Atkinson et al., 2000). Fibroblasts grown within the CGC sponge express a significantly increased collagen synthesis, compared with fibroblasts embedded in a collagen gel and monolayer culture of fibroblasts (Berthod et al., 1993).

14.4.1.4 Gelatin-based scaffolds

Gelatin-based materials such as gelatin–glucan (Lee et al., 2003), gelatin–hyaluronate (Choi et al., 1999a), and gelatin–chitosan–hyaluronic acid (Mao et al., 2003) matrices have been developed for skin tissue engineering. The denatured type of collagen, gelatin is nonantigenic, fibroblast attractant, and macrophage

activator and promotes epithelialization and granulation tissue formation (Choi et al., 1999a, 1999b, 2001; Hong et al., 2001). Glucan is antibacterial, antiviral, and anticoagulant and promotes wound healing activity (Douwes, 2005). Hyaluronic acid is added to improve the biological and mechanical properties of these scaffolds (Mao et al., 2003).

14.4.1.5 Fibrin-based scaffolds

Fibrin matrix has been used for in vitro construction of new human cartilage, skin, and bone (Ruszymah, 2004). Bioseed, developed by BioTissue Technologies, is a skin substitute, composed of fibrin sealant with cultured autologous human keratinocytes. Fibrin glue matrix gives sufficient adherence stability to the grafted keratinocytes in an actively proliferating state. Further advantages are easy repetition and grafting, as well as a reduction in operating time and costs (Kaiser et al., 1994). Extracellular protein-based scaffolds such as fibrin and plasma have been used to construct complete autologous oral mucosa equivalent and the results showed reasonable outcome for intraoral grafting with good handling characteristics and no contraction (Peña et al., 2012, 2010; Llames et al., 2014). However, the epithelium showed only monolayer keratinocytes and negative expression of the proliferation marker, ki67 which indicates to a poor differentiation.

As already mentioned, natural materials possess many advantages that have made them popular as scaffolds for tissue engineering. However, these materials have some disadvantages. Many of these materials are isolated from human or animal tissue and are not available in large quantities. They suffer from large batch-to-batch variations and are typically expensive. Additionally, these materials exhibit a limited range of physical properties. These drawbacks have led some researchers to consider using synthetic materials to fabricate matrices for use in tissue engineering of skin and oral mucosa.

14.4.1.6 Synthetic scaffolds

Polycarbonate permeable membranes are used in commercially available partial-thickness epithelial models (SkinEthic and MatTek tissue models). Successful use of a biodegradable segmented copolymer of poly (ethyleneglycolterephthalate)−poly (butylenes terephthalate) (PEGT/PBT) in skin tissue engineering have been reported (El-Ghalbzouri et al., 2004). This synthetic scaffold has good mechanical properties and there is no risk of transmitting disease. However, incorporation of fibroblast-populated collagen or fibrin into the pores of the scaffold is required for better results. Porous polylactic glycolic acid scaffold has also been used to construct a lining mucosa in a tissue-engineered prosthesis mucosa for replacement of a tracheal defect (Kim et al., 2004). A dermal scaffold composed of knitted poly (lactic-co-glycolic acid: PLGA) (10:90)-poly (-caprolactone) mesh has shown superior results in terms of cell distribution and tissue formation compared to three

natural scaffolds including equine collagen foam, AlloDerm, and Chitosan (Ng et al., 2004).

Biodegradable electrospun polymer scaffolds have been used for oral mucosa tissue engineering (Selim et al., 2011; Moharamzadeh et al., 2008b). The electrospun polymer structure allows optimal fibroblast infiltration into the scaffold. However, the epithelial invasion into the connective tissue layer can also occur if the scaffold has large pore size on the epithelial side. Bilayer and trilayer electrospun scaffolds with compact epithelial surface and more porous connective tissue layer have been fabricated to allow favorable fibroblast infiltration from the connective tissue side and prevent keratinocyte invasion from the epithelial side (Bye et al., 2013).

Further investigations are underway using 3D-printing technology to develop and optimize 3D-printed synthetic scaffolds for oral mucosa tissue engineering.

14.4.1.7 Hybrid scaffolds

A skin substitute based on a semi-synthetic scaffold made of benzyl ester of hyaluronan (HYAFF and Laserskin) has been developed. This scaffold has good in vitro and in vivo biocompatibility and controlled biodegradability (Zacchi et al., 1998). A hybrid scaffold of PLGA-collagen has been used for dermal tissue engineering but it has shown more contraction compared to collagen-hyaluronic acid foam (Ng et al., 2005).

In a study comparing three different types of dermal scaffolds, a more efficient connective tissue formation was observed using a compression molded/salt leached PEGT/PBT copolymer in comparison to lyophilized crosslinked collagen, and collagen-PEGT/PBT hybrid scaffolds. It was also shown that the thickness, porosity, and interconnecting pore size are important parameters in the ability of synthetic scaffolds to control connective tissue formation (Wang et al., 2005).

14.4.2 Cells

Another important factor that must be considered in oral mucosa and skin reconstruction is the type and origin of fibroblasts and keratinocytes. Fibroblasts are usually isolated from the connective tissue layer of the skin or oral mucosal biopsy and are used in early passages for tissue engineering because the ECM production by dermal fibroblasts decreases as the passage number increases (Takeda et al., 1992; Khorramizadeh et al., 1999). Normal oral keratinocytes can be obtained from different sites of the oral cavity such as hard palate (Cho et al., 2000), gingival (Yoshizawa et al., 2004), or buccal mucosa (Bhargava et al., 2004). Normal human keratinocytes should also be used in very early passages but immortalized human keratinocytes such as HaCaT cells (Boelsma et al., 1999) or TR146 tumor-derived cells (Schmalz et al., 2000) can be used in extended passages in reconstruction of oral mucosal test models. However, epidermal differentiation of transformed keratinocytes is not perfect, as the ultimate steps of terminal differentiation do not occur

(Boelsma et al., 1999) and tumor-derived cells are not normal and not viable for clinical use. Immortalized human oral keratinocyte cell line (OKF6/TERT-2) has been cultured on fibroblast-populated collagen gel to produce 3D tissue-engineered oral mucosal models (Dongari-Bagtzoglou and Kashleva, 2007). The use of this cell line resulted in a multilayer epithelial structure organized similarly to the cells in native oral mucosa with presence of a stratum basale, having one layer of columnar to round cells, a relatively flattened stratum spinosum and stratum granulosum, and a nonkeratinizing stratum corneum.

14.4.3 Culture environment

Commonly used culture medium for oral mucosa reconstruction is Dulbecco's modified Eagle medium (DMEM)-Ham's F-12 medium (3:1), supplemented with fetal calf serum, glutamine, EGF, hydrocortisone, adenine, insulin, transferrin, triiodothyronine, fungizone, penicillin, and streptomycin.

Izumi et al. (2000) developed and characterized a tissue-engineered human oral mucosal equivalent using a serum-free culture method. In this study they eliminated the use of serum and irradiated mouse fibroblast feeder layers in order to minimize the exposure of human graft recipients to xenogenetic DNA or slow viruses that might be present in irradiated mouse 3T3 cells and serum, respectively. Yoshizawa et al. (2004) employed the same technique to produce human conjunctiva and oral mucosa equivalents.

It has been demonstrated that perfusion of keratinocytes with medium further enhances cell viability and proliferation of oral keratinocytes cultured in a porous 3D matrix of collagen-GAG cross-linked with glutaraldehyde (Navarro et al., 2001).

Serum-free culture medium with low calcium has been used to culture basal keratinocytes. Various procedures have been used to induce epithelial keratinocyte differentiation in 3D cultures including the addition of calcium at mM concentration, concomitant addition of serum and calcium, and lowering the incubation temperature of the cells. It has been demonstrated that a synergic stimulation by calcium, serum, confluence, and lower incubation temperature amplified the differentiation rate (Borowiec et al., 2013).

14.5 Applications of tissue-engineered oral mucosa

There are generally two major applications for tissue-engineered oral mucosa: (1) clinical applications and (2) as in vitro test systems and models. It is important to realize that tissue engineering approaches are different for each purpose. As an example, for clinical applications such as grafting, transplantation, and guided tissue regeneration a biodegradable scaffold with optimal mechanical properties is desirable because it will be replaced by the host tissue and it must resist natural forces in the oral cavity, while a nonbiodegradable scaffold may result in a foreign body reaction. Also transmission of infection and tissue rejection are major issues.

Tumor derived or virally transformed cell lines are precluded from clinical use and high standards of tissue production and quality control are essential. However, these are less of a problem for an in vitro test model. Indeed the need for reproducibility and lack of batch-to-batch variability may make the use of tumor and virally transformed cell lines desirable in this case. The scaffold should have maximum biostability to maintain its structure throughout the testing procedure. Depending on the biological endpoint, both epithelium only and full-thickness oral mucosal models may have a use for in vitro tests.

14.5.1 In vivo clinical applications

14.5.1.1 Intraoral grafting

Compared to the transplantation of autologous keratinocytes alone, full-thickness engineered mucosa grafting results in better and faster wound healing of oral tissues. Long-term clinical follow-up of transplanted engineered oral mucosa has established this technique as an excellent additional tool in oral and maxillofacial surgery (Lauer and Schimming, 2002).

Full-thickness engineered human oral mucosa can be used in periodontal peri-implant reconstruction and maxillofacial reconstructive surgery. Izumi et al. (2003b) reported a 100% take rate with intraoral grafting of engineered oral mucosa. In their study the engineered grafts showed clinical changes indicating vascular ingrowth and had cytologic evidence of the persistence of grafted cultured keratinocytes on the surface. The graft enhanced the maturation of the underlying submucosal layer associated with rapid epithelial coverage. In vitro labeling of cultured and subsequently grafted gingival keratinocytes have shown that the transplanted keratinocytes integrate into the newly formed mucosal epithelium (Lauer and Schimming, 2001). It has also been reported that the presence of an intact and viable epithelium influences secondary in vivo remodeling within the connective tissue layer of transplanted engineered oral mucosa by synthesis and release of cytokines, enzymes, and growth factors (Izumi et al., 2003c).

Treatment of patients with cleft palate is often complicated due to the shortage of oral soft tissue and the presence of scarred tissue from previous surgeries. Tissue-engineered palatal mucosa have been developed for potential use in treatment of patients with cleft palate (Ophof et al., 2008; Luitaud et al., 2007a). Cryopreservation of labial mucosa harvested during initial lip surgery has been reported to be a useful approach to preserve keratinocytes for reconstruction of the mucosa at a later stage (Xiong et al., 2010).

Tissue-engineered oral mucosa has also been employed in the field of periodontal plastic surgery for the treatment of patients with inadequate attached gingiva (Rouabhia and Allaire, 2010). In a randomized clinical trial, cultured gingival grafts on biodegradable scaffolds have been shown to be safe and capable of generating keratinized attached gingival tissue (Mohammadi et al., 2007). The same group reported successful application of tissue-engineered gingival graft for regeneration of peri-implant gingival tissue at a lower premolar implant with insufficient

attached keratinized gingiva (Mohammadi et al., 2011). It has been shown that the presence of elastin in the connective tissue layer of the engineered oral mucosa modulates the keratinization of the overlying epithelium (Hsieh et al., 2010). A tissue-engineered bilayered live cell therapy was examined as an alternative to free gingival grafts in subjects with oral mucosal defects. In all treated cases, all sites healed uneventfully, with complete epithelialization by 14 days postoperative and no significant adverse events. The treated sites matched the surrounding tissues in both texture and color (Nevins, 2010).

Ex vivo production of large size oral mucosa equivalent up to 15 cm^2 was successfully achieved by Kato et al. (2015) through optimizing the cell seeding density of oral keratinocytes and thickness of Alloderm to about 5.3×10^5 cells/cm^2 and 508.0 μm, respectively. The feasibility of OM fabrication in such reproducible way enables for using it in treatment of various congenital and acquired intraoral defects.

Hemifacial microsomia and ankyloglossia is an example of facial congenital anomaly that has been treated by grafting of autologous plasma-based engineered OM and yields a satisfactory outcome in pediatric patient. However, postoperative wound shrinkage and recurrence of ankyloglossia entailed repetition of grafting procedure (Llames et al., 2014). Likewise, cleft palate could be augmented by full-thickness palatal OM based on collagen matrix (Luitaud et al., 2007b).

Good clinical results have also been achieved using OM equivalent in both minor and extensive oral reconstructive surgery. The efficacy, safety, rapid integration, and vascularization of ex vivo-produced OM used for augmenting mucogingival defects was successfully demonstrated by clinical trial (Izumi et al., 2013). An extensive intraoral defects, on the other hand, such as mandibular defect following segmental mandibulectomy due to carcinoma or extreme atrophic maxilla showed an optimal functional and esthetic outcomes following treatment by fibula flap prelaminated with tissue-engineered oral mucosa (Sieira Gil et al., 2015).

14.5.1.2 Extraoral grafting

Compared to skin, the oral mucosa exhibits rapid healing, reduced scarring, resistance to infection and does not contain dermal appendages such as hair follicles or sweat glands. This means that although it is not available in the same quantities as skin, it has more desirable properties for grafting and replacement of lost epithelia in a number of extraoral applications.

Tissue-engineered oral mucosa equivalents have been developed for extraoral applications. Bhargava et al. (2004) reported successful culture of a full-thickness tissue-engineered buccal mucosa based on DED with good mechanical properties for substitution urethroplasty. Tissue-engineered buccal mucosa is considered the gold standard for substitution urethroplasty in patients with long urethral strictures and has been extensively investigated by several research groups in the past decade (Morey, 2005; Patterson et al., 2011; Bhargava et al., 2008; Osman et al., 2014; Xie et al., 2014). However, some concerns are raised about stricture recurrence and fibrosis which may attributed to the contractive nature of fibroblasts or the matrix on which they cultured (Osman et al., 2015).

The application of a tissue-engineered human oral mucosal equivalent based on an acellular allogenic dermis for the treatment of a burn wound with satisfactory outcome has been reported (Iida et al., 2005).

Autologous transplantation of cultivated oral epithelium on human amniotic membrane has been suggested as a feasible method for ocular surface reconstruction (Nakamura et al., 2003). Yoshizawa et al. (2004) developed and characterized human conjunctiva and oral mucosa equivalents and suggested their use as graft materials for eyelid reconstruction.

Oral mucosa epithelia transplant has been found very useful in enhancing reepithelization and minimize inflammation in sever burned cornea, however, the long-term graft survival is largely depends on progenitor stem cells in the basal layer (Chen et al., 2009).

Esophageal stenosis is one of the major complications of aggressive endoscopic resection. In animal experimental models, tissue-engineered oral mucosal grafts were effective in promoting reepithelialization and suppressing inflammation which causes esophageal scarring and stenosis after endoscopic submucosal dissection (Takagi et al., 2010; Yang and Soetikno, 2007). The suitability of oral mucosa epithelial sheet was also investigated for treatment of esophageal ulcer after endoscopic submucosal dissection and the findings indicated to the possibility of using epithelial sheet to promote wound healing and reepithelialization and prevent esophageal stenosis (Takagi et al., 2011).

Although encouraging progress has been made to date, commercializing engineered oral mucosa for clinical applications is restricted due to the limitations in manufacturing facilities, governmental regulation issues (Ram-Liebig et al., 2015), and low profitability. Since the best cells for the patients are their own cells, using allogenic cells is not desirable. Especially when most of the indications for many engineered oral mucosal grafts are not as urgent as the use of skin substitutes in life-threatening situations such as extensive burns. Therefore tissue-engineered oral mucosa for clinical applications can only be sold as a service with limited profitability.

14.5.2 In vitro applications

In vitro applications of 3D oral mucosal models include biocompatibility testing and oral biology studies such as oral disease modeling. Oral mucosal biocompatibility testing has been discussed below and the oral disease modeling will be discussed separately in Chapter 16, Periodontal soft tissue reconstruction.

14.5.2.1 Oral mucosal biocompatibility testing

Conventional cytotoxicity assays use monolayer cultures of cells, either monocultures or cocultures. However, it is increasingly recognized that these assays are not particularly physiologically relevant. It has also been shown that when experiments are moved into 3Ds there is often a cytoprotective effect observed with TC50 values higher for 3D models than the traditional 2D models (Sun et al., 2006).

Commercially available 3D oral mucosal biocompatibility test models have been used to assess the biological effects of different types of dental materials including bonding adhesives (Vande Vannet and Hanssens, 2007), orthodontic wires (Vande Vannet et al., 2007), and other metals used in dentistry such as nickel (Trombetta et al., 2005). MatTek's split-thickness 3D buccal mucosal model (EpiOral) has been used to investigate the influence of ethanol and ethanol-containing mouthwashes on permeability of oral mucosa in vitro (Koschier et al., 2011). In our previous studies a full-thickness 3D human oral mucosal model was developed and characterized for biocompatibility assessment of dental materials (Moharamzadeh et al., 2008b). The use of this model permitted biocompatibility testing of experimental dental composite resins in a direct contact format with the surface of the engineered oral mucosa (Moharamzadeh et al., 2008a). This test set-up highly resembled the clinical situation and provided useful and relevant information on the interaction of the oral mucosa with resin-based dental materials with different monomer compositions.

A similar model was used for biological evaluation of alcohol-containing antiseptic mouthwashes (Moharamzadeh et al., 2009). In this study the use of a 3D model allowed a wide range of biological endpoints to be recorded including basic histology, the Alamar Blue and MTT tissue viability assays, transmission electron microscopy analysis of the mucosa and the measurement of release of the proinflammatory cytokine IL-1β. Histology enabled visualization and direct assessment of toxicity and damage to the epithelium by the test agent, which was quantified using tissue viability assays. The TEM allowed us to detect any alteration to the epithelium, the basement membrane apparatus and the connective tissue layer in an ultrastructural scale.

Several other studies have indicated the use of engineered oral mucosal models based on collagen membranes and synthetic polymers as in vitro test models to evaluate biological effects of biomaterials. Mostefaoui et al. (2002) using a reconstructed human oral mucosal model on a bovine collagen membrane, examined the effects of dentifrices on tissue structure and proinflammatory mediator released by epithelial cells. Schmalz et al. studied mucosal irritancy of metals used in dentistry by introducing these materials onto 3D fibroblast-keratinocyte coculture on nylon mesh (1997) and also a 3D culture of TR146 cells grown on polycarbonate filters (2000). Similar epithelial model has been used by several investigators to evaluate the effects $HgCl_2$ (Khawaja et al., 2002) and different surfactants (Hagi-Pavli et al., 2004; Lundqvist et al., 2002) on epithelial viability and cytokine release from the epithelium.

These in vitro models seem promising for mucotoxicity evaluation of dental biomaterials since they reflect the clinical situation better than single layer cell culture test models. Therefore they can reduce the need for animal testing and be more specific. Furthermore, such models allow investigators to study multiple responses of the epithelium or mucosa to different stimuli. This is particularly valuable in testing responses to different biomaterials, oral healthcare products as well as in studies to investigate the response of the oral epithelium or mucosa to bacteria and other disease processes.

3D tissue-engineered oral mucosal model has also been developed for the purpose of investigation of the implant−soft tissue interface (Chai et al., 2010). This

model consisted of both epithelium and connective tissue layers. Further experiments (Chai, Brook, Emanuelsson, et al., 2012) showed there was evidence of hemidesmosome-like structures formed at the implant−oral mucosa interface under transmission electron microscopy examination. Besides qualitative analysis of the implant−soft tissue interface, this oral mucosal model also allowed quantitative analysis of the biological seal of the Ti-oral mucosa interface based on permeability and attachment tests (Chai, Brook, Palmquist, et al., 2012) as well as the analysis of the contour of soft tissue attached to the implant (Chai et al., 2013).

This newly developed model provides more useful information than the monolayer cell culture systems for the investigation of the implant−soft tissue interface. The 3D model provides a wide range of information that it would otherwise only be possible to be obtained from animal experiments. The soft tissue response to various aspects of implant surfaces such as the implant materials, surface topography, chemical composition, and surface geometry could be evaluated using this in vitro model. Nevertheless, it must be appreciated that the oral mucosal model is not yet able to fully substitute for the in vivo situation.

14.6 Future trends

Significant progress has been made in tissue engineering of human oral mucosa for various in vitro and in vivo applications in the past decades. Developments in oral mucosa tissue engineering seem to follow advances in skin technology in terms of scaffold fabrication and engineering strategies. 3D bioprinting technology has been used for skin engineering in recent years and has demonstrated the advantages of being more flexible, reproducible, resembling the native skin morphologically and biologically, and enabling simultaneous incorporation of various cell types (Lee et al., 2014; Rimann et al., 2016; Koch et al., 2012). Due to the structural similarity between skin and oral mucosa, this technique could be translated to generate oral mucosal equivalents in vitro.

References

Almela, T., Brook, I.M., Moharamzadeh, K., 2016. Development of three-dimensional tissue engineered bone-oral mucosal composite models. J. Mater. Sci. Mater. Med. 27, 65.

Arenholt-Bindslev, D., Jepsen, A., Maccallum, D.K., Lillie, J.H., 1987. The growth and structure of human oral keratinocytes in culture. J. Invest. Dermatol. 88, 314−319.

Atkinson, M.E., Jowett, A., White, F.H., 2000. Principles of Anatomy and Oral Anatomy for Dental Students. Cava Cadavers, Taddington.

Atula, S., Grenman, R., Syrjanen, S., 1997. Fibroblasts can modulate the phenotype of malignant epithelial cells in vitro. Exp. Cell Res. 235, 180−187.

Bae, S., Sun, S., Aghaloo, T., Oh, J.E., Mckenna, C.E., Kang, M.K., et al., 2014. Development of oral osteomucosal tissue constructs in vitro and localization of

fluorescently-labeled bisphosphonates to hard and soft tissue. Int. J. Mol. Med. 34, 559–563.

Berthod, F., Hayek, D., Damour, O., Collombel, C., 1993. Collagen synthesis by fibroblasts cultured within a collagen sponge. Biomaterials. 14, 749–754.

Bhargava, S., Chapple, C.R., Bullock, A.J., Layton, C., Macneil, S., 2004. Tissue-engineered buccal mucosa for substitution urethroplasty. BJU Int. 93, 807–811.

Bhargava, S., Patterson, J.M., Inman, R.D., Macneil, S., Chapple, C.R., 2008. Tissue-engineered buccal mucosa urethroplasty-clinical outcomes. Eur. Urol. 53, 1263–1269.

Black, A.F., Bouez, C., Perrier, E., Schlotmann, K., Chapuis, F., Damour, O., 2005. Optimization and characterization of an engineered human skin equivalent. Tissue Eng. 11, 723–733.

Boelsma, E., Verhoeven, M.C., Ponec, M., 1999. Reconstruction of a human skin equivalent using a spontaneously transformed keratinocyte cell line (HaCaT). J. Invest. Dermatol. 112, 489–498.

Borowiec, A.S., Delcourt, P., Dewailly, E., Bidaux, G., 2013. Optimal differentiation of in vitro keratinocytes requires multifactorial external control. PLoS One. 8, e77507.

Bye, F.J., Bissoli, J., Black, L., Bullock, A.J., Puwanun, S., Moharamzadeh, K., et al., 2013. Development of bilayer and trilayer nanofibrous/microfibrous scaffolds for regenerative medicine. Biomater. Sci. 1, 942.

Chai, W., Brook, I., Emanuelsson, L., Palmquist, A., van Noort, R., Moharamzadeh, K., 2012. Ultrastructural analysis of implant-soft tissue interface on a three-dimensional tissue-engineered oral mucosal model. J. Biomed. Mater. Res. A 100, 269–77.

Chai, W.L., Brook, I.M., Palmquist, A., van Noort, R., Moharamzadeh, K., 2012. The biological seal of the implant-soft tissue interface evaluated in a tissue-engineered oral mucosal model. J. R. Soc. Interface. 9, 3528–3538.

Chai, W.L., Moharamzadeh, K., Brook, I.M., Emanuelsson, L., Palmquist, A., van Noort, R., 2010. Development of a novel model for the investigation of implant-soft tissue interface. J. Periodontol. 81, 1187–1195.

Chai, W.L., Moharamzadeh, K., van Noort, R., Emanuelsson, L., Palmquist, A., Brook, I.M., 2013. Contour analysis of an implant–soft tissue interface. J. Periodontal. Res. 48, 663–670.

Chakrabarty, K.H., Dawson, R.A., Harris, P., Layton, C., Babu, M., Gould, L., et al., 1999. Development of autologous human dermal-epidermal composites based on sterilized human allodermis for clinical use. Br. J. Dermatol. 141, 811–823.

Chen, H.-C.J., Chen, H.-L., Lai, J.-Y., Chen, C.-C., Tsai, Y.-J., Kuo, M.-T., et al., 2009. Persistence of transplanted oral mucosal epithelial cells in human cornea. Invest. Ophthalmol. Visual Sci. 50, 4660.

Cho, K.H., Ahn, H.T., Park, K.C., Chung, J.H., Kim, S.W., Sung, M.W., et al., 2000. Reconstruction of human hard-palate mucosal epithelium on de-epidermized dermis. J. Dermatol. Sci. 22, 117–124.

Choi, Y.S., Hong, S.R., Lee, Y.M., Song, K.W., Park, M.H., Nam, Y.S., 1999a. Studies on gelatin-containing artificial skin: II. Preparation and characterization of cross-linked gelatin-hyaluronate sponge. J. Biomed. Mater. Res. 48, 631–639.

Choi, Y.S., Hong, S.R., Lee, Y.M., Song, K.W., Park, M.H., Nam, Y.S., 1999b. Study on gelatin-containing artificial skin: I. Preparation and characteristics of novel gelatin-alginate sponge. Biomaterials. 20, 409–417.

Choi, Y.S., Lee, S.B., Hong, S.R., Lee, Y.M., Song, K.W., Park, M.H., 2001. Studies on gelatin-based sponges. Part III: a comparative study of cross-linked gelatin/alginate, gelatin/hyaluronate and chitosan/hyaluronate sponges and their application as a wound dressing in full-thickness skin defect of rat. J. Mater. Sci. Mater. Med. 12, 67–73.

Dongari-Bagtzoglou, A., Kashleva, H., 2007. Development of a highly reproducible three-dimensional organotypic model of the oral mucosa. Nat. Protoc. 1, 2012−2018.

Douwes, J., 2005. (1-- > 3)-Beta-D-glucans and respiratory health: a review of the scientific evidence. Indoor Air. 15, 160−169.

Eaglstein, W.H., Iriondo, M., Laszlo, K., 1995. A composite skin substitute (graftskin) for surgical wounds. A clinical experience. Dermatol. Surg. 21, 839−843.

El-Ghalbzouri, A., Lamme, E.N., van Blitterswijk, C., Koopman, J., Ponec, M., 2004. The use of PEGT/PBT as a dermal scaffold for skin tissue engineering. Biomaterials. 25, 2987−2996.

El Ghalbzouri, A., Lamme, E., Ponec, M., 2002. Crucial role of fibroblasts in regulating epidermal morphogenesis. Cell Tissue Res. 310, 189−199.

Enoch, S., Moseley, R., Stephens, P., Thomas, D.W., 2008. The oral mucosa: a model of wound healing with reduced scarring. Oral Surg. 1 (1), 11−21.

Fusenig, N.E., 1994. Epithelial-mesenchymal interactions regulate keratinocyte growth and differentiation in vitro. In: Leigh, I.M., Lane, E.B., Watt, F.M. (Eds.), The Keratinocyte Handbook. University Press, Cambridge.

Gentzkow, G.D., Iwasaki, S.D., Hershon, K.S., Mengel, M., Prendergast, J.J., Ricotta, J.J., et al., 1996. Use of dermagraft, a cultured human dermis, to treat diabetic foot ulcers. Diabet. Care. 19, 350−354.

Ghosh, M.M., Boyce, S., Layton, C., Freedlander, E., Mac Neil, S., 1997. A comparison of methodologies for the preparation of human epidermal-dermal composites. Ann. Plast. Surg. 39, 390−404.

Glim, J.E., Egmond, M., Niessen, F.B., Everts, V., Beelen, R.H.J., 2013. Detrimental dermal wound healing: What can we learn from the oral mucosa? Wound Repair Regener. 21, 648−660.

Hafemann, B., Ensslen, S., Erdmann, C., Niedballa, R., Zuhlke, A., Ghofrani, K., et al., 1999. Use of a collagen/elastin-membrane for the tissue engineering of dermis. Burns. 25, 373−384.

Hagi-Pavli, E., Williams, D., Thornhill, M.H., Adams, G., Smith, S., Cruchley, A.T., 2004. The effect of different surfactants on epithelial hoeostasis. J. Dent. Res. 83, (Special issue A), abst 1230.

Heck, E.L., Bergstresser, P.R., Baxter, C.R., 1985. Composite skin graft: frozen dermal allografts support the engraftment and expansion of autologous epidermis. J. Trauma. 25, 106−112.

Herson, M.R., Mathor, M.B., Altran, S., Capelozzi, V.L., Ferreira, M.C., 2001. In vitro construction of a potential skin substitute through direct human keratinocyte plating onto decellularized glycerol-preserved allodermis. Artif. Organs. 25, 901−906.

Hildebrand, H.C., Hakkinen, L., Wiebe, C.B., Larjava, H.S., 2002. Characterization of organotypic keratinocyte cultures on de-epithelialized bovine tongue mucosa. Histol. Histopathol. 17, 151−163.

Hong, S.R., Lee, S.J., Shim, J.W., Choi, Y.S., Lee, Y.M., Song, K.W., et al., 2001. Study on gelatin-containing artificial skin IV: a comparative study on the effect of antibiotic and EGF on cell proliferation during epidermal healing. Biomaterials. 22, 2777−2783.

Hsieh, P.C., Jin, Y.T., Chang, C.W., Huang, C.C., Liao, S.C., Yuan, K., 2010. Elastin in oral connective tissue modulates the keratinization of overlying epithelium. J. Clin. Periodontol. 37, 705−711.

Iida, T., Takami, Y., Yamaguchi, R., Shimazaki, S., Harii, K., 2005. Development of a tissue-engineered human oral mucosa equivalent based on an acellular allogeneic dermal matrix: A preliminary report of clinical application to burn wounds. Scand. J. Plast. Reconstr. Surg. Hand Surg. 39, 138−146.

Izumi, K., Feinberg, S.E., Iida, A., Yoshizawa, M., 2003a. Intraoral grafting of an ex vivo produced oral mucosa equivalent: a preliminary report. Int. J. Oral Maxillofac. Surg. 32, 188–197.

Izumi, K., Feinberg, S.E., Iida, A., Yoshizawa, M., 2003b. Intraoral grafting of an ex vivo produced oral mucosa equivalent: a preliminary report. Int. J. Oral Maxillofac. Surg. 32, 188–197.

Izumi, K., Feinberg, S.E., Terashi, H., Marcelo, C.L., 2003c. Evaluation of transplanted tissue-engineered oral mucosa equivalents in severe combined immunodeficient mice. Tissue Eng. 9, 163–174.

Izumi, K., Neiva, R.F., Feinberg, S.E., 2013. Intraoral grafting of tissue-engineered human oral mucosa. Int. J. Oral Maxillofac. Implants. 28, e295–e303.

Izumi, K., Takacs, G., Terashi, H., Feinberg, S.E., 1999. Ex vivo development of a composite human oral mucosal equivalent. J. Oral Maxillofac. Surg. 57, 571–577, discussion 577-8.

Izumi, K., Terashi, H., Marcelo, C.L., Feinberg, S.E., 2000. Development and characterization of a tissue-engineered human oral mucosa equivalent produced in a serum-free culture system. J. Dent. Res. 79, 798–805.

Kaiser, H.W., Stark, G.B., Kopp, J., Balcerkiewicz, A., Spilker, G., Kreysel, H.W., 1994. Cultured autologous keratinocytes in fibrin glue suspension, exclusively and combined with STS-allograft (preliminary clinical and histological report of a new technique). Burns. 20, 23–29.

Kato, H., Marcelo, C.L., Washington, J.B., Bingham, E.L., Feinberg, S.E., 2015. Fabrication of large size ex vivo-produced oral mucosal equivalents for clinical application. Tissue Eng. C Methods. 21, 872–880.

Khawaja, N.A., Lundqvist, C.K., Cruchley, A.T., Williams, D.M., Thornhill, M.H., 2002. HgCl2 Induces IL-6 and IL-8 production in a stratified oral mucosal culture system. J. Dent. Res. 8, A-496.

Khorramizadeh, M.R., Tredget, E.E., Telasky, C., Shen, Q., Ghahary, A., 1999. Aging differentially modulates the expression of collagen and collagenase in dermal fibroblasts. Mol. Cell Biochem. 194, 99–108.

Kim, J., Suh, S.W., Shin, J.Y., Kim, J.H., Choi, Y.S., Kim, H., 2004. Replacement of a tracheal defect with a tissue-engineered prosthesis: early results from animal experiments. J. Thorac. Cardiovasc. Surg. 128, 124–129.

Koch, L., Deiwick, A., Schlie, S., Michael, S., Gruene, M., Coger, V., et al., 2012. Skin tissue generation by laser cell printing. Biotechnol. Bioeng. 109, 1855–1863.

Koschier, F., Kostrubsky, V., Toole, C., Gallo, M.A., 2011. In vitro effects of ethanol and mouthrinse on permeability in an oral buccal mucosal tissue construct. Food Chem. Toxicol. 49, 2524–2529.

Krejci, N.C., Cuono, C.B., Langdon, R.C., Mcguire, J., 1991. In vitro reconstitution of skin: fibroblasts facilitate keratinocyte growth and differentiation on acellular reticular dermis. J. Invest. Dermatol. 97, 843–848.

Lauer, G., 1994. Autografting of feeder-cell free cultured gingival epithelium. Method and clinical application. J. Craniomaxillofac. Surg. 22, 18–22.

Lauer, G., Schimming, R., 2001. Tissue-engineered mucosa graft for reconstruction of the intraoral lining after freeing of the tongue: a clinical and immunohistologic study. J. Oral Maxillofac. Surg. 59, 169–175, discussion 175-7.

Lauer, G., Schimming, R., 2002. Clinical application of tissue-engineered autologous oral mucosa transplants. Mund Kiefer Gesichtschir. 6, 379–393.

Lee, D.Y., Ahn, H.T., Cho, K.H., 2000. A new skin equivalent model: dermal substrate that combines de-epidermized dermis with fibroblast-populated collagen matrix. J. Dermatol. Sci. 23, 132–137.

Lee, S.B., Jeon, H.W., Lee, Y.W., Lee, Y.M., Song, K.W., Park, M.H., et al., 2003. Bioartificial skin composed of gelatin and (1-- > 3), (1-- > 6)-beta-glucan. Biomaterials. 24, 2503–2511.

Lee, V., Singh, G., Trasatti, J.P., Bjornsson, C., Xu, X., Tran, T., et al., 2014. Design and fabrication of human skin by three-dimensional bioprinting. Tissue Eng. C-Methods. 20, 473–484.

Liu, J., Mao, J., Chen, L., 2011. Epithelial-mesenchymal interactions as a working concept for oral mucosa regeneration. Tissue Eng. B: Rev. 17, 25–31.

Livesey, S.A., Herndon, D.N., Hollyoak, M.A., Atkinson, Y.H., Nag, A., 1995. Transplanted acellular allograft dermal matrix. Potential as a template for the reconstruction of viable dermis. Transplantation. 60, 1–9.

Llames, S., Recuero, I., Romance, A., Garcia, E., Pena, I., Del Valle, A., et al., 2014. Tissue-engineered oral mucosa for mucosal reconstruction in a pediatric patient with hemifacial microsomia and ankyloglossia. Cleft Palate-Craniofac. J. 51, 246–251.

Luitaud, C., Laflamme, C., Semlali, A., Saidi, S., Grenier, G., Zakrzewski, A., et al., 2007a. Development of an engineering autologous palatal mucosa-like tissue for potential clinical applications. J. Biomed. Mater. Res. B: Appl. Biomater. 83, 554–561.

Luitaud, C., Laflamme, C., Semlali, A., Saidi, S., Grenier, G., Zakrzewski, A., et al., 2007b. Development of an engineering autologous palatal mucosa-like tissue for potential clinical applications. J. Biomed. Mater. Res. B. 83B, 554–561.

Lundqvist, C.K., Thornhill, M.H., Williams, D.M., Smith, S., Cruchley, A.T., 2002. The effect of sodium lauryl sulphate on epithelial homeostasis. J. Dent. Res. 8, (special issue A), abst 3054.

Ma, L., Gao, C., Mao, Z., Zhou, J., Shen, J., Hu, X., et al., 2003. Collagen/chitosan porous scaffolds with improved biostability for skin tissue engineering. Biomaterials. 24, 4833–4841.

MacCallum, D.K., Lillie, J.H., 1990. Evidence for autoregulation of cell division and cell transit in keratinocytes grown on collagen at an air–liquid interface. Skin Pharmacol. 3, 86–96.

Mak, K., Manji, A., Wiebe, C., Larjava, H., Häkkinen, L., Gallant-Behm, C., et al., 2009. Scarless healing of oral mucosa is characterized by faster resolution of inflammation and control of myofibroblast action compared to skin wounds in the red Duroc pig model. J. Dermatol. Sci. 56, 168–180.

Mao, J.S., Liu, H.F., Yin, Y.J., Yao, K.D., 2003. The properties of chitosan-gelatin membranes and scaffolds modified with hyaluronic acid by different methods. Biomaterials. 24, 1621–1629.

Masuda, I., 1996. An in vitro oral mucosal model reconstructed from human normal gingival cells. Kokubyo Gakkai Zasshi. 63, 334–353.

McKay, I., Woodward, B., Wood, K., Navsaria, H.A., Hoekstra, H., Green, C., 1994. Reconstruction of human skin from glycerol-preserved allodermis and cultured keratinocyte sheets. Burns. 20 (Suppl 1), S19–S22.

Merne, M., Syrjanen, S., 2003. The mesenchymal substrate influences the epithelial phenotype in a three-dimensional cell culture. Arch. Dermatol. Res. 295, 190–198.

Mohammadi, M., Mofid, R., Shokrgozar, M.A., 2011. Peri-implant soft tissue management through use of cultured gingival graft: a case report. Acta Med. Iran. 49, 319–324.

Mohammadi, M., Shokrgozar, M.A., Mofid, R., 2007. Culture of human gingival fibroblasts on a biodegradable scaffold and evaluation of its effect on attached gingiva: a randomized, controlled pilot study. J. Periodontol. 78, 1897–1903.

Moharamzadeh, K., Brook, I.M., Scutt, A.M., Thornhill, M.H., van Noort, R., 2008a. Mucotoxicity of dental composite resins on a tissue-engineered human oral mucosal model. J. Dent. 36, 331–336.

Moharamzadeh, K., Brook, I.M., van Noort, R., Scutt, A.M., Smith, K.G., Thornhill, M.H., 2008b. Development, optimization and characterization of a full-thickness tissue engineered human oral mucosal model for biological assessment of dental biomaterials. J. Mater. Sci. Mater. Med. 19, 1793–1801.

Moharamzadeh, K., Brook, I.M., van Noort, R., Scutt, A.M., Thornhill, M.H., 2007. Tissue-engineered oral mucosa: a review of the scientific literature. J. Dent. Res. 86, 115–124.

Moharamzadeh, K., Colley, H., Murdoch, C., Hearnden, V., Chai, W.L., Brook, I.M., et al., 2012. Tissue-engineered oral mucosa. J. Dent. Res. 91, 642–650.

Moharamzadeh, K., Franklin, K.L., Brook, I.M., van Noort, R., 2009. Biologic assessment of antiseptic mouthwashes using a three-dimensional human oral mucosal model. J. Periodontol. 80, 769–775.

Morey, A.F., 2005. Tissue-engineered buccal mucosa for substitution urethroplasty. J. Urol. 174, 1858.

Moriyama, T., Asahina, I., Ishii, M., Oda, M., Ishii, Y., Enomoto, S., 2001. Development of composite cultured oral mucosa utilizing collagen sponge matrix and contracted collagen gel: a preliminary study for clinical applications. Tissue Eng. 7, 415–427.

Mostefaoui, Y., Claveau, I., Ross, G., Rouabhia, M., 2002. Tissue structure, and IL-1beta, IL-8, and TNF-alpha secretions after contact by engineered human oral mucosa with dentifrices. J. Clin. Periodontol. 29, 1035–1041.

Nakamura, T., Endo, K., Cooper, L.J., Fullwood, N.J., Tanifuji, N., Tsuzuki, M., et al., 2003. The successful culture and autologous transplantation of rabbit oral mucosal epithelial cells on amniotic membrane. Invest Ophthalmol. Vis. Sci. 44, 106–116.

Navarro, F.A., Mizuno, S., Huertas, J.C., Glowacki, J., Orgill, D.P., 2001. Perfusion of medium improves growth of human oral neomucosal tissue constructs. Wound Repair Regen. 9, 507–512.

Nevins, M.L., 2010. Tissue-engineered bilayered cell therapy for the treatment of oral mucosal defects: a case series. Int. J. Periodont. Restorative Dent. 30, 31–39.

Ng, K.W., Khor, H.L., Hutmacher, D.W., 2004. In vitro characterization of natural and synthetic dermal matrices cultured with human dermal fibroblasts. Biomaterials. 25, 2807–2818.

Ng, K.W., Tham, W., Lim, T.C., Werner Hutmacher, D., 2005. Assimilating cell sheets and hybrid scaffolds for dermal tissue engineering. J. Biomed. Mater. Res. A. 75, 425–438.

Nimni, M.E., 1995. Collagen: molecular structure and biomaterial properties. In: Wise, E.A. (Ed.), Encyclopedic Handbook of Biomaterials and Bioengineering Part A: Materials. Marcel Dekker Inc, New York.

Ojeh, N.O., Frame, J.D., Navsaria, H.A., 2001. In vitro characterization of an artificial dermal scaffold. Tissue Eng. 7, 457–472.

Okazaki, M., Yoshimura, K., Suzuki, Y., Harii, K., 2003. Effects of subepithelial fibroblasts on epithelial differentiation in human skin and oral mucosa: heterotypically recombined organotypic culture model. Plast Reconstr. Surg. 112, 784–792.

Ophof, R., Maltha, J.C., Kuijpers-Jagtman, A.M., Von Den Hoff, J.W., 2008. Implantation of tissue-engineered mucosal substitutes in the dog palate. Eur. J. Orthod. 30, 1–9.

Ophof, R., van Rheden, R.E., Von Den, H.J., Schalkwijk, J., Kuijpers-Jagtman, A.M., 2002. Oral keratinocytes cultured on dermal matrices form a mucosa-like tissue. Biomaterials. 23, 3741–3748.

Osman, N.I., Hillary, C., Bullock, A.J., Macneil, S., Chapple, C.R., 2015. Tissue engineered buccal mucosa for urethroplasty: progress and future directions. Adv. Drug Deliv. Rev. 82-83, 69–76.

Osman, N.I., Patterson, J.M., Macneil, S., Chapple, C.R., 2014. Long-term follow-up after tissue-engineered buccal mucosa urethroplasty. Eur. Urol. 66, 790–791.
Patterson, J.M., Bullock, A.J., Macneil, S., Chapple, C.R., 2011. Methods to reduce the contraction of tissue-engineered buccal mucosa for use in substitution urethroplasty. Eur. Urol. 60, 856–861.
Peña, I., Junquera, L.M., Llorente, S., de Villalaín, L., de Vicente, J.C., Llames, S., 2012. Clinical outcomes after the use of complete autologous oral mucosa equivalents: preliminary cases. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 113, e4–e11.
Peña, I., Junquera, L.M., Meana, Á., García, E., García, V., de Vicente, J.C., 2010. In vitro engineering of complete autologous oral mucosa equivalents: characterization of a novel scaffold. J. Periodontal. Res. 45, 375–380.
Purdue, G.F., 1997. Dermagraft-TC pivotal efficacy and safety study. J. Burn Care Rehabil. 18, S13–S14.
Ralston, D.R., Layton, C., Dalley, A.J., Boyce, S.G., Freedlander, E., Mac Neil, S., 1999. The requirement for basement membrane antigens in the production of human epidermal/dermal composites in vitro. Br. J. Dermatol. 140, 605–615.
Ram-Liebig, G., Bednarz, J., Stuerzebecher, B., Fahlenkamp, D., Barbagli, G., Romano, G., et al., 2015. Regulatory challenges for autologous tissue engineered products on their way from bench to bedside in Europe. Adv. Drug Deliv. Rev. 82-83, 181–191.
Rennekampff, H.O., Kiessig, V., Griffey, S., Greenleaf, G., Hansbrough, J.F., 1997. Acellular human dermis promotes cultured keratinocyte engraftment. J. Burn Care Rehabil. 18, 535–544.
Rimann, M., Bono, E., Annaheim, H., Bleisch, M., Graf-Hausner, U., 2016. Standardized 3D bioprinting of soft tissue models with human primary cells. J. Lab. Autom. 21, 496–509.
Rosdy, M., Clauss, L.C., 1990. Terminal epidermal differentiation of human keratinocytes grown in chemically defined medium on inert filter substrates at the air-liquid interface. J. Invest. Dermatol. 95, 409–414.
Rosdy, M., Pisani, A., Ortonne, J.P., 1993. Production of basement membrane components by a reconstructed epidermis cultured in the absence of serum and dermal factors. Br. J. Dermatol. 129, 227–234.
Rouabhia, M., Allaire, P., 2010. Gingival mucosa regeneration in athymic mice using in vitro engineered human oral mucosa. Biomaterials. 31, 5798–5804.
Rouabhia, M., Deslauriers, N., 2002. Production and characterization of an in vitro engineered human oral mucosa. Biochem. Cell Biol. 80, 189–195.
Ruszymah, B.H., 2004. Autologous human fibrin as the biomaterial for tissue engineering. Med. J. Malaysia. 59 (Suppl B), 30–31.
Saintigny, G., Bonnard, M., Damour, O., Collombel, C., 1993. Reconstruction of epidermis on a chitosan cross-linked collagen-GAG lattice: effect of fibroblasts. Acta Derm. Venereol. 73, 175–180.
Schmalz, G., Arenholt-Bindslev, D., Hiller, K.A., Schweikl, H., 1997. Epithelium-fibroblast co-culture for assessing mucosal irritancy of metals used in dentistry. Eur. J. Oral. Sci. 105, 86–91.
Schmalz, G., Schweikl, H., Hiller, K.A., 2000. Release of prostaglandin E2, IL-6 and IL-8 from human oral epithelial culture models after exposure to compounds of dental materials. Eur. J. Oral Sci. 108, 442–448.
Selim, M., Bullock, A.J., Blackwood, K.A., Chapple, C.R., Macneil, S., 2011. Developing biodegradable scaffolds for tissue engineering of the urethra. BJU Int. 107, 296–302.

Sieira Gil, R., Pagés, C.M., Díez, E.G., Llames, S., Fuertes, A.F., Vilagran, J.L., 2015. Tissue-engineered oral mucosa grafts for intraoral lining reconstruction of the maxilla and mandible with a fibula flap. J. Oral Maxillofac. Surg. 73, 195.e1-195.e16.

Smola, H., Stark, H.J., Thiekotter, G., Mirancea, N., Krieg, T., Fusenig, N.E., 1998. Dynamics of basement membrane formation by keratinocyte-fibroblast interactions in organotypic skin culture. Exp. Cell Res. 239, 399−410.

Sun, T., Jackson, S., Haycock, J.W., Macneil, S., 2006. Culture of skin cells in 3D rather than 2D improves their ability to survive exposure to cytotoxic agents. J. Biotechnol. 122, 372−381.

Takagi, R., Murakami, D., Kondo, M., Ohki, T., Sasaki, R., Mizutani, M., et al., 2010. Fabrication of human oral mucosal epithelial cell sheets for treatment of esophageal ulceration by endoscopic submucosal dissection. Gastrointest. Endosc. 72, 1253−1259.

Takagi, R., Yamato, M., Murakami, D., Kondo, M., Ohki, T., Sasaki, R., et al., 2011. Fabrication and validation of autologous human oral mucosal epithelial cell sheets to prevent stenosis after esophageal endoscopic submucosal dissection. Pathobiology. 78, 311−319.

Takeda, K., Gosiewska, A., Peterkofsky, B., 1992. Similar, but not identical, modulation of expression of extracellular matrix components during in vitro and in vivo aging of human skin fibroblasts. J. Cell Physiol. 153, 450−459.

Trombetta, D., Mondello, M.R., Cimino, F., Cristani, M., Pergolizzi, S., Saija, A., 2005. Toxic effect of nickel in an in vitro model of human oral epithelium. Toxicol. Lett. 159, 219−225.

Vaissiere, G., Chevallay, B., Herbage, D., Damour, O., 2000. Comparative analysis of different collagen-based biomaterials as scaffolds for long-term culture of human fibroblasts. Med. Biol. Eng. Comput. 38, 205−210.

Vande Vannet, B., Hanssens, J.L., Wehrbein, H., 2007. The use of three-dimensional oral mucosa cell cultures to assess the toxicity of soldered and welded wires. Eur. J. Orthod. 29, 60−66.

Vande Vannet, B.M., Hanssens, J.L., 2007. Cytotoxicity of two bonding adhesives assessed by three-dimensional cell culture. Angle Orthodont. 77, 716−722.

Wang, H., Pieper, J., Peters, F., van Blitterswijk, C.A., Lamme, E.N., 2005. Synthetic scaffold morphology controls human dermal connective tissue formation. J. Biomed. Mater. Res. A. 74, 523−532.

Xie, M., Xu, Y., Song, L., Wang, J., Lv, X., Zhang, Y., 2014. Tissue-engineered buccal mucosa using silk fibroin matrices for urethral reconstruction in a canine model. J. Surg. Res. 188, 1−7.

Xiong, X., Jia, J., He, S., Zhao, Y., 2010. Cryopreserved lip mucosa tissue derived keratinocytes can fabricate tissue engineered palatal mucosa equivalent. J. Biomed. Mater. Res. B: Appl. Biomater. 94, 165−170.

Yang, G.P., Soetikno, R.M., 2007. Treatment of oesophageal ulcerations using endoscopic transplantation of tissue-engineered autologous oral mucosal epithelial cell sheets in a canine model. Gut. 56, 313−314.

Yoshizawa, M., Feinberg, S.E., Marcelo, C.L., Elner, V.M., 2004. Ex vivo produced human conjunctiva and oral mucosa equivalents grown in a serum-free culture system. J. Oral Maxillofac. Surg. 62, 980−988.

Zacchi, V., Soranzo, C., Cortivo, R., Radice, M., Brun, P., Abatangelo, G., 1998. In vitro engineering of human skin-like tissue. J. Biomed. Mater. Res. 40, 187−194.

Tissue-engineered models of oral soft tissue diseases

15

Keyvan Moharamzadeh, Helen Colley,
Vanessa Hearnden and Craig Murdoch
University of Sheffield, Sheffield, United Kingdom

15.1 Introduction

Tissue-engineered models of human oral mucosa have been developed and optimized to investigate the biology of different oral diseases, their progression and the evaluation of the effects of various treatment modalities. We have previously published an article (Moharamzadeh et al., 2012) outlining the progress made with regards to 3D tissue-engineered models of oral diseases including oral cancer, oral infection models, drug delivery systems, and imaging diagnostic technologies. This chapter includes an updated version of our previous paper covering the recent studies using tissue-engineered human oral mucosa for modeling of human oral diseases.

15.2 In vitro models of dysplasia and oral cancer

The organotypic skin model, first developed by Fusenig et al. (1983), has been developed and used to study processes involved in oral carcinogenesis. In vitro models of oral carcinogenesis at various stages of malignant transformation have been developed drawing on the ease of isolation of normal oral epithelial cells and the use of well-established immortalized dysplastic and neoplastic cell lines (Costea et al., 2005; Colley et al., 2011). Histomorphological and immunohistochemical characterization of these in vitro models show that they replicate changes in proliferation rates (Costea et al., 2005; Colley et al., 2011) and differentiation phenotype associated with malignant transformation and reflect the in vivo situation. Gaballah et al. (2008) using clinically isolated keratinocytes and fibroblasts developed models of mild, moderate, and severe dysplasia on collagen scaffolds. By devising a dysplasia-scoring system, architectural and cytological changes were used to grade the severity of dysplasia enabling a comparison to be made between tissue-engineered models (using different growth conditions) to the original lesion. However, models produced using mortal dysplastic keratinocytes failed to match the original dysplastic nature of the clinical lesion and the severity of the dysplasia also increased with passage number suggesting a phenotypic drift (Gaballah et al., 2008).

Tumor growth and invasion is not just controlled by epithelial cells alone but is an interplay between a number of cell types, the extra-cellular matrix and the tumor

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00015-3
© 2017 Elsevier Ltd. All rights reserved.

microenvironment. As the most abundant stromal cell, the role of fibroblasts in three-dimensional (3D) models of oral squamous cell carcinoma (OSCC) has received attention. Stromal fibroblasts significantly increase epithelial proliferation in vitro (Costea et al., 2005) and have been shown to be essential for tumor progression (Gaggioli et al., 2007). Marsh et al (2011) showed increased invasion of OSCC cells into the extra-cellular matrix in the presence of myofibroblasts compared to normal oral fibroblasts, with no tumor invasion in models absent of fibroblasts.

Tissue-engineered models of oral cancer have also been used to investigate the role of proinvasive signals in cancer progression. Duong et al. (2005) used an organotypic tumor model to study the effects of cell migration factors urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor (PAI-1) on the invasion of cancer cells through a reconstituted basement membrane into an underlying stroma. They found that uPA significantly promoted OSCC invasion whilst treatment with PAI-1 (the major inhibitor of uPA) inhibited the migratory effect. Culture under hypoxic conditions also promoted invasion by simultaneously increasing levels of uPA, the matrix metalloproteinases MMP-2 and MPP-9, and reducing PAI-1 levels. Daly et al. (2008) investigated two stromal cell-derived signaling molecules, hepatocyte growth factor (HGF), and stromal derived factor-1 (SDF-1/CXCL12) to determine their role in stimulating invasion in vitro. They found that both HGF and SDF-1 stimulated OSCC invasion in fibroblast-free organotypic cultures. However, no difference in the degree of invasion was seen when fibroblasts were present. In contrast, another report found exposing models to concentrations of HGF along with other growth factors and cytokines (GM-CSF, stem cell factor, epidermal growth factor, keratinocyte growth factor, transforming growth factor-α and interleukin-1) did not activate cellular migration of dysplastic oral keratinocyte (DOK) cells (a spontaneously immortalized cell line isolated from a dysplastic lesion) into a collagen matrix. These latter findings contradict what is typically seen in monolayer migration assays but support claims that soluble factors are not accountable for fibroblast-stimulated invasion but that fibroblasts act to remodel the matrix to enable collective invasion of carcinoma cells (Gaggioli et al., 2007). Despite being more physiological relevant than monolayers, the basic structure of the connective tissue component and the reconstituted basement membrane in organotypic models allows only a somewhat simplistic representation of the native stromal microenvironment (Wolf et al., 2009). Studies suggest that the extracellular matrix has dual roles acting not only as a barrier that invading malignant cells need to transverse but also acts as an anchorage point, allowing invading cells to attach (Gaggioli et al., 2007). Furthermore, contrary to the well-established view that focal breaks in the basement membrane correlates to malignant progression studies using the organotypic model suggest that expression of basement membrane proteins (laminin-332, type iv collagen, and fibronectin) is enhanced with cancer progression and that the basement membrane is both degraded and synthesized to support the invading front (Kulasekara et al., 2009). Mucosal models that utilize a more complex, native, connective tissue containing a defined basement membrane to investigate oral dysplasia and invasive carcinoma may provide a greater insight into the molecular mechanisms controlling premalignant dysplasia and invasion in vivo. Different models have been developed to replicate the different stages of

carcinogenesis; dysplasia, carcinoma in situ and early invasive carcinoma (Colley et al., 2011) using deepidermized dermis as there is evidence that this is more representative of native dermis. Essentially, well-characterized oral squamous cell lines were seeded as cell suspensions or as multicellular tumor spheroids with oral fibroblasts onto a deepidermized acellular dermis as this is more representative of native dermis (Wolf et al., 2009). Further to this Nurmenniemi et al. (2009) used human uterine leiomyoma tissue as a scaffold which, by nature contains a variety of cell types including endothelial cells, lymphocytes, macrophages, and extra-cellular matrix components and has been utilized to more realistically mimic the tumor microenvironment in studies investigating OSCC invasion and metastases; however, the use of uterine leiomyoma tissue is not widely available and uterine tissue may not accurately reflect oral tissue (Teppo et al., 2013; Alahuhta et al., 2015).

Overall, such models could facilitate study of the molecular processes involved in malignant transformation, invasion, and tumor growth as well as in vitro testing of new treatments, diagnostic tests, and drug delivery systems for OSCC.

15.3 Tissue-engineered models of radiotherapy-induced oral mucositis

Tissue-engineered oral mucosa has been used to investigate radiotherapy-induced oral mucositis which is a severe and often dose-limiting side-effect of cancer therapy that occurs in patients receiving radiotherapy for head and neck cancers and bone marrow transplant.

Colley et al. (2013) evaluated the cell damage and cytokine release from 3D oral mucosal models as well as monolayer cultures of normal oral keratinocytes, fibroblasts, and endothelial cells following exposure to 20 Gy irradiation. Compared to nonirradiated cells the viability of all monolayer cell cultures was significantly reduced 72 h postirradiation and levels of interleukin (IL)-6 and CXCL8 release were increased. The viability of irradiated tissue-engineered oral mucosal models was significantly reduced following radiation exposure. Histologically, irradiated mucosal models displayed thinner epithelium, increased apoptosis, and more extensive damage than nonirradiated models. IL-6, CXCL8, and granulocyte macrophage colony-stimulating factor release were reduced whereas IL-1α levels were increased in irradiated mucosal models compared to controls.

Previous studies using in vitro 3D oral mucosal models composed of oral keratinocytes seeded onto AlloDerm without fibroblasts have also reported similar results after exposure to different doses of radiation (Tobita et al., 2010).

15.4 In vitro three-dimensional models of bisphosphonate and medication-related osteonecrosis

A full-thickness 3D tissue-engineered osteo-mucosal model has been developed to investigate the localization of bisphosphonates in oral soft and hard tissues

(Bae et al., 2014). This in vitro tissue model consisted of vital rat bone overlain with engineered oral mucosa containing oral keratinocytes cultured on a fibroblast-populated collagen hydrogel. Following treatment with fluorescently labeled bisphosphonate the osteo-mucosal constructs exhibited fluorescent signals both in the bone and the epithelium. The engineered osteo-mucosal model used in this study seems to be a relevant and suitable alternative to animal models for the study of bisphosphonate and medication-related osteonecrosis. There is a need for further studies in this area to develop complex oral tissue models with normal human bone and oral mucosal cells to evaluate the effects of different therapeutic agents on the treatment of bisphosphonate and medication-related osteonecrosis of jaw which can be a debilitating condition.

15.5 Imaging and spectroscopic diagnostic techniques

Diagnosis of many oral mucosa conditions requires surgical biopsies and subsequent histological and immunohistochemical analysis. While well-established and trusted, these procedures have their limitations, including the delay between biopsy extraction and diagnosis, pain for the patient, labor intensive procedures and local trauma caused to the tissues. A noninvasive point-of-care diagnostic imaging system could dramatically improve patient outcomes especially in conditions such as OSCC where early detection, monitoring of disease progression, and definition of surgical boundaries are highly sought after. Many different imaging and spectroscopic modalities have been proposed for use in this area (optical coherence tomography (OCT) and Raman spectroscopy) while other commercially available tests based on fluorescence (VELscope) and optical illumination (Trimira Identafi 3000) are already available. Tissue-engineered oral mucosa has great value during the development stages of novel imaging systems as new imaging technologies can be tested without the need for patients or technology specially adapted and tested for in vivo use. For example, the potential for OCT as a method to detect OSCC has been evaluated (Smith et al., 2011). In this study OCT was shown to successfully differentiate between connective tissue and epithelial components. Recent advances in the OCT technology have improved image resolution enabling full-thickness of oral mucosal models to be visualized. When tissue-engineered models of OSCC and dysplasia were imaged using these OCT systems, epithelial disruption could be detected in the diseased compared to normal models (Boadi et al., 2015). Other work has focused on the use of scanning acoustic microscopy to characterize tissue-engineered models, in particular surface irregularities (Winterroth et al., 2011), which can help with quality control and monitoring of tissue-engineered oral mucosa production. More recently, Raman spectroscopic fiber-optic probe system has been used for noninvasive real-time monitoring of the fabrication of tissue-engineered oral mucosa constructs at the production and clinical implantation stages (Khmaladze et al., 2015).

15.6 Oral candidiasis disease modeling

Tissue-engineered oral mucosal models have been increasingly used to study the interaction of the fungus *Candida albicans* with oral tissue as a means to replicate oral candidiasis. Early experiments in this area of research relied on the use of reconstituted human oral epithelial (RHOE) models; where the buccal squamous cell carcinoma cell line (TR146) is cultured on a polycarbonate transwell insert at an air-to-liquid interface to form a multilayered epithelium. This simple in vitro epithelial model has generated much data on host-Candida interactions such as Candida biofilm development (Dongari-Bagtzoglou et al., 2009), the transcription factors important in the yeast to hyphal transition that is crucial for tissue penetration and damage (Cleary et al., 2010), the host signal transduction mechanisms activated and the types of cytokines released upon stimulation with *C. albicans* (Moyes et al., 2010; Schaller et al., 2006). However, because RHOE models are based on cancer cells, questions have been raised about the accuracy of this model in replicating the histological features of the normal oral mucosa and whether data generated using this model is representative or normal keratinocytes. Mostefaoui et al. (2004) were the first to use tissue-engineered oral mucosa to investigate oral candidiasis in vitro and the use of this model is becoming increasingly popular. These more complex models are based on normal or immortalized oral keratinocytes cultured on top of an oral fibroblast-containing collagen hydrogel or human deepithelized dermis at an air-to-liquid interface to form multilayered epithelium that show remarkably similar levels of keratinocyte differentiation, basal cell proliferation, and cytokeratin expression to normal oral mucosa (Dongari-Bagtzoglou and Kashleva, 2006; Yadev et al., 2011). Moreover, we have shown that *C. albicans*-infected tissue-engineered oral mucosal models display increased cytokine (CXCL8, IL-1β) release and β-defensin 2 expression compared to RHOE models making them more representative models of in vivo oral candidiasis (Yadev et al., 2011). Tissue-engineered oral mucosal models have been used to show that *C. albicans*-secreted aspartyl proteases are required for E-cadherin degradation at epithelial cell junctions during hyphal invasion of oral tissue (Villar et al., 2007) and that several *C. albicans* genes (Hwp1, Hyr1, Ssa1, IPT1, ECM33) and their products are crucial for adhesion to and invasion of the oral epithelium during oral candidiasis (Sun et al., 2010; Dwivedi et al., 2011; Rouabhia et al., 2011, 2012).

Recently, tissue-engineered oral mucosal models were used to examine the in vitro inhibitory activity of probiotic products derived from the bacterium *Bacillus subtilis* against *C. albicans*. In these experiments coculture of *B. subtilis* with *C. albicans* on the mucosal surface resulted in reduced numbers of candida cells and less tissue damage compared to models treated with Candida alone (Zhao et al., 2016), suggesting the potential usefulness of bacterial-derived products as antifungal agents. Another recent development is the use of oral mucosal models in mixed species experiments, in particular those of *C. albicans* with common oral bacteria such as *Streptococcus oralis*.

Diaz et al. observed that *C. albicans* and several streptococci species formed a synergistic partnership where *C. albicans* promoted the ability of streptococci to

form biofilms on the surface of tissue-engineered oral mucosal models. In addition, the presence of streptococci increased the ability of *C. albicans* to invade oral mucosal models under conditions that mimic salivary flow (Diaz et al., 2012). Using mucosal models based on immortalized oral keratinocytes Xu et al. showed that *S. oralis* augmented *C. albicans* invasion through epithelial junctions by decreasing epithelial E-cadherin levels by synergistically increasing expression of μ-calpain, a proteolytic enzyme that specifically targets E-cadherin (Xu et al., 2016). The use of complex oral mucosal and gingival models along in conjunction with complex multispecies mixed biofilms containing oral bacterial and Candida species will lead the way for future research into oral candidiasis.

15.7 Oral mucosal models of bacterial infection

The oral mucosa is in constant contact with numerous commensal and potentially pathogenic microorganisms. Consequently, tissue-engineered oral mucosal models have been extensively used to investigate the interaction of oral microbes with the oral epithelium in an attempt to understand the mechanisms of symbiosis and pathogenesis. Although some studies have examined the interaction of *Fusobacterium nucleatum* with in vitro models of the oral mucosa, most work to date has focused on the invasiveness and tissue destruction capacity of the Gram-negative anaerobic bacterium *Porphyromonas gingivalis* that is an important etiological agent in chronic periodontitis and has recently been associated with the development several systemic conditions such as atherosclerosis and cardiovascular disease. Not only were strains of *P. gingivalis* and *F. nucleatum* found to be internalized by the upper layers of oral keratinocytes in infection models, they were also observed in the deeper layers and in the connective tissue, suggesting that these bacteria can penetrate through the epithelium during infection (Gursoy et al., 2010). Moreover, Andrian et al. (2007) found that *P. gingivalis* up-regulated expression of MMP-2 and -9 in infection models that may contribute to the extensive tissue destruction observed in periodontitis.

We have recently optimized a full-thickness organotypic oral mucosal model based on fibroblast-populated collagen hydrogel overlain with multilayers of oral keratinocytes to study *P. gingivalis* invasion of the epithelium (Pinnock et al., 2014). Significant differences in the response of oral mucosal models to *P. gingivalis* infection were found when compared to monolayer cultures of oral epithelial cells. Intracellular survival (threefold) and bacterial release (fourfold) of *P. gingivalis* were significantly increased in mucosal models compared with monolayer cultures, which may be due to the multilayered nature and exfoliation of epithelial cells in these organotypic models. Furthermore, marked differences in the cytokine profile between infected organotypic models and monolayer cultures were observed.

New mucosal models have been generated based on the use of primary gingival epithelial cells in order to more specifically replicate the gingival sites where

periodontal disease arises. These models show increased keratinization replicating the gingival epithelium and so may provide even greater insight into host–pathogen interactions (Bao et al., 2014). Moreover, these models have been incubated with multibacterial species biofilms in order to mimic a more realistic diseased site rather than the traditional single species infection models (Bao et al., 2014). These studies have provided a wealth of information concerning the epithelial secretome and alteration of epithelial junctions in response to mature, disease-causing oral biofilms (Bostanci et al., 2015; Belibasakis et al., 2015; Thurnheer et al., 2016). The data generated by the use of these more realistic and complex oral models in host–pathogen studies may provide a greater understanding of the host responses to microbial biofilms than simple monolayer cultures and may expedite the development of novel therapies to treat periodontal and other oral diseases.

15.8 Immune response studies

Immunological effects of oral healthcare ingredients have been characterized using in vitro reconstructed split-thickness human epithelial model (Hagi-Pavli et al., 2014). Commercially available SkinEthic model of oral epithelium used in this study to assess the differential release of inflammatory cytokines was 3D tissue culture model consisting of a continuous oral keratinocyte tumor-derived cell line, TR146 grown in defined culture medium on 0.5 cm^2 polycarbonate membranes. Another recent study has attempted to produce full-thickness immune-competent tissue-engineered model of human oral mucosa by incorporating Langerhans-like MUTZ-3 cell line to the epithelial layer of the reconstructed human gingiva (Kosten et al., 2016).

It is important to realize that the allergic reactions and immune response have very complex mechanisms involving many different cell types within the oral mucosa including cellular elements of blood. Results obtained from the 3D oral mucosal models without the vascular system and lacking most of the components of the immune system must be interpreted with caution.

15.9 Drug delivery systems

Efficient and effective delivery of drugs into and across the oral mucosa is a challenge for treatment of many conditions affecting the oral mucosa including OSCC, mucositis, and lichen planus. The oral mucosa also has potential as a site for the systemic delivery of drugs which could provide an alternative to parental delivery (Hearnden et al., 2011). An effective test model is required in order to develop delivery systems capable of transmucosal or intramucosal delivery in an optimal manner with minimal local tissue damage, drug delivery to the desired location with ideal speed and minimal drug degradation. There is very little evidence in the literature of tissue-engineered oral mucosa being utilized in this manner. However,

commercially available epithelium-only models developed by MatTek Corp. (NJ, United States) have been shown to reliably test the permeability of different materials across the oral epithelium (Klausner et al., 2002). We have shown (Hearnden et al., 2009) that fluorescently labeled polymersomes, a nanoscale polymer-based delivery system, can be tracked through tissue-engineered oral mucosa using laser scanning confocal microscopy enabling progression of polymersomes to be tracked over time. The polymersomes were able to reach the basal cells of the tissue-engineered epithelium and there was evidence of polymersomes crossing into the connective tissue component, something which is often obstructed by the permeability barrier. The use of tissue-engineered models allows the evaluation of epithelial drug delivery systems without the need for animal models and enables initial toxicity testing prior to in vivo trials. The potential for these kinds of experiments is a clearly advantageous application of tissue-engineered oral mucosa.

15.10 Future trends

Tissue-engineered models of human oral mucosa have enabled us to investigate many of the oral diseases affecting the oral soft tissues only as described above. Recent advances in tissue engineering have permitted in vitro reconstruction of multilayered complex oral tissues containing both bone and oral mucosa (Almela et al., 2016). Development and optimization of these complex osteo-mucosal systems would prepare the grounds for studies investigating the diseases involving both alveolar bone and oral mucosa. These include studies of bone invasion of oral cancer, development of in vitro models of osteoradionecrosis, periodontal disease, and peri-implantitis.

References

Alahuhta, I., Aikio, M., Vayrynen, O., Nurmenniemi, S., Suojanen, J., Teppo, S., et al., 2015. Endostatin induces proliferation of oral carcinoma cells but its effect on invasion is modified by the tumor microenvironment. Exp. Cell Res. 336, 130−140.

Almela, T., Brook, I.M., Moharamzadeh, K., 2016. Development of three-dimensional tissue engineered bone-oral mucosal composite models. J. Mater. Sci. Mater. Med. 27, 65.

Andrian, E., Mostefaoui, Y., Rouabhia, M., Grenier, D., 2007. Regulation of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases by Porphyromonas gingivalis in an engineered human oral mucosa model. J. Cell Physiol. 211, 56−62.

Bae, S., Sun, S., Aghaloo, T., Oh, J.E., Mckenna, C.E., Kang, M.K., et al., 2014. Development of oral osteomucosal tissue constructs in vitro and localization of fluorescently-labeled bisphosphonates to hard and soft tissue. Int. J. Mol. Med. 34, 559−563.

Bao, K., Akguel, B., Bostanci, N., 2014. Establishment and characterization of immortalized gingival epithelial and fibroblastic cell lines for the development of organotypic cultures. Cells Tissues Organs. 199, 228−237.

Belibasakis, G.N., Kast, J.I., Thurnheer, T., Akdis, C.A., Bostanci, N., 2015. The expression of gingival epithelial junctions in response to subgingival biofilms. Virulence. 6, 704−709.

Boadi, J., Fernandes, J., Mittar, S., Hearnden, V., Lu, Z., Macneil, S., et al., 2015. Imaging of 3-D tissue engineered models of oral cancer using 890 and 1300 nm optical coherence tomography. Sovremennye Tehnol. Med. Mod. Technol. Med. 7, 60−67.

Bostanci, N., Bao, K., Wahlander, A., Grossmann, J., Thurnheer, T., Belibasakis, G.N., 2015. Secretome of gingival epithelium in response to subgingival biofilms. Mol. Oral Microbiol. 30, 323−335.

Cleary, I.A., Mulabagal, P., Reinhard, S.M., Yadev, N.P., Murdoch, C., Thornhill, M.H., et al., 2010. Pseudohyphal regulation by the transcription factor Rfg1p in Candida albicans. Eukaryot. Cell. 9, 1363−1373.

Colley, H.E., Eves, P.C., Pinnock, A., Thornhill, M.H., Murdoch, C., 2013. Tissue-engineered oral mucosa to study radiotherapy-induced oral mucositis. Int. J. Radiat Biol. 89, 907−914.

Colley, H.E., Hearnden, V., Jones, A.V., Weinreb, P.H., Violette, S.M., Macneil, S., et al., 2011. Development of tissue engineered models of oral dysplasia and early invasive oral squamous cell carcinoma. Br. J. Cancer. 105, 1582−1592.

Costea, D.E., Johannessen, A.C., Vintermyr, O.K., 2005. Fibroblast control on epithelial differentiation is gradually lost during in vitro tumor progression. Differentiation. 73, 134−141.

Daly, A.J., Mcilreavey, L., Irwin, C.R., 2008. Regulation of HGF and SDF-1 expression by oral fibroblasts—implications for invasion of oral cancer. Oral Oncol. 44, 646−651.

Diaz, P.I., Xie, Z., Sobue, T., Thompson, A., Biyikoglu, B., Ricker, A., et al., 2012. Synergistic interaction between Candida albicans and commensal oral streptococci in a novel in vitro mucosal model. Infect Immun. 80, 620−632.

Dongari-Bagtzoglou, A., Kashleva, H., 2006. Development of a highly reproducible three-dimensional organotypic model of the oral mucosa. Nat. Protoc. 1, 2012−2018.

Dongari-Bagtzoglou, A., Kashleva, H., Dwivedi, P., Diaz, P., Vasilakos, J., 2009. Characterization of mucosal Candida albicans biofilms. PLoS One. 4, e7967.

Duong, H.S., Le, A.D., Zhang, Q., Messadi, D.V., 2005. A novel 3-dimensional culture system as an in vitro model for studying oral cancer cell invasion. Int. J. Exp. Pathol. 86, 365−374.

Dwivedi, P., Thompson, A., Xie, Z., Kashleva, H., Ganguly, S., Mitchell, A.P., et al., 2011. Role of Bcr1-activated genes Hwp1 and Hyr1 in Candida albicans oral mucosal biofilms and neutrophil evasion. PLoS One. 6, e16218.

Fusenig, N.E., Breitkreutz, D., Dzarlieva, R.T., Boukamp, P., Bohnert, A., Tilgen, W., 1983. Growth and differentiation characteristics of transformed keratinocytes from mouse and human skin in vitro and in vivo. J. Invest Dermatol. 81, 168s−175ss.

Gaballah, K., Costea, D.E., Hills, A., Gollin, S.M., Harrison, P., Partridge, M., 2008. Tissue engineering of oral dysplasia. J. Pathol. 215, 280−289.

Gaggioli, C., Hooper, S., Hidalgo-Carcedo, C., Grosse, R., Marshall, J.F., Harrington, K., et al., 2007. Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. Nat. Cell Biol. 9, 1392−1400.

Gursoy, U.K., Pollanen, M., Kononen, E., Uitto, V.J., 2010. Biofilm formation enhances the oxygen tolerance and invasiveness of Fusobacterium nucleatum in an oral mucosa culture model. J. Periodontol. 81, 1084−1091.

Hagi-Pavli, E., Williams, D.M., Rowland, J.L., Thornhill, M., Cruchley, A.T., 2014. Characterizing the immunological effects of oral healthcare ingredients using an in vitro reconstructed human epithelial model. Food Chem. Toxicol. 74, 139−148.

Hearnden, V., Lomas, H., Macneil, S., Thornhill, M., Murdoch, C., Lewis, A., et al., 2009. Diffusion studies of nanometer polymersomes across tissue engineered human oral mucosa. Pharmaceut. Res. 26, 1718–1728.

Hearnden, V., Sankar, V., Hull, K., Juras, D.V., Greenberg, M., Kerr, A.R., et al., 2011. New developments and opportunities in oral mucosal drug delivery for local and systemic disease. Adv. Drug Deliv. Rev. 2011, 1.

Khmaladze, A., Kuo, S., Kim, R.Y., Matthews, R.V., Marcelo, C.L., Feinberg, S.E., et al., 2015. Human oral mucosa tissue-engineered constructs monitored by Raman fiber-optic probe. Tissue Eng. C: Methods. 21, 46–51.

Klausner, M., Kubilus, J., Song, Y., Michniak, B., 2002. Barrier function comparison between *in vitro* skin, oral (buccal) and ocular tissue models-implications for drug delivery studies. Mattek Tech. Reference274. Available: http://www.mattek.com/pages/abstracts/abstractview/article/274-barrier-function-comparison-between-in-vitro-skin-oral-buccal-and-ocular-tissue-models-im/.

Kosten, I.J., Spiekstra, S.W., de Gruijl, T.D., Gibbs, S., 2016. MUTZ-3 langerhans cell maturation and CXCL12 independent migration in reconstructed human gingiva. ALTEX. 33 (4), 423–434.

Kulasekara, K.K., Lukandu, O.M., Neppelberg, E., Vintermyr, O.K., Johannessen, A.C., Costea, D.E., 2009. Cancer progression is associated with increased expression of basement membrane proteins in three-dimensional in vitro models of human oral cancer. Arch. Oral. Biol. 54, 924–931.

Marsh, D., Suchak, K., Moutasim, K.A., Vallath, S., Hopper, C., Jerjes, W., et al., 2011. Stromal features are predictive of disease mortality in oral cancer patients. J. Pathol. 223, 470–481.

Moharamzadeh, K., Colley, H., Murdoch, C., Hearnden, V., Chai, W.L., Brook, I.M., et al., 2012. Tissue-engineered oral mucosa. J. Dent. Res. 91, 642–650.

Mostefaoui, Y., Claveau, I., Rouabhia, M., 2004. In vitro analyses of tissue structure and interleukin-1beta expression and production by human oral mucosa in response to Candida albicans infections. Cytokine. 25, 162–171.

Moyes, D.L., Runglall, M., Murciano, C., Shen, C., Nayar, D., Thavaraj, S., et al., 2010. A biphasic innate immune MAPK response discriminates between the yeast and hyphal forms of Candida albicans in epithelial cells. Cell Host Microbe. 8, 225–235.

Nurmenniemi, S., Sinikumpu, T., Alahuhta, I., Salo, S., Sutinen, M., Santala, M., et al., 2009. A novel organotypic model mimics the tumor microenvironment. Am. J. Pathol. 175, 1281–1291.

Pinnock, A., Murdoch, C., Moharamzadeh, K., Whawell, S., Douglas, C.W., 2014. Characterisation and optimisation of organotypic oral mucosal models to study Porphyromonas gingivalis invasion. Microbes Infect. 16, 310–319.

Rouabhia, M., Mukherjee, P.K., Lattif, A.A., Curt, S., Chandra, J., Ghannoum, M.A., 2011. Disruption of sphingolipid biosynthetic gene IPT1 reduces Candida albicans adhesion and prevents activation of human gingival epithelial cell innate immune defense. Med. Mycol. 49, 458–466.

Rouabhia, M., Semlali, A., Chandra, J., Mukherjee, P., Chmielewski, W., Ghannoum, M.A., 2012. Disruption of the ECM33 gene in Candida albicans prevents biofilm formation, engineered human oral mucosa tissue damage and gingival cell necrosis/apoptosis. Mediators Inflamm. 2012, 398207.

Schaller, M., Zakikhany, K., Naglik, J.R., Weindl, G., Hube, B., 2006. Models of oral and vaginal candidiasis based on in vitro reconstituted human epithelia. Nat. Protoc. 1, 2767–2773.

Smith, L.E., Hearnden, V., Smallwood, R., Matcher, S., Thornhill, M.H., 2011. Evaluating the use of optical coherence tomography for the detection of epithelial cancers in vitro. J. Biomed. Opt. 16, 116015.

Sun, J.N., Solis, N.V., Phan, Q.T., Bajwa, J.S., Kashleva, H., Thompson, A., et al., 2010. Host cell invasion and virulence mediated by Candida albicans Ssa1. PLoS Pathog. 6, e1001181.

Teppo, S., Sundquist, E., Vered, M., Holappa, H., Parkkisenniemi, J., Rinaldi, T., et al., 2013. The hypoxic tumor microenvironment regulates invasion of aggressive oral carcinoma cells. Exp. Cell Res. 319, 376–389.

Thurnheer, T., Bostanci, N., Belibasakis, G.N., 2016. Microbial dynamics during conversion from supragingival to subgingival biofilms in an in vitro model. Mol. Oral Microbiol. 31, 125–135.

Tobita, T., Izumi, K., Feinberg, S.E., 2010. Development of an in vitro model for radiation-induced effects on oral keratinocytes. Int. J. Oral Maxillofac. Surg. 39, 364–370.

Villar, C.C., Kashleva, H., Nobile, C.J., Mitchell, A.P., Dongari-Bagtzoglou, A., 2007. Mucosal tissue invasion by Candida albicans is associated with E-cadherin degradation, mediated by transcription factor Rim101p and protease Sap5p. Infect. Immun. 75, 2126–2135.

Winterroth, F., Hollman, K.W., Kuo, S., Izumi, K., Feinberg, S.E., Hollister, S.J., et al., 2011. Comparison of scanning acoustic microscopy and histology images in characterizing surface irregularities among engineered human oral mucosal tissues. Ultras. Med. Biol. 37, 1734–1742.

Wolf, K., Alexander, S., Schacht, V., Coussens, L.M., von Andrian, U.H., van Rheenen, J., et al., 2009. Collagen-based cell migration models in vitro and in vivo. Semin. Cell Dev. Biol. 20, 931–941.

Xu, H., Sobue, T., Bertolini, M., Thompson, A., Dongari-Bagtzoglou, A., 2016. Streptococcus oralis and Candida albicans synergistically activate mu-Calpain to degrade E-cadherin from oral epithelial junctions. J. Infect. Dis. 214, 925–934.

Yadev, N.P., Murdoch, C., Saville, S.P., Thornhill, M.H., 2011. Evaluation of tissue engineered models of the oral mucosa to investigate oral candidiasis. Microb. Pathog. 50, 278–285.

Zhao, C., Lv, X., Fu, J., He, C., Hua, H., Yan, Z., 2016. In vitro inhibitory activity of probiotic products against oral Candida species. J. Appl. Microbiol. 121, 254–262.

This page intentionally left blank

Periodontal soft tissue reconstruction

16

Lisetta Lam, Ryan S.B. Lee and Saso Ivanovski
Griffith University, Gold Coast, Australia

16.1 Introduction

The periodontium is a complex structure composed of both soft (gingiva, periodontal ligament) and hard (alveolar bone, cementum) tissues. Periodontal tissue loss is most commonly associated with the bacterial plaque-induced inflammatory condition, periodontitis, but can also occur as a result of other causes, such as mechanical trauma. While the regeneration of the full periodontal complex involves the reconstitution of both soft and hard tissues, in certain cases, surgical procedures are carried out for the sole purpose of reconstructing the soft tissues surrounding the tooth. This chapter will focus on biomaterials that are utilized for soft tissue reconstruction.

Periodontal soft tissue reconstruction involves the correction and restoration of anatomical, developmental, traumatic, or plaque disease-induced mucogingival defects. The primary aim of the mucogingival surgical therapies is the complete restoration of the anatomy of the mucogingival complex and the regeneration of the attachment apparatus of the tooth, ultimately restoring esthetics and relieving dental hypersensitivity (Trombelli et al., 2005). Periodontal soft tissue surgery can also be used to thicken and increase the gingival tissue dimensions at mucogingival defect sites prior to prosthetic restoration procedures, creating a favorable periodontal environment (Thoma et al., 2009; Ramachandra et al., 2014; Oates et al., 2003).

16.2 Autologous grafts

Autogenous graft is considered as a gold standard material for mucogingival procedures due to its clinical predictability. There are two main types of autogenous grafts—the free gingival graft (FGG) (Fig. 16.1) and the connective tissue graft (CTG) (Fig. 16.2). FGGs include an epithelial and a connective tissue component and are primarily used to increase the width of keratinized tissue, while CTGs only consist of subcutaneous tissue and are used for the treatment of recession defects, usually in conjunction with coronally repositioned flaps. Both FGGs and CTGs have been shown to be effective in the treatment of mucogingival defects, but they also have some notable limitations (Cairo et al., 2012; Camargo et al., 2001; Alghamdi et al., 2009).

A disadvantage of autogenous grafts is that the amount of graft material that can be harvested is limited, as the grafts are commonly harvested from the palatal and

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00016-5
© 2017 Elsevier Ltd. All rights reserved.

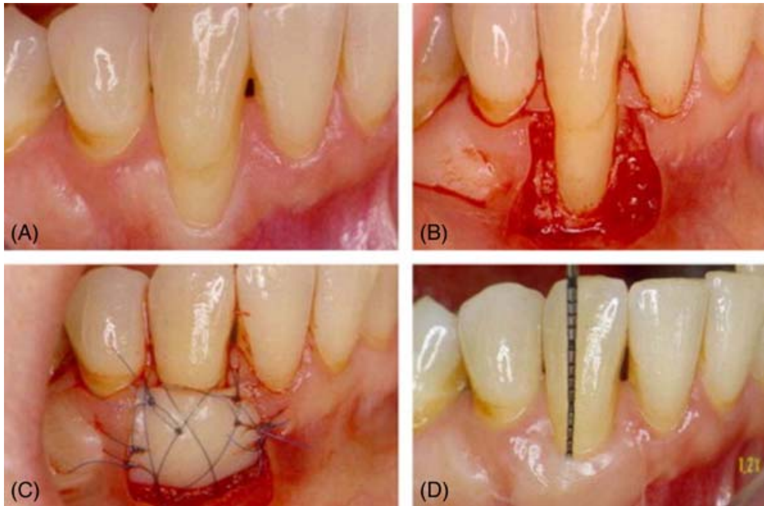


Figure 16.1 Free gingival graft. (A) Mandibular incisors with recession and lack of attached gingiva, (B) preparation of the recipient site, (C) the graft is sutured to the recipient bed, and (D) 2 months postoperative appearance.
Adapted from: Alghamdi, H., Babay, N., Sukumaran, A., 2009. Surgical management of gingival recession: a clinical update. Saudi Dent. J., 21, 83−94 with permission.

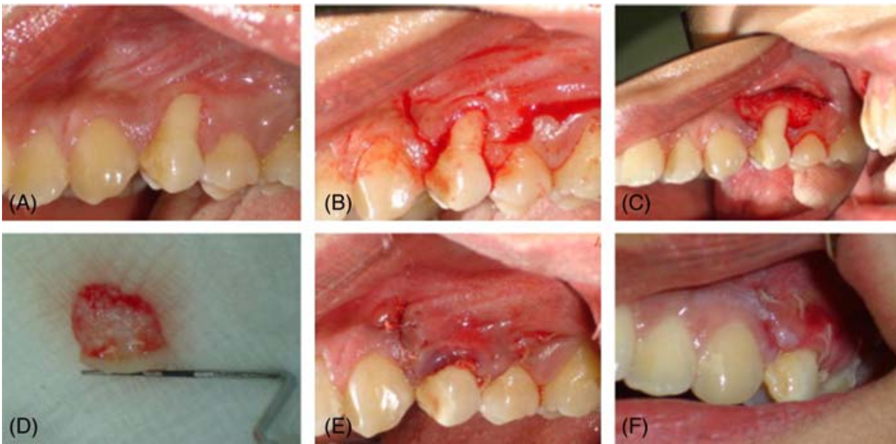


Figure 16.2 Connective tissue flap. (A) Recession at maxillary first premolar, (B) vertical incision, (C) partial thickness flap reflection, (D) the harvested graft tissue, (E) the graft sutured in position, and (F) 2 weeks healing.
Adapted from: Alghamdi, H., Babay, N., Sukumaran, A., 2009. Surgical management of gingival recession: a clinical update. Saudi Dent. J., 21, 83−94 with permission.

retromolar regions. Therefore the quantity and quality of tissue that can be harvested depends on the anatomy of the donor sites.

Another noteworthy disadvantage of autologous grafts is that the harvesting procedure itself is often associated with considerable donor site morbidity and postoperative complications (Herford et al., 2010). Indeed, the following postoperative complications have been identified and reported (Ramachandra et al., 2014; Griffin et al., 2006):

- Hemorrhage from the donor site
- Permanent anesthesia and paresthesia of portions of the palate
- Prolonged and pronounced pain/discomfort at the donor site
- Infection at the palatal donor site
- Increased chairside time for harvesting the graft from the palate
- Occurrence of surgical cyst following subepithelial CTG

16.3 General considerations for biomaterials in periodontal soft tissue reconstruction

Due to the limited availability and postoperative morbidity associated with autogenous graft harvesting procedures, alternative biomaterials have been proposed. The main advantages of utilizing such biomaterials are unlimited availability, decreased surgical time and reduced postoperative discomfort/pain due to the lack of a donor site (Lima et al., 2015).

For a biomaterial to be effective, it has to meet certain criteria based on organ and tissue properties and the specific goals of reconstruction (Hanker and Giammara, 1988; Pachence, 1996). The following characteristics, or design criteria for soft tissue regeneration devices have been proposed: biocompatibility, space maintenance, cell occlusion/guidance, tissue integration, ease of use, and biological activity (Scantlebury, 1993; Gottlow, 1993; Hardwick et al., 1995). Within this framework, there is a diverse range of biomaterials available in the marketplace. In general, these can be categorized as allogenic, xenograft, autologous, alloplastic, and tissue engineered materials, based on their original sources (Table 16.1).

Table 16.1 Categories of periodontal soft tissue biomaterials

Periodontal soft tissue biomaterials				
Allogenic materials, e.g., AlloDerm®, Puros Dermis®	Xenogeneic materials, e.g., extracellular matrix (ECM) membrane, bilayer collagen matrix	Autologous materials, e.g., platelet-rich fibrin (PRF) membrane	Alloplastic materials, e.g., three-dimensional (3D) printed scaffolds	Tissue-engineered material, e.g., living cell construct

16.4 Allogenic materials

16.4.1 Acellular dermal matrix allograft

The acellular dermal matrix allograft (ADMA) has been widely studied and used as a substitute for autogenous grafts in periodontal soft tissue surgeries, although it was originally developed for the treatment of full-thickness burn wounds (Yan et al., 2006; Barros et al., 2004; Cummings et al., 2005; De Queiroz Côrtes et al., 2004). This allograft is aseptically obtained from human donor skin. The preparation of this allograft involves a multistep proprietary process in which the epidermis and cellular components of the dermis have been removed, but the basement membrane and extracellular matrix components and collagenous scaffolding have been maintained (Chambrone and Chambrone, 2006; Zucchelli and Mounssif, 2015; Tal et al., 2002; Scarano et al., 2009). The remaining tissue is washed in detergent solutions to remove microbial contaminants and immunogenic material, and is then cryoprotected by a rapid freeze drying proprietary process to preserve the biochemical and structural integrity of the remaining material (Zucchelli and Mounssif, 2015). Preservation of the ultrastructural integrity of the collagen and elastic matrices is important to avoid induction of inflammatory responses in the recipient tissue (Wainwright et al., 1996; Rhee et al., 1998; Wainwright, 1995). The resultant immunologically inert allograft may serve as an architectural framework to support fibroblast migration and revascularization from the host tissues (Scarano et al., 2009).

The healing process associated with ADMA is similar to that observed with autogenous grafts, and the material been shown to consistently integrate into the host tissue (Sullivan and Atkins, 1968). The allograft acts as a bioactive scaffold for vascular endothelial cells and fibroblasts, thus facilitating their repopulation of the connective tissue matrix. It also encourages epithelial cells to migrate from the adjacent tissue margins (Wong et al., 2008; Jhaveri et al., 2010). Since the structural integrity of the material is maintained, it is revascularized via preserved vascular channels within the material. According to a clinical and histological study using ADMA for keratinized tissue augmentation in human subjects, the ADMA was substituted and reepithelialized within 10 weeks with complete integration of the ADMA with the host's gingival tissue (Scarano et al., 2009). In addition, this nonimmunogenic freeze-dried allograft did not elicit any antihuman leukocyte antigen-antibody activity (Scarano et al., 2009).

The use of ADMA in periodontal soft tissue reconstruction possesses distinct advantages, including: avoidance of the palatal donor site; treatment of multiple gingival recessions in one visit; unlimited amounts of tissue availability; high quality of donor tissue; and the ability to match or improve upon the results observed with autogenous palatal tissue grafts. Additionally, it offers a higher case acceptance rate and less postoperative discomfort (Silc and Petrungaro, 2013).

Various ADMA products are available. AlloDerm® (BioHorizons, Birmingham, AL, United States) has been in use for over 20 years and is the most widely researched allograft (Fig. 16.3) (Ramachandra et al., 2014; Silc and Petrungaro,

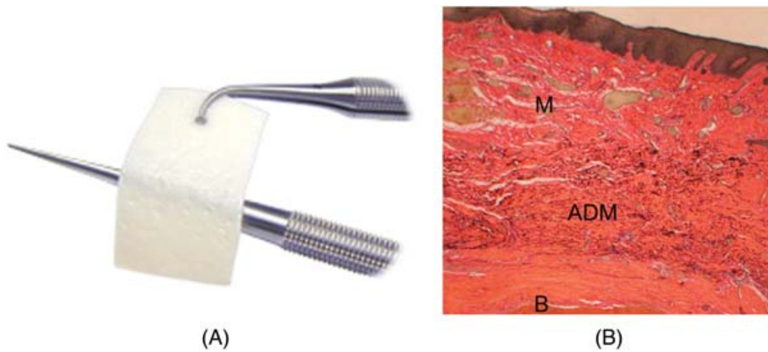


Figure 16.3 (A) AlloDerm (BioHorizons, Birmingham, AL, United States); (B) Histological specimen of acellular dermal matrix demonstrating mucosal tissue (M) overlying the area of graft placement (ADM) and osseous crest (B).
Adapted from: (A) BioHorizons; (B) Cummings, L.C., Kaldahl, W.B., Allen, E.P., 2005. Histologic evaluation of autogenous connective tissue and acellular dermal matrix grafts in humans. J. Periodontol. 76, 178−186 with permission.

Figure 16.4 Puros Dermis (Zimmer Dental, Carlsbad, CA).
Adapted from: Zimmer Dental brochure with permission.

2013). It is an ADMA derived from donated human skin from tissue banks accredited by the American Association of Tissue Banks. This allograft is a freeze-dried, cell free, dermal matrix comprised of a structurally integrated basement membrane complex and an extracellular matrix of collagen bundles and elastic fibers (Agarwal et al., 2015). AlloDerm® was introduced into periodontics as a substitute for autogenous CTG or FGG in root coverage procedures and for increasing the width of attached gingiva around teeth (Callan, 1990; Shulman, 1996) and implants (Callan and Silverstein, 1998). The material has two sides, a basement membrane and a dermal side, and it is used as a subgingival soft tissue graft in the treatment of gingival recession (Achauer et al., 1998; Tal, 1999; Ramachandra et al., 2014; Cummings et al., 2005).

Puros® Dermis (Zimmer Dental, Carlsbad, CA) is another ADMA that was introduced as an alternate to autogenous soft tissue graft for root coverage in the treatment of gingival recession (Fig. 16.4) (Barker et al., 2010). The Puros

Dermis® allograft retains the natural collagen matrix and mechanical properties of native dermis as a result of the company's proprietary Tutoplast process (Hinton et al., 1992). While preserving the collagen matrix, as well as the tissue structural integrity and biomechanical properties, this process removes the cellular components, inactivating bacterial, viral, and prion contamination and eliminating antigenicity. Barker et al. (2010) compared the treatment of localized tissue recession using AlloDerm® and Puros Dermis® in a split-mouth design randomized controlled trial. There was no difference in amount of root coverage, probing depth or keratinized tissue among the two ADMA materials. Both materials were successful in achieving over 80% of root coverage (Barker et al., 2010). Puros ADMA has also been used to increase the thickness of the mucosa at implant placement (Farina and Zaffe, 2015). It was shown that while histologically there was no significant difference between grafted and nongrafted sites, clinically Puros Dermis® was able to significantly increase mucosal thickness around implants especially in sites with thin gingival biotype (Farina and Zaffe, 2015).

16.5 Xenogeneic materials

16.5.1 Extracellular matrix (ECM) membrane

16.5.1.1 MucoMatrixX

MucomatrixX (Dentegris International, Duisburg) is a collagen tissue matrix derived from animal dermis (Fig. 16.5). It is manufactured by a multistep preparation process to remove all the potential immunogenic components from the dermis (Ramachandra et al., 2014). The final product consists of a 3D stable matrix of collagen and elastin suitable to be used as a soft tissue graft. Photomicrographic assessment of the MucoMatrixX revealed that the collagen tissue matrix closely resembles the connective tissue of the normal healthy gingiva. However, the material has not been clinically validated with only one case report describing its use. This report described a single case of MucoMatrixX used as a soft tissue graft in combination with a coronally advanced flap for root coverage purposes

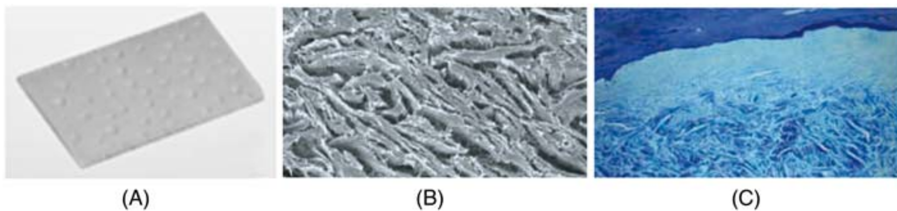


Figure 16.5 (A) MucomatrixX (Dentegris International, Duisburg); (B) REM image of MucoMatrixX; (C) histology of MucoMatrixX 6 months after implantation showing optimal integration without inflammatory reaction.
Adapted from: (A and B) Dentegris International brochure with permission.

(Ramachandra et al., 2014). No other studies were found to support the use of MucoMatrixX in periodontal soft tissue reconstruction.

16.5.1.2 DynaMatrix

DynaMatrix (Keystone Dental, MA) is an ECM membrane obtained from the submucosa of the small intestine of pigs using a process that retains the natural composition of matrix molecules such as collagens (types I, III, IV, and VI), glycosaminoglycans, glycoproteins, proteoglycans, and growth factors, which are known to play important roles in tissue repair and remodeling (Fig. 16.6) (Badylak, 1993; Hodde et al., 2007a; Nevins et al., 2010). Its 3D architecture, composition, and bioactivity are retained following treatment with an oxidizing agent, lyophilization, and after sterilization with ethylene oxide gas (Hodde et al., 2007a; Hodde et al., 2007b).

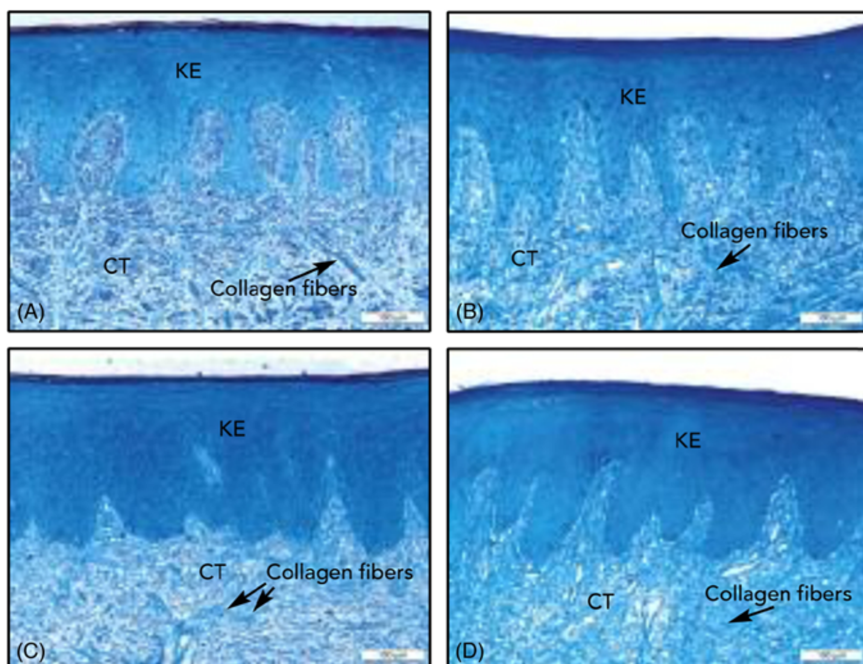


Figure 16.6 Biopsies of both autogenous gingival graft (A and C) and DynaMatrix membrane sites (B and D) appear to be similar histologically, with mature connective tissue covered by keratinized epithelium. There was a small band of dense orthokeratinization at the top of the epithelium in all specimens. The size and appearance of the rete pegs were comparable within the individual. *KE*, keratinized epithelium; *CT*, connective tissue. Adapted from: Nevins, M., Nevins, M.L., Camelo, M., Camelo, J.M.B., Schupbach, P., Kim, D.M., 2010. The clinical efficacy of DynaMatrix extracellular membrane in augmenting keratinized tissue. Int. J. Periodont. Restorative Dent. 30 with permission.

The principle behind using ECM membranes in soft tissue reconstruction is that implantation of this type of graft material can improve the natural wound healing environment as it mimics the natural structure and composition of the surrounding tissue at the recipient site (Hodde et al., 2007a). While synthetic materials may be fabricated to mimic the 3D architecture of the surrounding tissues, biologic scaffolds derived from ECM can be implanted in their natural forms to achieve the same result. The challenge in preparing these biologic scaffold materials for clinical use, however, lies in retaining the natural structure and composition of the ECM while ensuring its safety for clinical implantation (Hodde et al., 2007a).

Processing methods used to achieve clinical safety often include steps that subject the ECM to acids, enzymes, or other chemical treatments. These steps can denature the biomaterial, eliminate its inherent bioactivity, and prevent its ability to interact with the patient's cells. For example, crosslinking agents such as glutaraldehyde or hexamethylene diisocyanate are often used to increase membrane strength and reduce antigenicity. However, these compounds may also reduce the material's biocompatibility, inhibit the ability of cells to interact with the treated material and may also cause cutaneous sensitization and lead to calcification (Hodde et al., 2007a). Enzymes such as trypsin, amylase, and neuramidase are often used to reduce rejection potential because they semi-selectively remove matrix components from the finished product. Such treatments, however, also remove potentially valuable matrix constituents, such as growth factors and glycosaminoglycans (Hodde et al., 2007a). Chemicals such as hydrogen peroxide or peracetic acid are often used as disinfectants, but these oxidize the biomaterial and may reduce the structural integrity of the collagen fibers. Oxidative modification of glycosaminoglycans fractures and impairs their ability to interact with growth factors and other essential matrix components, while oxidation of proteins often renders them inactive, eliminating their bioactive properties (Uchiyama et al., 1990; Hodde et al., 2007a).

Preclinical studies have documented that the DynaMatrix membrane stimulates epidermal cell differentiation and basement formation (Lindberg and Badylak, 2001), supports angiogenesis in vitro and in vivo (Nihsen et al., 2008), and supports cellular adherence and stimulates differentiation and proliferation (Lindberg and Badylak, 2001; Badylak et al., 1999, 2001). Thus these properties may be able to facilitate keratinization over the membrane similar to that observed with autogenous graft healing. It was proposed that the epithelium populates the DynaMatrix membrane through migration of cells from the denuded epithelium, thus inducing secondary epithelialization by "creeping over" the wound bed. Indeed, the scaffold has been shown to facilitate the repopulation of fibroblasts, blood vessels, and epithelium from the surrounding tissues (Wei et al., 2002). It appears that the DynaMatrix membrane is readily incorporated into the recipient bed because of the essential biologic components of healing that it contains: matrix scaffold (ECM) and signals (growth factors and ECM cell receptor-mediated binding sites) (Hodde et al., 2007a). Growth factors such as fibroblast growth factor-2, transforming growth factor-1, and CTG factor are important stimulators of angiogenesis, capillary ingrowth, and tissue regeneration (Inkinen et al., 2003; Presta et al., 2005).

In terms of periodontal soft tissue reconstruction, Nevins et al. (2010) conducted a randomized, controlled split-mouth study to compare the efficacy and feasibility of DynaMatrix with that of an autogenous gingival graft in increasing the width of attached keratinized tissue (Nevins et al., 2010). Patients received the autogenous gingival graft (control) on one side and the DynaMatrix membrane (test) on the contralateral side. After 13 weeks, both test and control sites achieved a clinically significant increase in the amount of keratinized gingiva, and the DynaMatrix membrane-treated sites blended well with the surrounding tissue, with a better appearance when compared to the autogenous gingival grafted sites. The biopsy specimens of both test and control sites appeared to be similar histologically, with mature connective tissue covered by keratinized epithelium (Fig. 16.6). The results of both clinical and histologic evaluations have suggested a potential application of an ECM membrane in achieving gingival augmentation (Nevins et al., 2010). Further large-scale, multicenter, long-term clinical trials are needed to validate its efficacy (Kim and Neiva, 2015).

16.5.2 Bilayer collagen matrix

Mucograft (Geistlich Pharma AG, Wolhusen, Switzerland) is a resorbable porcine collagen matrix manufactured into a bilayered structure designed for periodontal soft tissue augmentation (Fig. 16.7) (Sanz et al., 2009; Ghanaati et al., 2011; Lima et al., 2015). The matrix is composed of pure collagen type I and type III without further crosslinking or chemical treatment and is obtained by a proprietary standardized manufacturing process, sterilized by gamma irradiation and is carefully purified to avoid antigenic reactions (Ghanaati et al., 2011).

The thin, smooth, and low-porosity compact layer, which consists of compact collagen fibers with occlusive cellular properties, allows tissue adherence as a prerequisite for favorable wound healing. This layer, made from porcine peritoneum, not only protects against bacterial infiltration during open healing conditions, it also contains adequate elastic properties to accommodate suturing. The second layer, derived from porcine skin, consists of a thick, porous, 3D spongy collagen structure, which is placed next to the host tissues to facilitate tissue adherence, organization of the blood clot and promote neoangiogenesis and tissue integration (Ghanaati et al., 2011; Ramachandra et al., 2014; Camelo et al., 2012). The porosity is obtained through defined parameters and a controlled lyophilization process. Both layers are combined through a biophysical interweaving process without any chemical manipulation. The volume fraction of pores in the matrix is 90% and the size distribution for these pores ranges from 5 to 200 μm, with smaller pores being primarily located on the compact layer and larger pores found in the spongy layer (Ghanaati et al., 2011).

The design rationale for this highly porous collagen matrix is to allow it to serve as a scaffold for soft tissue integration from the adjacent gingival tissues, thus promoting the ingrowth and repopulation of fibroblasts and blood vessels and the epithelialization with keratinized gingiva. The more porous layer is rapidly infiltrated by host mesenchymal cells, while the layer designed to be a barrier allowed cell

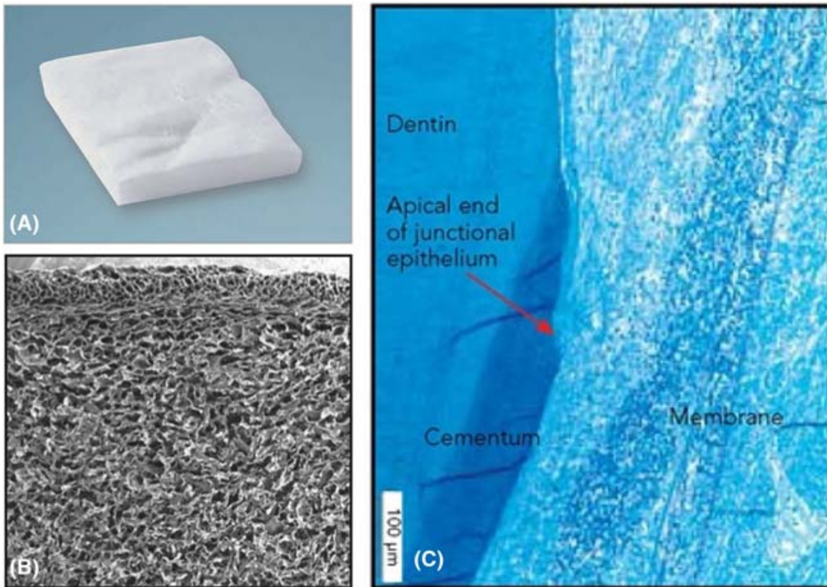


Figure 16.7 (A) Geistlich Mucograft 3D Collagen Matrix; (B) scanning electronic microscopy of Mucograft (right); (C) histology of the Mucograft membrane in combination with CAF for treatment of gingival recession showing active resorption and dissolution, with no evidence of inflammatory cell infiltrate.
Adapted from: (B) Geistlich brochure; (C) Camelo, M., Nevins, M., Nevins, M.L., Schupbach, P., Kim, D.M., 2012. Treatment of gingival recession defects with xenogenic collagen matrix: a histologic report. Int. J. Periodont. Restorative Dent. 32 with permission.

attachment and host tissue integration, but at the same time remain impermeable to invading cells for the first 30 days (Ghanaati et al., 2011). Histological evaluation of this matrix showed acceptable tissue integration, even in open (nonsubmerged) healing conditions. At 1 week the matrix could be easily identified in a highly inflamed and vascularized connective tissue. At 1 month, only remnants of the matrix could be identified close to areas of crestal bone resorption whereas inflammation was mainly limited to an area underneath the sulcular and junctional epithelia. At 3 months the matrix was no longer identifiable and a dense fiber-rich connective tissue was observed. After 6 months the collagen matrix is fully integrated into the surrounding tissues without signs of inflammation (Jung et al., 2011; Vignoletti et al., 2015). Areas of regeneration also demonstrated a similar appearance to that of the surrounding natural soft tissues, both in terms of texture and color, which makes its use preferable in esthetic areas that are difficult to match with palatal FGGs (Nevins et al., 2011; Schmitt et al., 2015; Chambrone and Tatakis, 2015). The tissue reaction associated with Mucograft appears to be favorable, as unlike a typical foreign body response, it is not associated with the presence of multinucleated giant cells, lymphocytes, or persistently inflamed tissue.

This matrix has been extensively studied in clinical trials as an alternative to harvesting of autogenous CTGs from the palate. It has been used to address a variety of indications involving the loss of connective tissue structures, including recession coverage and regeneration of keratinized mucosa around teeth and implants (Sanz et al., 2009; Herford et al., 2010; McGuire and Scheyer, 2010; Nevins et al., 2011; Cardaropoli et al., 2012; Fu et al., 2012; Thoma et al., 2012; Schmitt et al., 2015). The collagen matrix, when used as a soft tissue substitute for increasing the width of keratinized tissue or mucosa, appears to be as effective and predictable as a FGG. Two clinical trials reported similar results with a consistent increase in the width of keratinized tissue ranging between 2.5 and 3 mm (Sanz et al., 2009; Lorenzo et al., 2012). Additionally, the use of the collagen matrix significantly reduced surgical time when compared with autologous grafting, and avoided the need for a tissue harvesting procedure and hence significantly reduced postoperative morbidity (Sanz et al., 2009; Lima et al., 2015; Zucchelli and Mounssif, 2015; Fu et al., 2012).

Overall, this bilayered matrix elicits a favorable tissue reaction, demonstrates potential as a scaffold for preferential tissue ingrowth, and achieves a desirable therapeutic outcome when applied in periodontal soft tissue reconstruction around teeth and implants (Ghanaati et al., 2011), especially when the primary aim is to increase the width of keratinized tissue. However, its effectiveness as a CTG alternative in root coverage procedures has not been convincingly demonstrated.

16.6 Autogenous materials

16.6.1 Platelet-rich fibrin membrane

Platelet-rich fibrin (PRF) membrane is defined as an autogenous leukocyte and platelet-rich fibrin biomaterial that is prepared from the patient's own blood and can be used as a membrane for root coverage purposes (Fig. 16.8) (Toffler et al.,

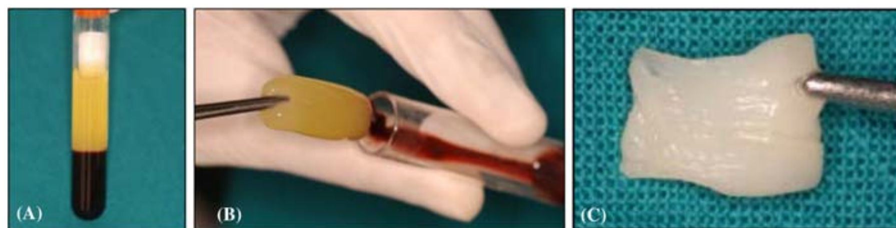


Figure 16.8 (A) Blood centrifugation immediately after collection allows the position of a structured and resistant fibrin clot; (B) PRF clot removal; (C) autologous PRF membrane obtained by driving out the serum from the clot.
Adapted from: Jankovic, S., Aleksic, Z., Klokkevold, P., Lekovic, V., Dimitrijevic, B., Barrie Kenney, E., et al., 2012. Use of platelet-rich fibrin membrane following treatment of gingival recession: a randomized clinical trial. Int. J. Periodontics Restorative Dent. 32, 165 with permission.

2009; Dohan et al., 2010; Kumar and Shubhashini, 2013). It belongs to a new generation of platelet concentrates that can be prepared using simplified processing methods, and was first developed in France by Choukroun et al. in 2001 to accelerate soft and hard tissue healing in oral and maxillofacial surgery (Kumar and Shubhashini, 2013).

The PRF membrane preparation technique requires neither anticoagulant nor bovine thrombin (Kumar and Shubhashini, 2013; Toffler et al., 2009). The PRF production protocol attempts to accumulate platelets and released cytokines in a fibrin clot (Naik et al., 2013). This clot incorporates many wound healing promoters present in the initial blood harvest. It can be used directly as a clot or, after compression, as a mechanically stable membrane that can be readily handled (Toffler et al., 2009; Dohan et al., 2010).

There are widespread applications of this autologous biomaterial in oral, maxillofacial, ear, nose, and throat and plastic surgery (Kumar and Shubhashini, 2013). In periodontal surgery, PRF membranes may be utilized in combination with graft materials to expedite healing in lateral sinus floor elevations (Choukroun et al., 2006b; Inchingolo et al., 2010), ridge augmentations (Toffler et al., 2009), maxillary reconstructions (Toffler et al., 2009), regeneration following cyst ablation (Choukroun et al., 2006a), guided bone regeneration (Del Corso et al., 2010), and socket preservation (Zhao et al., 2011). In terms of periodontal soft tissue reconstruction, PRF membranes have been used in conjunction with different root coverage techniques for the treatment of gingival recession. Anilkumar et al. (2009) used PRF membrane along with a laterally displaced flap technique for the treatment of an isolated recession defect and reported complete root coverage with excellent gingival tissue status after 6 months. On the other hand, Aroca et al. (2009) reported that the addition of a PRF membrane positioned under a modified coronally advanced flap (MCAF) provided inferior root coverage but achieved additional gain in gingival/mucosal thickness at 6 months compared to MCAF alone (Aroca et al., 2009).

The effectiveness of PRF in the treatment of gingival recession is debatable due to a lack of long-term follow-up studies and contradicting results; however, the biggest advantage of PRF as a membrane is that it is both autologous in nature and can be prepared chairside (Barker et al., 2010).

16.7 Tissue engineering

16.7.1 Living cellular construct

Tissue engineering involves the use of living cells, manipulated through their extracellular environment and even genetically, to develop biological substitutes for implantation into the body to regenerate the lost biological function of the respective tissue (Nerem and Sambanis, 1995; Bartold et al., 2016; Ivanovski et al., 2014). It involves the interplay between three components: (1) the implanted and cultured cells that will create the new tissue; (2) a biomaterial to act as an ECM,

carrier or scaffold to support the cells and facilitate their delivery; and (3) the appropriate levels and sequencing of regulatory signaling molecules that instruct the cells to form the desired tissue type (Bartold et al., 2016, 2000, 2006).

In recent years, 3D tissue-engineered mucosal soft tissue constructs have been developed, optimized, and characterized. Different biomaterials have been used in the past decades as connective tissue scaffolds, involving a variety of cell-culture techniques and tissue engineering approaches to reconstruct soft tissues (Moharamzadeh et al., 2012; Ivanovski et al., 2014). Based on this knowledge, tissue engineering technology has also been applied in periodontal soft tissue reconstruction. Indeed, in mucogingival procedures for gingival augmentation, the surgically denuded periosteum presents in a similar condition to that encountered in dermatological wound exposure (Pini Prato et al., 2003). The exposed recipient site, which is the source of blood supply, can be covered with cultivated keratodermal cells, instead of a traditional epithelial-CTG (Pini Prato et al., 2003).

Recent investigations have explored the possibility of using live, tissue-engineered constructs (or living cell constructs, LCC), as alternatives to palatal autografts, allogeneic grafts synthetic biomaterials for mucogingival surgery (Nevins, 2010). LCC is composed of a device component (collagen matrix) and biologic components (cells and their products). It is a bilayered construct of allogeneic, viable neonatal cells comprised of a lower fibroblast layer and an upper keratinocyte layer (Milstone et al., 2000; Kim and Neiva, 2015). The LCC produces cytokines and cell growth factors (e.g., vascular endothelial growth factor, platelet-derived growth factor, bone morphogenetic protein 2, and transforming growth factor β) involved not only in cell proliferation but also angiogenesis, which is a critical component of wound healing in tissue regeneration (Milstone et al., 2000; McGuire et al., 2011). The two layers of the construct appear to act synergistically, presenting a broader array of cytokines than either layer would present alone (Nevins, 2010).

Although the mechanism of action of the LCC has not been fully elucidated, it has been postulated that it improves the wound environment through growth factor interactions, matrix deposition and degradation, wound coverage, and the provision of responsive cells (McGuire et al., 2011; Morelli et al., 2011). It has been established that LCC does not function as an autograft, which normally vascularizes, integrates, and persists in situ, retaining the characteristics of the tissue of origin; instead, the LCC appears to stimulate the patient's own cells to regenerate site-appropriate tissue through the modulation and improvement of secondary intention healing (McGuire et al., 2011; Waymack et al., 2000; Muhart et al., 1999; McGuire et al., 2008).

Despite being comprised of allogenic cells, numerous studies have determined that the LCC does not elicit an immune reaction (McGuire et al., 2011; Waymack et al., 2000; Muhart et al., 1999; Griffiths et al., 2004; McGuire et al., 2008). Because the LCC is no longer present after the first few weeks, LCC appears to act as a wound healing therapy rather than a graft, perhaps guiding the patient's own cells to the treatment site to develop new tissues (Nevins et al., 2010).

McGuire et al. conducted two randomized, within-patient (split-mouth), controlled studies that investigated LCC (CelTx, Organogenesis, Canton, MA) derived from human allogenic fibroblasts, keratinocytes, bovine collagen, and human

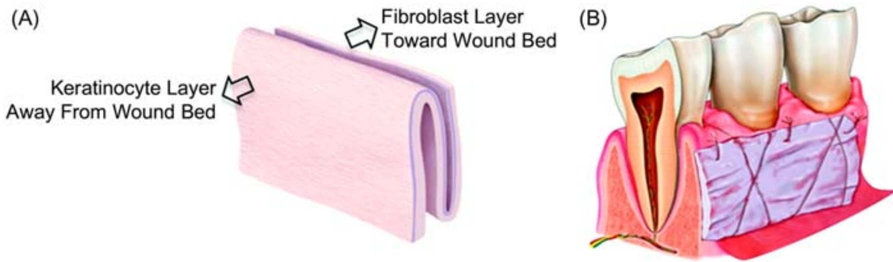


Figure 16.9 (A) The LCC arranged in a Z-fold fashion; (B) placed over an oral defect in which the mucosal and keratinized gingiva was removed (B).
Adapted from: McGuire, M.K., Scheyer, E.T., Nevins, M.L., Neiva, R., Cochran, D.L., Mellonig, J.T., et al., 2011. Living cellular construct for increasing the width of keratinized gingiva: results from a randomized, within-patient, controlled trial. J. Periodontol. 82, 1414−1423 with permission.

extracellular matrix proteins as an alternative to autogenous FGG for increasing the width of keratinized gingiva (McGuire et al., 2011, 2008). In these studies, the LCC was prepared in a Z-fold with the keratinocyte cell layer facing out (away from wound bed) and fibroblast layer facing toward the wound bed (Fig. 16.9). LCC was found to predictably generate a clinically significant zone of keratinized tissue ≥ 1 mm around teeth by 6 months. In addition, more patients preferred treatment with the LCC therapy over autograft surgery with a palatal donor site (McGuire et al., 2011, 2008).

In a case series of using LCC as an alternative to autogenous FGG for treatment of mucogingival defects, histological evaluation of the treatment sites at 3 to 7 weeks revealed clinically normal gingival tissue consisting of parakeratinized epithelium with a very sparse and diffuse chronic inflammatory response within the connective tissue (Nevins, 2010). DNA persistence analysis indicated that only each subject's DNA was present in the healing sites at the time of biopsy, demonstrating a lack of LCC persistence (Nevins, 2010). Scheyer et al. (2014) also provided histologic findings showing that LCC-treated sites resembled gingiva rather than alveolar mucosa. Additionally, Morelli et al. (2011) demonstrated that, during early wound healing events, expression of angiogenic-related biomarkers is upregulated in sites treated with LCC compared with autogenous FGGs.

The mechanism of action of LCC is still unknown (Morelli et al., 2011). However, it is speculated to modulate healing by secondary intention of the surrounding soft tissues. Living cellular constructs act as a temporary wound covering that is eventually replaced by host cells. DNA of allogeneic fibroblasts and keratinocytes placed over wounds was no longer present after 6 weeks of healing (Griffiths et al., 2004; Morelli et al., 2011). It is postulated that the population of live fibroblasts and keratinocytes improves the wound environment through growth factor interactions, matrix deposition and degradation, wound coverage, and a provision of responsive cells, leading to a clinically beneficial outcome (Sabolinski et al., 1996).

Tissue-engineered products such as LCC may have their role in gingival augmentation procedures (Kim and Neiva, 2015). Nonetheless, long-term follow-up studies are recommended, and at present, the use of tissue-engineered soft tissue graft substitutes are limited by high production costs, a small window of utilization, and the complex nature of manufacturing tissue-engineered products containing living cells (Kim and Neiva, 2015; Nevins et al., 2010; Pini Prato et al., 2000, 2003; McGuire et al., 2008; McGuire and Nunn, 2005; Mohammadi et al., 2007).

16.8 Alloplastic materials

16.8.1 Three-dimensional printing scaffolds

When tissue engineering approaches are applied to the reconstruction of complex tissue structures such as the periodontium, biomaterials can serve as 3D templates and synthetic extracellular matrix environments for the regenerative process (Tevlin et al., 2014; Obregon et al., 2015). 3D bioprinted tissues and biological structures have been proposed as promising alternative biomaterials in regenerative medicine and dentistry (Obregon et al., 2015). Different 3D bioprinting methods and different classes of biomaterials (polymer hydrogels, ceramics, composites, and cell aggregates) may be used for manufacturing of scaffolds, as well as craniofacial tissue analogs. 3D printing of biomaterials represent a promising tools, allowing customization to the desired size, configuration, and architecture of a given defect (Rasperini et al., 2015). While the fabrication of scaffolds upon which cells attach, migrate, and proliferate is already in use, printing of all the components that form a tissue (living cells and matrix materials together) to produce individualized tissue constructs is still in its early stages (Obregon et al., 2015).

Rasperini et al. reported the first human case of treatment of a large periodontal soft and osseous defect with a 3D-printed bioresorbable patient-specific polymer scaffold incorporating a signaling growth factor (Rasperini et al., 2015). A customized scaffold was 3D printed by selective laser sintering using medical-grade polycaprolactone (PCL) to fit the periosseous defect according to the prototyped model derived from the patient's cone beam computed tomography scan (Fig. 16.10). The scaffold's internal region consisted of extended pegs oriented perpendicularly to the root for the support and guidance of periodontal ligament formation, perforations for fixation, and an internal compartment for delivery of recombinant human platelet-derived growth factor BB (rhPDGF-BB). The 3D scaffold was immersed in rhPDGF-BB for 15 min and then surgically delivered into the defect and fixed with ultrasound-activated resorbable poly-D and L-lactic acid pins (SonicWeld, KLS Martin Group, Tuttlingen, Germany). The scaffold remained in situ for 12 months, unfortunately after which it became exposed and was removed. The authors attributed the failure to the prolonged resorption rate of the material and recommended the use of a more rapidly resorbing matrix with a healing window of less than 1 year combined with a less bulky design to minimize wound dehiscence, exposure, and subsequent microbial contamination (Rasperini et al., 2015). In light of these

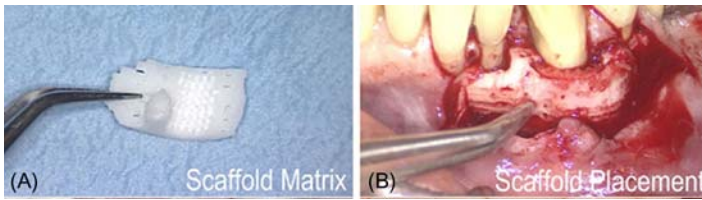


Figure 16.10 (A) Customized scaffold was 3D printed using medical-grade PCL to fit the periosseous defect using a prototyped model of the defect from the patient's cone beam computed tomography scan. (B) Placement of the 3D-printed scaffold into ridge defect following full-thickness flap exposure.
Adapted from: Rasperini, G., Pilipchuk, S., Flanagan, C., Park, C., Pagni, G., Hollister, S., et al., 2015. 3D-printed bioresorbable scaffold for periodontal repair. J. Dent. Res. 94, 153S−157S with permission.

preliminary results the use of customized 3D-printed bioresorbable scaffold to treat a periodontal defect warrants further study for more personalized oral regenerative clinical applications.

16.9 Conclusion

The field of periodontal biomaterials has expanded greatly over the last 40 years and will continue to advance in line with increasing understanding of the multiple disciplines relevant to this area, including clinical and biomaterial sciences, as well as cellular and molecular biology. Many techniques and biomaterials are available for reconstruction of periodontal soft tissue defect. Currently only Mucograft® and Alloderm® have been extensively investigated in randomized controlled clinical trials and have been relatively successful as an alternative to autogenous grafts for periodontal or mucogingival surgery. This review of the literature underlines the importance of design and manipulation of biomaterial composition and morphology, in order to meet the unique challenges involved in the reconstruction of periodontal soft tissue defects. This is especially important in light of the rapidly expanding research, development, and clinical testing in the fields of 3D printing and tissue engineering.

References

Achauer, B.M., Vanderkam, V.M., Celikoz, B., Jacobson, D.G., 1998. Augmentation of facial soft-tissue defects with Alloderm dermal graft. Ann. Plast. Surg. 41, 503−507.

Agarwal, C., Kumar, A.T., Mehta, D.S., 2015. Comparative evaluation of free gingival graft and AlloDerm® in enhancing the width of attached gingival: a clinical study. Contemp. Clin. Dent. 6, 483.

Alghamdi, H., Babay, N., Sukumaran, A., 2009. Surgical management of gingival recession: a clinical update. Saudi Dent. J. 21, 83–94.

Anilkumar, K., Geetha, A., Umasudhakar, T.R., Vijayalakshmi, R., Pameela, E., 2009. Platelet-rich-fibrin: a novel root coverage approach. J. Indian Soc. Periodontol. 13, 50.

Aroca, S., Keglevich, T., Barbieri, B., Gera, I., Etienne, D., 2009. Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: a 6-month study. J. Periodontol. 80, 244–252.

Badylak, S., Liang, A., Record, R., Tullius, R., Hodde, J., 1999. Endothelial cell adherence to small intestinal submucosa: an acellular bioscaffold. Biomaterials. 20, 2257–2263.

Badylak, S.F., 1993. Small intestinal submucosa (SIS): a biomaterial conducive to smart tissue remodeling. Tissue Engineering. Springer, Boston.

Badylak, S.F., Park, K., Peppas, N., Mccabe, G., Yoder, M., 2001. Marrow-derived cells populate scaffolds composed of xenogeneic extracellular matrix. Exp. Hematol. 29, 1310–1318.

Barker, T.S., Cueva, M.A., Rivera-Hidalgo, F., Beach, M.M., Rossmann, J.A., Kerns, D.G., et al., 2010. A comparative study of root coverage using two different acellular dermal matrix products. J. Periodontol. 81, 1596–1603.

Barros, R.R., Novaes Jr, A.B., Grisi, M.F., Souza, S.L., Taba Jr, M., Palioto, D.B., 2004. A 6-month comparative clinical study of a conventional and a new surgical approach for root coverage with acellular dermal matrix. J. Periodontol. 75, 1350–1356.

Bartold, P., Gronthos, S., Ivanovski, S., Fisher, A., Hutmacher, D., 2016. Tissue engineered periodontal products. J. Periodontal. Res. 51, 1–15.

Bartold, P., Mcculloch, C.A., Narayanan, A.S., Pitaru, S., 2000. Tissue engineering: a new paradigm for periodontal regeneration based on molecular and cell biology. Periodontology 2000(24), 253–269.

Bartold, P.M., Xiao, Y., Lyngstaadas, S.P., Paine, M.L., Snead, M.L., 2006. Principles and applications of cell delivery systems for periodontal regeneration. Periodontology 2000 (41), 123–135.

Cairo, F., Cortellini, P., Tonetti, M., Nieri, M., Mervelt, J., Cincinelli, S., et al., 2012. Coronally advanced flap with and without connective tissue graft for the treatment of single maxillary gingival recession with loss of inter-dental attachment. A randomized controlled clinical trial. J. Clin. Periodontol. 39, 760–768.

Callan, D., 1990. Use of human freeze-dried demineralized bone prior to implantation. Part I. Pract. Perio Aest Dent. 2, 14–18.

Callan, D., Silverstein, L., 1998. Use of acellular dermal matrix for increasing keratinized tissue around teeth and implants. Pract. Periodont. Aesthet. Dent. 10, 731–734.

Camargo, P.M., Melnick, P.R., Kenney, E.B., 2001. The use of free gingival grafts for aesthetic purposes. Periodontology 2000(27), 72–96.

Camelo, M., Nevins, M., Nevins, M.L., Schupbach, P., Kim, D.M., 2012. Treatment of gingival recession defects with xenogenic collagen matrix: a histologic report. Int. J. Periodontics Restorative Dent. 32 (2), 167–173.

Cardaropoli, D., Tamagnone, L., Roffredo, A., Gaveglio, L., 2012. Treatment of gingival recession defects using coronally advanced flap with a porcine collagen matrix compared to coronally advanced flap with connective tissue graft: a randomized controlled clinical trial. J. Periodontol. 83, 321–328.

Chambrone, L., Tatakis, D.N., 2015. Periodontal soft tissue root coverage procedures: a systematic review from the AAP Regeneration Workshop. J. Periodontol. 86, S8–S51.

Chambrone, L.A., Chambrone, L., 2006. Subepithelial connective tissue grafts in the treatment of multiple recession-type defects. J. Periodontol. 77, 909–916.
Choukroun, J., Diss, A., Simonpieri, A., Girard, M.-O., Schoeffler, C., Dohan, S.L., et al., 2006a. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol. 101, e56–e60.
Choukroun, J., Diss, A., Simonpieri, A., Girard, M.-O., Schoeffler, C., Dohan, S.L., et al., 2006b. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol. 101, 299–303.
Cummings, L.C., Kaldahl, W.B., Allen, E.P., 2005. Histologic evaluation of autogenous connective tissue and acellular dermal matrix grafts in humans. J. Periodontol. 76, 178–186.
De Queiroz Côrtes, A., Martins, Â.G., Nociti Jr, F.H., Sallum, A.W., Casati, M.Z., Sallum, E. A., 2004. Coronally positioned flap with or without acellular dermal matrix graft in the treatment of Class I gingival recessions: a randomized controlled clinical study. J. Periodontol. 75, 1137–1144.
Del Corso, M., Toffler, M., Dohan Ehrenfest, D., 2010. Use of an autologous leukocyte and platelet-rich fibrin (L-PRF) membrane in post-avulsion sites: an overview of Choukroun's PRF. J. Implant. Adv. Clin. Dent. 1, 27–35.
Dohan, D., Corso, M., Diss, A., Mouhyi, J., Charrier, J., 2010. Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. J. Periodontol. 81, 546–555.
Farina, V., Zaffe, D., 2015. Changes in thickness of mucosa adjacent to implants using tissue matrix allograft: a clinical and histologic evaluation. Int J Oral Maxillofac Implants. 30 (4), 909–917.
Fu, J.-H., Su, C.-Y., Wang, H.-L., 2012. Esthetic soft tissue management for teeth and implants. J. Evidence Based Dent. Practice. 12, 129–142.
Ghanaati, S., Schlee, M., Webber, M.J., Willershausen, I., Barbeck, M., Balic, E., et al., 2011. Evaluation of the tissue reaction to a new bilayered collagen matrix in vivo and its translation to the clinic. Biomed. Mater. 6, 015010.
Gottlow, J., 1993. Guided tissue regeneration using bioresorbable and non-resorbable devices: initial healing and long-term results. J. Periodontol. 64, 1157–1165.
Griffin, T.J., Cheung, W.S., Zavras, A.I., Damoulis, P.D., 2006. Postoperative complications following gingival augmentation procedures. J. Periodontol. 77, 2070–2079.
Griffiths, M., Ojeh, N., Livingstone, R., Price, R., Navsaria, H., 2004. Survival of Apligraf in acute human wounds. Tissue Eng. 10, 1180–1195.
Hanker, J.S., Giammara, B.L., 1988. Biomaterials and biomedical devices. Science. 242, 885–892.
Hardwick, R., Hayes, B.K., Flynn, C., 1995. Devices for dentoalveolar regeneration: an up-to-date literature review. J. Periodontol. 66, 495–505.
Herford, A.S., Akin, L., Cicciu, M., Maiorana, C., Boyne, P.J., 2010. Use of a porcine collagen matrix as an alternative to autogenous tissue for grafting oral soft tissue defects. J. Oral Maxillofac. Surg. 68, 1463–1470.
Hinton, R., Jinnah, R.H., Johnson, C., Warden, K., Clarke, H.J., 1992. A biomechanical analysis of solvent-dehydrated and freeze-dried human fascia lata allografts A preliminary report. Am. J. Sports Med. 20, 607–612.
Hodde, J., Janis, A., Ernst, D., Zopf, D., Sherman, D., Johnson, C., 2007a. Effects of sterilization on an extracellular matrix scaffold: part I. Composition and matrix architecture. J. Mater. Sci.: Mater. Med. 18, 537–543.

Hodde, J., Janis, A., Hiles, M., 2007b. Effects of sterilization on an extracellular matrix scaffold: part II. Bioactivity and matrix interaction. J. Mater. Sci.: Mater. Med. 18, 545–550.

Inchingolo, F., Tatullo, M., Marrelli, M., Inchingolo, A., Scacco, S., Inchingolo, A., et al., 2010. Trial with platelet-rich fibrin and bio-oss used as grafting materials in the treatment of the severe maxillar bone atrophy: clinical and radiological evaluations. Eur. Rev. Med. Pharmacol. Sci. 14, 1075–1084.

Inkinen, K., Wolff, H., Lindroos, P., Ahonen, J., 2003. Connective tissue growth factor and its correlation to other growth factors in experimental granulation tissue. Connect. Tissue Res. 44, 19–29.

Ivanovski, S., Vaquette, C., Gronthos, S., Hutmacher, D., Bartold, P., 2014. Multiphasic scaffolds for periodontal tissue engineering. J. Dent. Res. 93, 1212–1221.

Jankovic, S., Aleksic, Z., Klokkevold, P., Lekovic, V., Dimitrijevic, B., Barrie Kenney, E., et al., 2012. Use of platelet-rich fibrin membrane following treatment of gingival recession: a randomized clinical trial. Int. J. Periodontics Restorative Dent. 32, 165.

Jhaveri, H.M., Chavan, M.S., Tomar, G.B., Deshmukh, V.L., Wani, M.R., Miller Jr, P.D., 2010. Acellular dermal matrix seeded with autologous gingival fibroblasts for the treatment of gingival recession: a proof-of-concept study. J. Periodontol. 81, 616–625.

Jung, R.E., Hürzeler, M.B., Thoma, D.S., Khraisat, A., Hämmerle, C.H., 2011. Local tolerance and efficiency of two prototype collagen matrices to increase the width of keratinized tissue. J. Clin. Periodontol. 38, 173–179.

Kim, D.M., Neiva, R., 2015. Periodontal soft tissue non–root coverage procedures: a systematic review from the AAP regeneration workshop. J. Periodontol. 86, S56–S72.

Kumar, R.V., Shubhashini, N., 2013. Platelet rich fibrin: a new paradigm in periodontal regeneration. Cell Tissue Bank. 14, 453–463.

Lima, R.S., Peruzzo, D.C., Napimoga, M.H., Saba-Chujfi, E., Santos-Pereira, S.A.D., Martinez, E.F., 2015. Evaluation of the biological behavior of Mucograft® in human gingival fibroblasts: an in vitro study. Braz. Dent. J. 26, 602–606.

Lindberg, K., Badylak, S.F., 2001. Porcine small intestinal submucosa (SIS): a bioscaffold supporting in vitro primary human epidermal cell differentiation and synthesis of basement membrane proteins. Burns. 27, 254–266.

Lorenzo, R., García, V., Orsini, M., Martin, C., Sanz, M., 2012. Clinical efficacy of a xenogeneic collagen matrix in augmenting keratinized mucosa around implants: a randomized controlled prospective clinical trial. Clin. Oral Implants Res. 23, 316–324.

McGuire, M.K., Nunn, M.E., 2005. Evaluation of the safety and efficacy of periodontal applications of a living tissue-engineered human fibroblast-derived dermal substitute. I. Comparison to the gingival autograft: a randomized controlled pilot study. J. Periodontol. 76, 867–880.

McGuire, M.K., Scheyer, E.T., 2010. Xenogeneic collagen matrix with coronally advanced flap compared to connective tissue with coronally advanced flap for the treatment of dehiscence-type recession defects. J. Periodontol. 81, 1108–1117.

McGuire, M.K., Scheyer, E.T., Nevins, M.L., Neiva, R., Cochran, D.L., Mellonig, J.T., et al., 2011. Living cellular construct for increasing the width of keratinized gingiva: results from a randomized, within-patient, controlled trial. J. Periodontol. 82, 1414–1423.

McGuire, M.K., Scheyer, E.T., Nunn, M.E., Lavin, P.T., 2008. A pilot study to evaluate a tissue-engineered bilayered cell therapy as an alternative to tissue from the palate. J. Periodontol. 79, 1847–1856.

Milstone, L., Asgari, M., Schwartz, P., Hardin-Young, J., 2000. Growth factor expression, healing, and structural characteristics of Graftskin (Apligraf). Wounds. 12, 12A–19A.

Mohammadi, M., Shokrgozar, M.A., Mofid, R., 2007. Culture of human gingival fibroblasts on a biodegradable scaffold and evaluation of its effect on attached gingiva: a randomized, controlled pilot study. J. Periodontol. 78, 1897−1903.

Moharamzadeh, K., Colley, H., Murdoch, C., Hearnden, V., Chai, W., Brook, I., et al., 2012. Tissue-engineered oral mucosa. J. Dent. Res. 91, 642−650.

Morelli, T., Neiva, R., Nevins, M., Mcguire, M., Scheyer, E., Oh, T.-J., et al., 2011. Angiogenic biomarkers and healing of living cellular constructs. J. Dent. Res. 90, 456−462.

Muhart, M., Mcfalls, S., Kirsner, R.S., Elgart, G.W., Kerdel, F., Sabolinski, M.L., et al., 1999. Behavior of tissue-engineered skin: a comparison of a living skin equivalent, autograft, and occlusive dressing in human donor sites. Arch. Dermatol. 135, 913−918.

Naik, B., Karunakar, P., Jayadev, M., Marshal, V.R., 2013. Role of Platelet rich fibrin in wound healing: A critical review. J. Conserv. Dent. 16, 284.

Nerem, R.M., Sambanis, A., 1995. Tissue engineering: from biology to biological substitutes. Tissue Eng. 1, 3−13.

Nevins, M., Nevins, M.L., Camelo, M., Camelo, J.M.B., Schupbach, P., Kim, D.M., 2010. The clinical efficacy of DynaMatrix extracellular membrane in augmenting keratinized tissue. Int. J. Periodontics Restorative Dent. 30 (2), 151−161.

Nevins, M., Nevins, M.L., Kim, S.-W., Schupbach, P., Kim, D.M., 2011. The use of mucograft collagen matrix to augment the zone of keratinized tissue around teeth: a pilot study. Int. J. Periodontics Restorative Dent. 31 (4), 367−373.

Nevins, M.L., 2010. Tissue-engineered bilayered cell therapy for the treatment of oral mucosal defects: a case series. Int. J. Periodontics Restorative Dent. 30 (1), 31−39.

Nihsen, E.S., Johnson, C.E., Hiles, M.C., 2008. Bioactivity of small intestinal submucosa and oxidized regenerated cellulose/collagen. Adv. Skin Wound Care. 21, 479−486.

Oates, T.W., Robinson, M., Gunsolley, J.C., 2003. Surgical therapies for the treatment of gingival recession. A systematic review. Ann. Periodontol. 8, 303−320.

Obregon, F., Vaquette, C., Ivanovski, S., Hutmacher, D., Bertassoni, L., 2015. Three-dimensional bioprinting for regenerative dentistry and craniofacial tissue engineering. J. Dent. Res. 94, 143S−152S.

Pachence, J.M., 1996. Collagen-based devices for soft tissue repair. J. Biomed. Mater. Res. 33, 35−40.

Pini Prato, G.P., Rotundo, R., Magnani, C., Soranzo, C., Muzzi, L., Cairo, F., 2003. An autologous cell hyaluronic acid graft technique for gingival augmentation: a case series. J. Periodontol. 74, 262−267.

Pini Prato, G.P., Rotundo, R., Mognoni, C., Soranzo, C., 2000. Tissue engineering technology for gingival augmentation procedures: a case report. Int. J. Periodontics Restorative Dent. 20 (6), 552−559.

Presta, M., Dell'era, P., Mitola, S., Moroni, E., Ronca, R., Rusnati, M., 2005. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine Growth Factor Rev. 16, 159−178.

Ramachandra, S.S., Rana, R., Reetika, S., Jithendra, K., 2014. Options to avoid the second surgical site: a review of literature. Cell Tissue Bank. 15, 297−305.

Rasperini, G., Pilipchuk, S., Flanagan, C., Park, C., Pagni, G., Hollister, S., et al., 2015. 3D-printed bioresorbable scaffold for periodontal repair. J. Dent. Res. 94, 153S−157S.

Rhee, P.H., Friedman, C.D., Ridge, J.A., Kusiak, J., 1998. The use of processed allograft dermal matrix for intraoral resurfacing: an alternative to split-thickness skin grafts. Arch. Otolaryngol. Head Neck Surg. 124, 1201−1204.

Sabolinski, M., Alvarez, O., Auletta, M., Mulder, G., Parenteau, N., 1996. Cultured skin as a ‘smart material’for healing wounds: experience in venous ulcers. Biomaterials. 17, 311–320.

Sanz, M., Lorenzo, R., Aranda, J.J., Martin, C., Orsini, M., 2009. Clinical evaluation of a new collagen matrix (Mucograft® prototype) to enhance the width of keratinized tissue in patients with fixed prosthetic restorations: a randomized prospective clinical trial. J. Clin. Periodontol. 36, 868–876.

Scantlebury, T.V., 1993. 1982–1992: a decade of technology development for guided tissue regeneration. J. Periodontol. 64, 1129–1137.

Scarano, A., Barros, R.R., Iezzi, G., Piattelli, A., Novaes Jr, A.B., 2009. Acellular dermal matrix graft for gingival augmentation: a preliminary clinical, histologic, and ultrastructural evaluation. J. Periodontol. 80, 253–259.

Scheyer, E.T., Nevins, M.L., Neiva, R., Cochran, D.L., Giannobile, W.V., Woo, S.-B., et al., 2014. Generation of site-appropriate tissue by a living cellular sheet in the treatment of mucogingival defects. J. Periodontol. 85, e57–e64.

Schmitt, C.M., Moest, T., Lutz, R., Wehrhan, F., Neukam, F.W., Schlegel, K.A., 2015. Long-term outcomes after vestibuloplasty with a porcine collagen matrix (Mucograft®) versus the free gingival graft: a comparative prospective clinical trial. Clin. Oral Implants Res. 27 (11), e125–e133.

Shulman, J., 1996. Clinical evaluation of an acellular dermal allograft for increasing the zone of attached gingiva. Pract. Periodontics Aesthetic Dent. 8, 201–208.

Silc, J.T., Petrungaro, P.S., 2013. Acellular dermal matrix grafts for root coverage procedures: review of products and introduction of a new technique. Compend. Contin. Educ. Dent. 34, 408–414.

Sullivan, H., Atkins, J.H., 1968. Free autogenous gingival grafts. 3. Utilization of grafts in the treatment of gingival recession. Periodontics. 6, 152–160.

Tal, H., 1999. Subgingival acellular dermal matrix allograft for the treatment of gingival recession: a case report. J. Periodontol. 70, 1118–1124.

Tal, H., Moses, O., Zohar, R., Meir, H., Nemcovsky, C., 2002. Root coverage of advanced gingival recession: A comparative study between acellular dermal matrix allograft and subepithelial connective tissue grafts. J. Periodontol. 73, 1405–1411.

Tevlin, R., Mcardle, A., Atashroo, D., Walmsley, G., Senarath-Yapa, K., Zielins, E., et al., 2014. Biomaterials for craniofacial bone engineering. J. Dent. Res. 93, 1187–1195.

Thoma, D.S., Benić, G.I., Zwahlen, M., Hämmerle, C.H., Jung, R.E., 2009. A systematic review assessing soft tissue augmentation techniques. Clin. Oral Implants Res. 20, 146–165.

Thoma, D.S., Sancho-Puchades, M., Ettlin, D.A., Hämmerle, C.H., Jung, R.E., 2012. Impact of a collagen matrix on early healing, aesthetics and patient morbidity in oral mucosal wounds—a randomized study in humans. J. Clin. Periodontol. 39, 157–165.

Toffler, M., Toscano, N., Holtzclaw, D., Corso, M., Ehrenfest, D.D., 2009. Introducing Choukroun’s platelet rich fibrin (PRF) to the reconstructive surgery milieu. J. Implant Adv. Clin. Dent. 1, 21–30.

Trombelli, L., Minenna, L., Farina, R., Scabbia, A., 2005. Guided tissue regeneration in human gingival recessions. J. Clin. Periodontol. 32, 16–20.

Uchiyama, H., Dobashi, Y., Ohkouchi, K., Nagasawa, K., 1990. Chemical change involved in the oxidative reductive depolymerization of hyaluronic acid. J. Biol. Chem. 265, 7753–7759.

Vignoletti, F., Nuñez, J., Sanctis, F., Lopez, M., Caffesse, R., Sanz, M., 2015. Healing of a xenogeneic collagen matrix for keratinized tissue augmentation. Clin. Oral Implants Res. 26, 545–552.

Wainwright, D., 1995. Use of an acellular allograft dermal matrix (AlloDerm) in the management of full-thickness burns. Burns. 21, 243−248.

Wainwright, D., Madden, M., Luterman, A., Hunt, J., Monafo, W., Heimbach, D., et al., 1996. Clinical evaluation of an acellular allograft dermal matrix in full-thickness burns. J. Burn Care Res. 17, 124−136.

Waymack, P., Duff, R.G., Sabolinski, M., 2000. The effect of a tissue engineered bilayered living skin analog, over meshed split-thickness autografts on the healing of excised burn wounds. Burns. 26, 609−619.

Wei, P.-C., Laurell, L., Lingen, M.W., Geivelis, M., 2002. Acellular dermal matrix allografts to achieve increased attached gingiva. Part 2. A histological comparative study. J. Periodontol. 73, 257−265.

Wong, A.K., Schonmeyer, B.H., Singh, P., Carlson, D.L., Li, S., Mehrara, B.J., 2008. Histologic analysis of angiogenesis and lymphangiogenesis in acellular human dermis. Plastic Reconstruct. Surg. 121, 1144−1152.

Yan, J.-J., Tsai, A.Y.-M., Wong, M.-Y., Hou, L.-T., 2006. Comparison of acellular dermal graft and palatal autograft in the reconstruction of keratinized gingiva around dental implants: a case report. Int. J. Periodontics Restorative Dent. 26 (3), 287−292.

Zhao, J.-H., Tsai, C.-H., Chang, Y.-C., 2011. Clinical and histologic evaluations of healing in an extraction socket filled with platelet-rich fibrin. J. Dent. Sci. 6, 116−122.

Zucchelli, G., Mounssif, I., 2015. Periodontal plastic surgery. Periodontology 2000. 68, 333−368.

Layered scaffolds for periodontal regeneration

17

Ourania-Menti Goudouri[1], *Eleana Kontonasaki*[2] *and*
Aldo R. Boccaccini[1]
[1]University of Erlangen-Nuremberg, Erlangen, Germany,
[2]Aristotle University of Thessaloniki, Thessaloniki, Greece

17.1 Introduction

Alveolar bone loss is a common finding associated with periodontitis (Armitage 2004; Chen and Jin, 2010), which is a highly prevalent disease in humans affecting 90% of the worldwide population (Pihlstrom et al., 2005). Only in the United States, it has been reported to affect more than 45% of the adult US population (141.0 million adults) in the years 2009–2012, with 8.9% diagnosed with severe periodontitis and a prevalence that was highest among adults with less than high school education, adults below 100% of the federal poverty levels, and current smokers (Eke et al., 2012). Additionally, a part of the alveolar process is missing in most cases of orofacial clefts (Mossey and Castilla, 2001), which are the most prevalent congenital craniofacial anomalies and occur in 1:700 births worldwide (Moreau et al., 2007; Mossey and Castilla, 2001).

To date the treatment of alveolus defects relies on the use of autologous bone grafts harvested from the iliac crest. Harvest autologous bone carries along several donor site complication risks, including iliac crest morbidity, chronic postoperative pain, infections, as well as nerve and vascular injuries (Kolomvos et al., 2010; Dimitriou et al., 2011; Ahlmann et al., 2002). Novel approaches in tissue engineering are based on the development of artificial extracellular matrices (ECM) capable of providing support and triggering the differentiation of stem cells via biochemical, mechanical, or topographical cues (Sundelacruz and Kaplan, 2009; Chan and Leong, 2008; Gelain and Gelain, 2008).

Although the use of bilayered scaffolds in interfacial tissue engineering has been well documented (Kon et al., 2014; Liu et al., 2013; Seo et al., 2014), the application of these in the field of periodontal tissue regeneration is still in its infancy. The idea behind the use of multilayered scaffolds in periodontal tissue regeneration originates in the realization of how simplistic—considering the complexity of the tissue to be regenerated—is the approach of introducing a filler material into a periodontal bony defect (Bartold et al., 2000).

Therefore,the aim of this book chapter is to offer an overview of the techniques used for the construction of bilayered scaffolds as well as their potential in regenerating periodontal tissues in vitro and in vivo.

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00017-7
© 2017 Elsevier Ltd. All rights reserved.

17.2 Structure of periodontium

Periodontium (Fig. 17.1) is a complex tissue and consists of root cementum alveolar bone, the periodontal ligament (PDL) and the gingiva (Ho et al., 2007; Chen and Jin, 2010; Reddy, 2005).

Cementum is a hard and thin, avascular mineralized tissue that covers the root surface and serves as an anchor of the Sharpey's fibers within the root surface, supporting the tooth. Histologically, cementum is classified into acellular and cellular, with accelular cementum covering the cervical two-thirds and cellular cementum the apical one-third of the root surface and the furcation area in molars (Hammarström et al., 1996). Cellular cementum consists of cementoblasts, cementum forming cells and cementocytes, although there is no clear answer yet whether these cells constitute a different cell line from bone cells (osteoblasts and osteocytes) (Bosshardt, 2005; MacNeil et al., 1998; Matthews et al., 2016). Depending on the origin of the collagenous fibers embedded in cementum, acellular cementum can be further subdivided to (1) acellular afibrillar cementum, (2) acellular extrinsic fiber cementum (AEFC), and (3) acellular intrinsic fiber cementum (AIFC), while cellular cementum to (1) cellular intrinsic fiber cementum (CIFC) and (2) cellular mixed (i.e., extrinsic and intrinsic) stratified fiber cementum (CMSC) (Bosshardt and Selvig, 1997). Intrinsic fibers lay in parallel to the root surface and are believed to be the result of repairing processes, while extrinsic fibers have a transverse

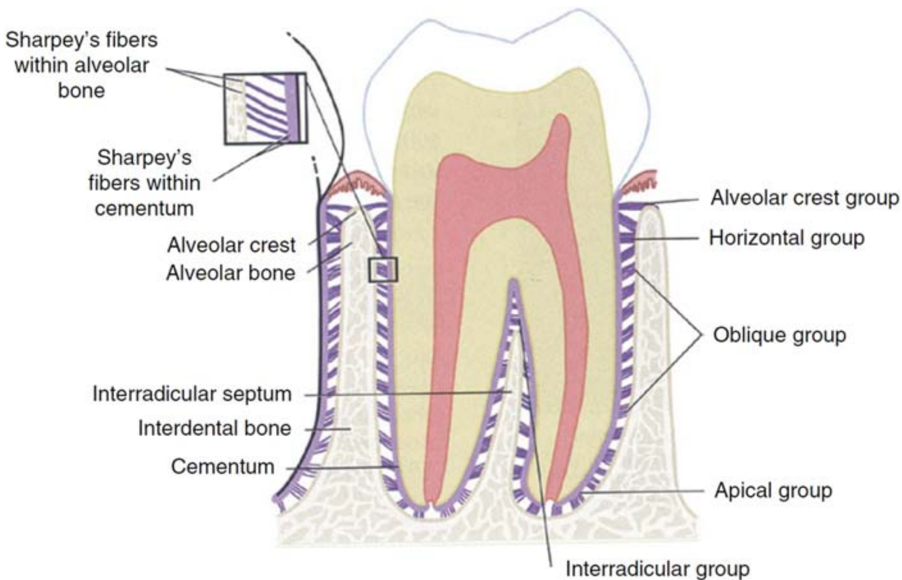


Figure 17.1 Schematic illustration of normal periodontium.
Reproduced from Bath-Balogh, M.M., Fehrenback, M.M., 2006. Dental Embryology, Histology, and Anatomy. Elsevier Inc. by permission from the publisher.

orientation, projecting to the PDL space, anchoring the tooth. AIFC cementum is considered to be CIFC developed slowly so that cementocytes are not embedded and CMSC constitutes a mixture of AEFC and CIFC with their fibers being laid in a different orientation and at different growing rates (Bosshardt and Schroeder, 1992; Xiong et al., 2013). Although the desired type of cementum following regenerative periodontal surgery is the acellular extrinsic fiber cementum, the cellular intrinsic fiber cementum is the most common finding after periodontal therapy (Xiong et al., 2013; Bosshardt and Schroeder, 1991). The inorganic part of cementum is inorganic hydroxyapatite comprising the 45−50% while the rest 50% is composed of collagen type I as the major component, collagen types III and XII (Reddy, 2005) and noncollagenous proteins such as proteoglycans, bone sialoprotein, osteonectin, osteopontin, and osteocalcin (Bosshardt et al., 1998; Matias et al., 2003).

PDL is a complex biological structure in which collagen fibers, blood vessels, and nerves are embedded in a matrix of polysaccharides, providing PDL with an atypical viscoelastic behavior under occlusal loads (Komatsu, 2010). PDL is a highly specialized tissue that participates in a variety of complex molecular and mechanical processes (Nanci, 2013). During tooth development, PDL develops from the dental follicle and by integrating its fibers within root cementum creates a stable biological connection with the dental root (Shimono et al., 2003; Foster et al., 2007). PDL fibrils are composed primarily of type I collagen and they are arranged in distinct fiber bundles (principal fibers) (Nanci, 2013; Nanci and Bosshardt, 2006; Edith et al., 2004) which can be categorized in different groups according to their different orientations, distributions, and functions (Chen and Jin, 2010; Lang et al., 2008). PDL fibrils and fibers are of nano- to microsized order (Beertsen et al., 1997; Schroeder, 1986a) and acquire their final arrangement after full eruption of the tooth and after the tooth gets its complete occlusal contact with its antagonist. This arrangement differs within the different parts of the root. In particular, the fibers lay horizontally in the coronal one-third of the root, they run obliquely from the occlusal surface to the alveolar bone in the middle third and apically from the cementum to the alveolar bone in the apical third of the root (Grant and Bernick, 1972; Bartold and Narayanan, 1998).

The main function of the PDL, which also presents the main challenge in the regeneration of periodontium, is to support the tooth by anchoring it to the mandible or maxilla, while absorbing part of the energy applied to the tooth during mastication (Chen and Jin, 2010; Nanci and Bosshardt, 2006). The resilient support of the tooth under ocllusal loads is achieved by the molecular structure of the type I collagen and the fibers network (McCulloch et al., 2000; Berkovitz, 1990) as well as its blood vessels and the extracellular matrix with proteoglycans, glycoproteins and bound water that may act as shock absorbers (Xiong et al., 2013; Berkovitz, 1990; Wills et al., 1972). Furthermore, PDL contains proprioceptive sensors that provide feedback during chewing, thus regulating the biting process (Hannam, 1982). Stimulation of the PDL from mechanical forces affects the synthesis of mechanoresponsive osteotropic cytokines and growth factors, which mediate numerous cellular and molecular events (Marchesan et al., 2011).

Gingiva comprises part of the masticatory oral mucosa that covers alveolar bone surrounding the tooth and is divided in three anatomical areas: marginal gingiva comprises the free edge of gingiva surrounding the tooth and covers the internal walls of gingival sulcus, attached gingiva is firmly bonded to the underlying periosteum of alveolar bone while interdental gingiva lies on the interproximal area between adjacent teeth (Solanki, 2012). Oral mucosa is a highly vascularized tissue with unique biomechanical properties (Chen et al., 2015). It consists of an outer layer of stratifying squamous epithelium and an underlying layer of fibrous connective tissue called lamina propria. Oral epithelium is comprised of four layers starting from the outside and going deeper (Winning and Townsend, 2000): (1) keratinized layer, (2) granular layer, (3) spinous layer, and (4) basal layer. The superficial hard keratinized epithelial layer protects the underlying tissues from mechanical, chemical, and microbial damage caused by the usual daily oral function. Within this layer a network of neighboring keratinocytes, dispersed in a matrix of viscous mucopolysaccharides provide adequate deformation and load bearing capacity to occlusal loads (Kydd and Daly, 1982). Keratinocytes undergo a continuous cycle of cell death being replaced by new cells originated from undifferentiated cells in the underlying tissues (Eckert and Rorke, 1989). Oral epithelium consists of other cell types, such as Langerhans' cells, Merkel cells, melanocytes, and inflammatory cells.

Gingival epithelium is topographically divided into three types (Schroeder and Listgarten, 1997; Solanki, 2012): (1) junctional epithelium, which serves as a protective seal of the periodontal tissues from the oral environment (Schroeder and Listgarten, 2003) and is a very thin nondifferentiated stratified epithelium with high cellular turnover (Shimono et al., 2003); (2) sulcular epithelium, which covers the internal walls of gingival sulcus and is nonkeratinized squamus epithelium (Newman et al., 2012) and (3) oral epithelium that extends from the muccogingival junction to the free gingival margins and is either keratinized or parakeratinized. The junctional epithelium faces both the lamina propria of the gingival and the tooth surface and is a dynamic tissue essential for protective and regenerative functions (Bosshardt and Lang, 2005).

Lamina propria consists of two layers, the papillary layer in contiguity to the epithelium and the reticular layer adjacent to the alveolar bone.

It is a fibrous connective tissue consisting of collagen fibers (about 60% by volume), fibroblasts (5%), vessels, nerves, and matrix (about 35%) (Newman et al., 2012). Gingival fibers are divided in collagenous, reticulus, and elastic (Newman et al., 2012). Randomly oriented collagen type I fibers form the bulk of the lamina propria and provide high tensile strength to the gingival tissue, while elastic fibers of oxytalan and elaunin are distributed among collagen fibers (Kydd and Daly, 1982; Chavrier et al., 1988). Cementum-oriented, these fibers run towards the gingiva margin and the external part of the labial periosteum and are responsible for the mechanical integrity of gingival tissue against mastication forces (Newman et al., 2012).

Alveolar bone is the part of the jaw bone that anchors the tooth. Alveolar bone is predominantly cancellous (or trabecular) bone surrounded by thin compact (or

cortical) bone (Saffar et al., 1997; Jiang et al., 2016). In the sockets, cancellous bone is usually limited in the apical third of the root area, where medullary spaces are smaller compared to those of the basal jaw bone. In the cervical area, cortical bone plates are firmly attached to the root and minimum or no cancellous bone interposes between the cortices and the inner alveolar wall. Along the root side a thin layer of compact bone called lamina dura is connected through PDL to the root cementum (Saffar et al., 1997). It is perforated by channels through which blood vessels and nerve fibers connect the marrow spaces to the PDL. The lamina dura is influenced by high occlusal loads, periodontal disease and various systematic diseases, and the presence of a radiographic crestal lamina dura is positively associated with clinical periodontal stability (White and Pharoah, 2009; Rams et al., 1994).

Alveolar bone is a highly mineralized tissue and like bone in other parts of human skeleton consists by weight of 25% mineralized tissue, 70% organic matrix (including cells 2−5%), and 15% water (Schroeder, 1986b; Sommerfeldt and Rubin, 2001). The mineral content is mostly poorly crystalline hydroxyapatite due to the incorporation of impurities, such as carbonate, sodium, zinc, and magnesium ions (LeGeros, 1991). It is a calcium-deficient apatite, with a Ca:P ratio less than 1.67, which is the theoretical value for pure hydroxyapatite, $Ca_5(PO_4)_3(OH)$ (LeGeros, 1991). This lack of stoichiometry makes bone hydroxyapatite resorbable, facilitating the bone remodeling process by osteoclasts. The organic matrix consists mostly of collagen type I (Miller and Parker, 1984) and various noncollagen proteins such as osteopontin, osteonectin, bone sialoporotein, etc. (Young et al., 1992). Osteoblasts, osteocytes, and osteoclasts are the basic cell types of alveolar bone, while other cell types such as adipocytes, endothelial cells, and immune competent cells such as macrophages are involved in the homeostasis and functions of the alveolar bone (Nijweide et al., 1986).

17.3 Requirements of a layered scaffold for periodontal regeneration

Periodontal tissue engineering is a challenging process. This is not only because the specific anatomical, morphological, and compositional characteristics of three different (PDL, alveolar bone, and cementum) tissues should be taken under consideration, but also because a gradual, continuous interface between the scaffolds should be created, resembling the physiological interfaces of alveolar bone/PDL and PDL/cementum.

First and foremost alveolus defects as well as periodontal pockets can vary in shape and size (Choi et al., 2012), introducing engineering challenges to the successful regeneration of the alveolar bone. The synthesis of personalized scaffolds according to individual needs and requirements is of major importance and it should be taken under consideration while selecting the fabrication method of the scaffolds.

Furthermore, periodontium involves tissues with anisotropic pore distribution as well a large span of pores sizes ranging from nano- to macropores (Ivanovski et al.,

2014). This complex hierarchical structure demands the compartmentalized synthesis of scaffolds aimed for the regeneration of hard and soft tissues, since no individual technique available to date can produce scaffolds with such an anisotropic pore distribution and size.

An additional requirement is the formation of appropriately oriented PDL fibers, which unravel from cementum anchoring the tooth to the newly formed alveolar bone. The fibrous nature of the PDL suggests the selection of methods that have been traditionally used for the fabrication of fibers, including electrospinning (Goudouri et al., 2016; Vaquette et al., 2012; Costa et al., 2014).

It is important to develop scaffolds that degrade appropriately to the tissue to be regenerated, while the maintenance of a mechanical stable interface during regeneration is of major importance. However, interfacial mechanics in periodontal tissue regeneration has not been adequately studied, while as mentioned by Ivanovski et al. (2014) although there has been an extensive focus on tissue formation, tissue function, including the restoration of physiologic loading and homeostasis, has been sparsely studied.

Finally, epithelial downgrowth along the root surface poses an important concern (Skoglund and Persson, 1985) and should therefore be eliminated. However, bilayered scaffolds can be combined with guided tissue regenerated (GTR) techniques, which have been widely used for the prevention of epithelial attachment as well as the prevention of oral bacteria to insert in the regeneration area (Bunyaratavej and Wang, 2001; Hammerle et al., 2002).

17.4 Current solutions available

17.4.1 Fabrication methods

17.4.1.1 Individual layers

The selection of a given processing method depends strongly on the morphology of the scaffolds needed in the end application, the specific anatomical characteristics of the tissue to be regenerated as well as on the inherent features of the process such as cost, simplicity, and versatility.

Concerning the scaffolds for the regeneration of the alveolar bone, it has been proved that the architecture of the ideal scaffold should support cell penetration, tissue ingrowth and vascularization, and nutrient delivery through an interconnected porous structure with high porosity and large pore diameters (Thavornyutikarn et al., 2014; Loh and Choong, 2013; Hollister, 2005). Therefore fused deposition modeling (FDM) (Ivanovski et al., 2014; Vaquette et al., 2012), foam replica technique, wet spinning (Requicha et al., 2013; Requicha et al., 2014), and CAD/CAM (Park et al., 2010; Park et al., 2012; Chan Ho Park et al., 2014) as well as particle leaching (Carlo Reis et al., 2011) have been selected by many researchers for the synthesis of scaffolds for the regeneration of the alveolar bone (Table 17.1).

Table 17.1 List of the materials and techniques used for the synthesis of bilayered scaffolds

Bone compartment	Ligament/gingiva compartment	Layer assembly	Citation
Starch & PCL (wet spinning)	Starch & PCL (solvent casting)	Chemical leaching with chloroform	Requicha et al. (2013), Requicha et al. (2014)
PGA & PCL (CAD/CAM)	PGA & PCL (CAD/CAM)	Chemical gluing with BioAct VSO	Park et al. (2010), Park et al. (2012), Park et al. (2014)
PCL & β-TCP (fused deposition modeling)	PCL (electrospinning)	Thermal leaching	Vaquette et al. (2012), Costa et al. (2014)
Gelatin-coated Mg-based silicate glass scaffolds (foam replica technique)	Gelatin (electrospinning)	Simultaneous cross-linking with EDC/NHS	–
HA & PCL (3D printing)	HA & PCL (3D printing)	Layer deposition of different microstructures	Lee et al. (2014)
CaP & PlGA (sugar leaching)	CaP & PlGA (solvent casting)	Assembly just prior to setting	Carlo Reis et al. (2011)

On the other hand, as already mentioned in Section 2.1, the PDL is a group of specialized collagen fibers of different diameters ranging from nano- to microsize that essentially connect the surface of the tooth root with the bony tooth socket. Whether they originate from bone or cementum, they unravel into smaller fibers, which join up with those of adjacent fibers to produce a meshwork of interconnected fibers oriented between bone and cementum (Nanci and Bosshardt, 2006; Nanci, 2013; Edith et al., 2004) (Fig. 17.2).

To mimic this fibrous network of the PDL, electrospinning is the technique of choice by several researchers (Vaquette et al., 2012; Ivanovski et al., 2014), since it is one of the most widely studied technique and have been used to fabricate nanofibrous scaffolds with diameters ranging in size from 50 nm to several microns, a size-scale that approaches the collagen fiber diameters observed in the native ECM (Baker and Handorf, 2009) (Fig. 17.3).

Region-specific scaffolds with different microstructures (Lee et al., 2014) have been also proposed for the regeneration of the periodontal apparatus. The proposed technique in this case is 3D printing, since it can provide a wider range of pore sizes than electrospinning or foam replica technique. An additional advantage of using 3D printing for the synthesis of both compartments is the fabrication of a gradual, continuous interface resembling the physiological tissue to be regenerated.

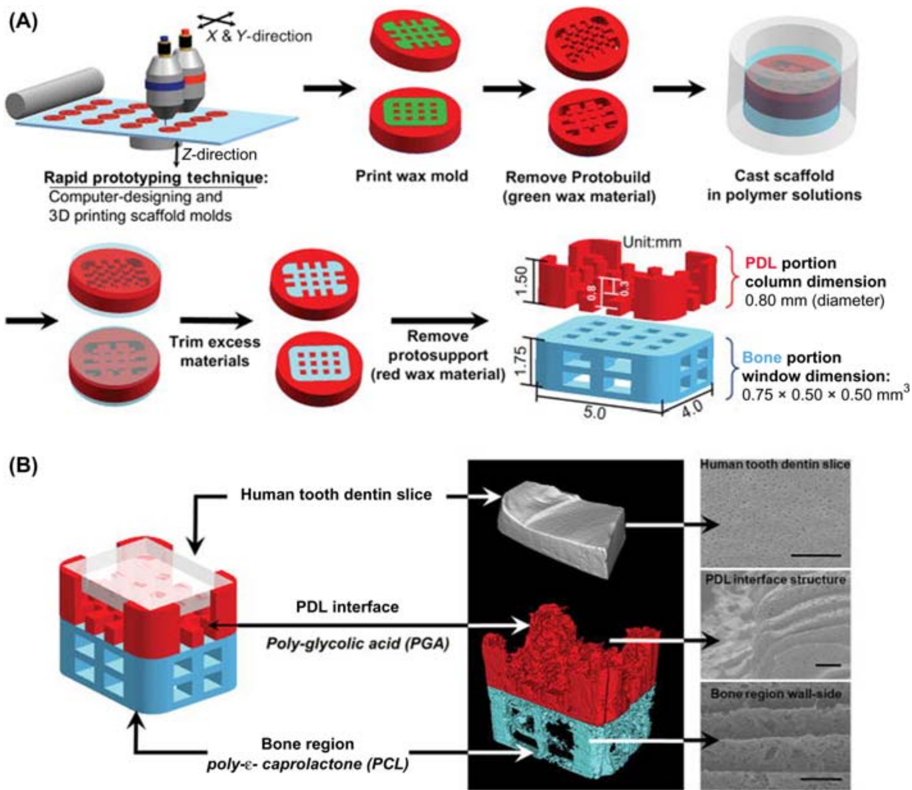


Figure 17.2 Schematic illustration of the 3-D wax printing system.
Reproduced from Park, C.H.C.H. et al., 2010. Biomimetic hybrid scaffolds for engineering human tooth-ligament interfaces. Biomaterials 31(23), 5945–5952. Available at: http://dx.doi.org/10.1016/j.biomaterials.2010.04.027 by permission from the publisher.

In the cases that the second layer of the scaffold is aimed to serve additionally as GTR barrier, solvent casting (Requicha et al., 2014, 2013; Carlo Reis et al., 2011) is a more appropriate technique, since it provides membranes with minimum porosity, prohibiting gingival epithelium growth into the periodontal defect.

17.4.1.2 Interface

The adhesion of the two layers follows the individual fabrication of the layers.

The two individual layers are usually assembled by chemical (Requicha et al., 2014, 2013) or thermal leaching (Costa et al., 2014; Vaquette et al., 2012) of one of the surfaces and attachment of the other layer by slight hand pressure. However, other techniques including chemical gluing and simultaneous cross-linking of both layers have been also used for assembling the individual layers.

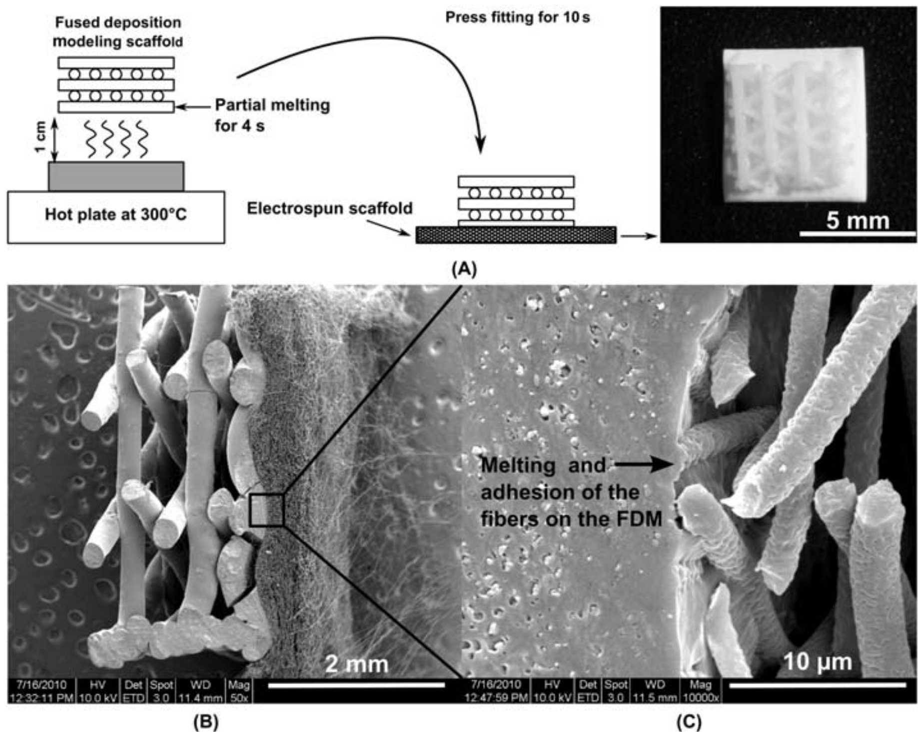


Figure 17.3 Fabrication of the biphasic scaffold. (A) Fabrication scheme, (B and C) cross-sectional views of the biphasic scaffold by scanning electron microscopy showing the fusion of the electrospun fibers (right hand side) onto the FDM component (left hand side). Reproduced from Vaquette, C.C. et al., 2012. A biphasic scaffold design combined with cell sheet technology for simultaneous regeneration of alveolar bone/periodontal ligament complex. Biomaterials 33(22), 5560−5573. Available at: http://dx.doi.org/10.1016/j.biomaterials.2012.04.038 by permission from the publisher.

17.4.2 Biological evaluation

The formation of mineralized tissue in the compartments that were designed to simulate cementum and alveolar bone has been proven in several in vitro and in vivo studies (Requicha et al., 2014, 2013; Lee et al., 2014; Park et al., 2010; Vaquette et al., 2012; Costa et al., 2014). However, the challenge in regenerating a complex structure like the periodontium is to stimulate the formation of collagenous fibers that insert into newly formed bone and cementum. The formation of such Sharpey's fiber-like structures has been proven in subcutaneous models (Lee et al., 2014; Vaquette et al., 2012; Costa et al., 2014) as well as in periodontal defect models in athymic rats (Park et al., 2012; Park et al., 2014) and mongrel dogs (Carlo Reis et al., 2011) (Fig. 17.4).

A significant result that has to be mentioned here is the use of cell sheet technology in combination with bilayered scaffolds. The regenerative potential of human PDL-cell sheets has been proven by several researchers (Flores et al., 2008; Iwata et al., 2009)

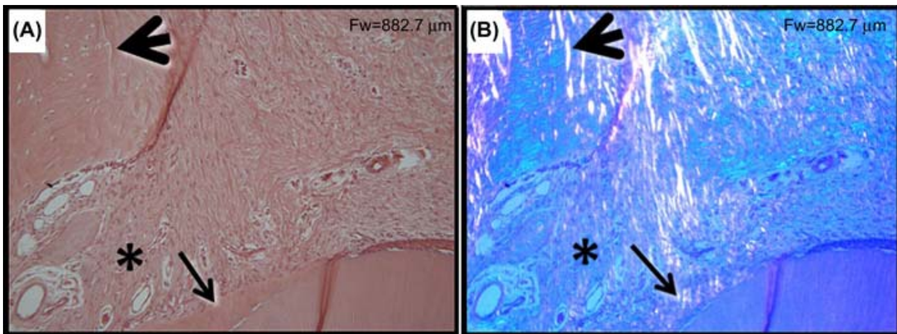


Figure 17.4 (A) New cementum (thin arrows) and periodontal ligament (*) formed along with the new alveolar bone (large arrows). (B) The collagen fibers of the periodontal ligament under polarized light, perpendicularly inserting in the new cementum, crossing the periodontal ligament and inserting in the bone on the opposite side.
Reproduced from Carlo Reis, E.C.E.C. et al., 2011. Periodontal regeneration using a bilayered PLGA/calcium phosphate construct. Biomaterials 32(35), 9244−9253. Available at: http://dx.doi.org/10.1016/j.biomaterials.2011.08.040 by permission from the publisher.

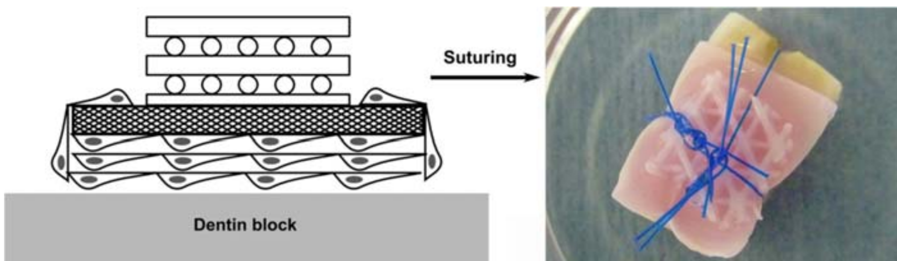


Figure 17.5 Description of biphasic scaffold assembling onto a dentin block.
Reproduced from Vaquette, C.C. et al., 2012. A biphasic scaffold design combined with cell sheet technology for simultaneous regeneration of alveolar bone/periodontal ligament complex. Biomaterials 33(22), 5560−5573. Available at: http://dx.doi.org/10.1016/j.biomaterials.2012.04.038 by permission from the publisher.

and has been already summarized in review papers (Iwata et al., 2013). However, the combination of cell sheet technology with bilayered scaffolds for the regeneration of the PDL has been only recently studied (Vaquette et al., 2012; Costa et al., 2014). In both studies the compartment of the bilayered scaffold aimed to simulate the PDL was attached to a PDL-cell sheet and then to a dentin block (Fig. 17.5).

The results of these studies indicated a periodontal fiber attachment to dentin only for the samples implanted with cell sheets. However, although the use of cell sheets was a crucial element for the attachment of the construct to dentin, the method bears many disadvantages, including high cost and time consumption, while results from large, clinical-relevant animal models are needed.

A detailed list of all in vitro and in vivo studies as well as their most significant results is presented in Table 17.2.

Table 17.2 **List of all in vitro and in vivo studies as well as their most significant results**

	Cell/animal kind	Implantation site	Results	Citation
In vitro	Canine adipose-derived stem cells	–	Osteogenic differentiation (markers: ALP, osteocalcin)	Requicha et al. (2013), Requicha et al. (2014)
	Bone marrow-derived stroma cell line (ST2)	–	Osteogenic differentiation (markers: ALP, osteocalcin, etc.)	
	Dental pulp stem/progenitor cells (DPSCs)	–	Mineralized tissue was formed in the phases that were designed to simulate dentin/cementum as well as the alveolar bone	Lee et al. (2014)
	PDL stem/progenitor cells (PDLSCs)		Spindle-shaped fibroblast-like cells were formed in the phase that was designed to simulate PDL	
	Alveolar bone stem/progenitor cells (ABSCs)			
	Osteoblasts Periodontal ligament cells	–	A cell sheet of PDL cells was formed after 7 days of culture under osteogenic conditions. No osteoblast cell sheet could be observed	Vaquette et al. (2012), Costa et al. (2014)
In vivo	Six week-old immunodeficient NIH III nude mice	Subcutaneous implantation	Cementum-like formation	Park et al. (2010)
	Ten-week-old immunodeficient mice (Harlan)	Subcutaneous implantation	Sharpey fiber-like structures inserting into cementum-like and bone-like tissue	Lee et al. (2014)
	Eight-week-old athymic rats	Subcutaneous implantation	Osteoblast seeded scaffolds presented high bone density, while the large pore size of the periodontal compartment permitted vascularization of the cell sheets	Vaquette et al. (2012), Costa et al. (2014)
	PDL-cell sheet technology		Periodontal fiber attachment to dentin for the samples implanted with cell sheets	
	Athymic rats	Periodontal defect model	The fiber-guiding scaffolds guide ligament cells with a polarized oblique orientation to the mineralized root surfaces in a predictable fashion similar to native ligament tissue	Park et al. (2012), Chan Ho Park et al. (2014)
	Healthy adult mongrel dogs	Periodontal defect model (induced periodontitis)	The birefringent collagen fibers were seen to emerge from the new cementum, crossing the periodontal ligament space and inserting into new bone	Carlo Reis et al. (2011)

17.5 Conclusions, limitations, and recommendations to readers

The major challenge in regenerating the periodontal apparatus is to create those conditions—related both to the selected materials and synthesis methodologies as well as the tissue regeneration techniques applied—for the creation of a complex mechanobiological substitute sufficient to compensate for the particular needs of each periodontal defect and adapt to the specific requirements of the host environment. In this perspective, layered scaffolds which allow the development of each periodontal tissue separately, but simultaneously integrating it into a single composite structure, could be proposed for periodontal tissue engineering. The engineering challenges for this achievement include the consideration of the particular tissue structural and functional characteristics but most importantly the cellular and molecular events that guide the development of this coordinated tissue engineering construct. Anisotropic pore size distribution of cementum and alveolar bone, multidirectional organization of the fibrous apparatus anchoring the tooth to the newly synthesized bone, appropriate degradation rates, maintenance and stability of the regenerated interface, adequate vaslcularization and innervation, as well as personalized needs concerning shape and dimensions, are only some of the major challenges towards the in vivo regeneration of the periodontal tissues' architecture and function. Interdisciplinary approaches including the selection of appropriate materials (layered or multiphase scaffolds) and stem cell population, in combination with GTR and cell sheet technology, drug delivery of growth factors and genetically modified cell therapy should be developed and combined for the complete restoration of the destructed periodontal architecture.

References

Ahlmann, E., et al., 2002. Comparison of anterior and posterior iliac crest bone grafts in terms of harvest-site morbidity and functional outcomes. J. Bone Joint Surg. Am. 84-A (5), 716−720. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12004011.

Armitage, G.C., 2004. Periodontal diagnoses and classification of periodontal diseases. Periodontology 2000(34), 9−21. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14717852.

Baker, B., Handorf, A., 2009. New directions in nanofibrous scaffolds for soft tissue engineering and regeneration. Expert Rev. Med. Dev. 6 (5), 515−532. Available at: http://www.expert-reviews.com/doi/abs/10.1586/erd.09.39.

Bartold, P., Narayanan, A., 1998. Biology of Periodontal Connective Tissue. Quintessence Publications, Inc, Chicago, USA.

Bartold, P.M., et al., 2000. Tissue engineering: a new paradigm for periodontal regeneration based on molecular and cell biology. Periodontology 2000(24), 253−269. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11276871.

Bath-Balogh, M., Fehrenback, M., 2006. Dental Embryology, Histology, and Anatomy. Elsevier Inc.

Beertsen, W., McCulloch, C.A., Sodek, J., 1997. The periodontal ligament: a unique, multifunctional connective tissue. Periodontology 2000(13), 20–40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9567922.

Berkovitz, B.K., 1990. The structure of the periodontal ligament: an update. Eur. J. Orthodont. 12 (1), 51–76. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2180728.

Bosshardt, D.D., 2005. Are cementoblasts a subpopulation of osteoblasts or a unique phenotype? J. Dent. Res. 84 (5), 390–406. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15840773.

Bosshardt, D.D., et al., 1998. Developmental appearance and distribution of bone sialoprotein and osteopontin in human and rat cementum. Anat. Rec. 250 (1), 13–33. Available at: http://www.ncbi.nlm.nih.gov/pubmed/458064.

Bosshardt, D.D., Lang, N.P., 2005. The junctional epithelium: from health to disease. J. Dent. Res. 84 (1), 9–20. Available at: http://jdr.sagepub.com/cgi/doi/10.1177/154405910508400102.

Bosshardt, D.D., Schroeder, H.E., 1991. Establishment of acellular extrinsic fiber cementum on human teeth. A light- and electron-microscopic study. Cell Tissue Res. 263 (2), 325–336. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2007256.

Bosshardt, D.D., Schroeder, H.E., 1992. Initial formation of cellular intrinsic fiber cementum in developing human teeth. A light- and electron-microscopic study. Cell Tissue Res. 267 (2), 321–335. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1600564.

Bosshardt, D.D., Selvig, K.A., 1997. Dental cementum: the dynamic tissue covering of the root. Periodontology 2000(13), 41–75. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9567923.

Bunyaratavej, P., Wang, H.-L., 2001. Collagen membranes: a review. J. Periodontol. 72 (2), 215–229.

Carlo Reis, E.C., et al., 2011. Periodontal regeneration using a bilayered PLGA/calcium phosphate construct. Biomaterials. 32 (35), 9244–9253. Available at: http://dx.doi.org/10.1016/j.biomaterials.2011.08.040.

Chan, B.P., Leong, K.W., 2008. Scaffolding in tissue engineering: general approaches and tissue-specific considerations. Eur. Spine J. 17 (S4), 467–479. Available at: http://link.springer.com/10.1007/s00586-008-0745-3.

Chavrier, C., et al., 1988. Distribution and organization of the elastic system fibres in healthy human gingiva. Ultrastructural and immunohistochemical study. Histochemistry. 89 (1), 47–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3366663.

Chen, F.-M., Jin, Y., 2010. Periodontal tissue engineering and regeneration: current approaches and expanding opportunities. Tissue Eng. B: Rev. 16 (2), 219–255. Available at: http://www.liebertonline.com/doi/abs/10.1089/ten.teb.2009.0562.

Chen, J., et al., 2015. Biomechanics of oral mucosa. J. R. Soc. Interface/The R. Soc. 12 (109), 20150325. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26224566.

Choi, H.S., et al., 2012. Influence of the alveolar cleft type on preoperative estimation using 3D CT assessment for alveolar cleft. Arch. Plast. Surg. 39 (5), 477.

Costa, P.F., et al., 2014. Advanced tissue engineering scaffold design for regeneration of the complex hierarchical periodontal structure. J. Clin. Periodontol. 41 (3), 283–294.

Dimitriou, R., et al., 2011. Complications following autologous bone graft harvesting from the iliac crest and using the RIA: a systematic review. Injury. 42 (Suppl 2), S3-15. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21704997.

Eckert, R.L., Rorke, E.A., 1989. Molecular biology of keratinocyte differentiation. Environ. Health Perspect. 80, 109–116. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2466639.

Eke, P.I., et al., 2012. Prevalence of periodontitis in adults in the United States: 2009 and 2010. J. Dent. Res. 91 (10), 914–920. Available at: http://jdr.sagepub.com/cgi/doi/10.1177/0022034512457373.

Flores, M.G., et al., 2008. Periodontal ligament cell sheet promotes periodontal regeneration in athymic rats. J. Clin. Periodontol. 35 (12), 1066–1072. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19040584.

Foster, B.L., et al., 2007. Advances in defining regulators of cementum development and periodontal regeneration. Curr. Top. Dev. Biol. 78, 47–126. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17338915.

Gelain, F., Gelain, F., 2008. Novel opportunities and challenges offered by nanobiomaterials in tissue engineering. Int. J. Nanomed.415. Available at: http://www.dovepress.com/novel-opportunities-and-challenges-offered-by-nanobiomaterials-in-tiss-peer-reviewed-article-IJN.

Goudouri, O.-M., et al., 2016. Sol-gel processing of novel bioactive Mg-containing silicate scaffolds for alveolar bone regeneration. J. Biomater. Appl. 30 (6), 740–749. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25972398.

Grant, D., Bernick, S., 1972. Formation of the periodontal ligament. J. Periodontol. 43 (1), 17–25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/4621364.

Hammarström, L., Alatli, I., Fong, C.D., 1996. Origins of cementum. Oral Dis. 2 (1), 63–69. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8957939.

Hammerle, C.H.F., Jung, R.E., Feloutzis, A., 2002. A systematic review of the survival of implants in bone sites augmented with barrier membranes (guided bone regeneration) in partially edentulous patients. J. Clin. Periodontol. 29 (s3), 226–231.

Hannam, 1982. The innervations of the Periodontal ligament. In: Berkovitz, B., Moxham, B., Newman, H. (Eds.), The Periodontal Ligament in Health and Disease. Mosbey-Wolfe, pp. 173–196.

Ho, S.P., et al., 2007. The tooth attachment mechanism defined by structure, chemical composition and mechanical properties of collagen fibers in the periodontium. Biomaterials. 28 (35), 5238–5245. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0142961207006758.

Hollister, S.J., 2005. Porous scaffold design for tissue engineering. Nat. Mater. 4 (July), 518–524. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16003400.

Ivanovski, S., et al., 2014. Multiphasic scaffolds for periodontal tissue engineering. J. Dent. Res. 93 (12), 1212–1221. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-84911907516&partnerID=tZOtx3y1.

Iwata, T., et al., 2013. Cell sheet engineering and its application for periodontal regeneration. Periodontology 2000. 51 (1), 1–18.

Iwata, T., et al., 2009. Periodontal regeneration with multi-layered periodontal ligament-derived cell sheets in a canine model. Biomaterials. 30 (14), 2716–2723. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19201461.

Jiang, N., et al., 2016. Periodontal ligament and alveolar bone in health and adaptation: tooth movement. Front. Oral Biol. 18, 1–8.

Kolomvos, N., et al., 2010. Iliac crest morbidity following maxillofacial bone grafting in children: a clinical and radiographic prospective study. J. Cranio-Maxillo-Facial Surg. 38 (4), 293–302. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19945294.

Komatsu, K., 2010. Mechanical strength and viscoelastic response of the periodontal ligament in relation to structure. J. Dent. Biomech.2010. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20948569.

Kon, E., et al., 2014. Clinical results of multilayered biomaterials for osteochondral regeneration. J. Exp. Orthopaed. 1 (1), 10. Available at: http://www.jeo-esska.com/content/1/1/10.

Kydd, W.L., Daly, C.H., 1982. The biologic and mechanical effects of stress on oral mucosa. J. Prosthetic Dent. 47 (3), 317–329. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7038105.

Lang, N., Lindhe, J., Karring, T., 2008. In: Lang, N., Lindhe, J. (Eds.), Clinical Periodontology and Implant Dentistry, 5th ed. Wiley Blackwell, NJ, USA.

Lee, C.H., et al., 2014. Three-dimensional printed multiphase scaffolds for regeneration of periodontium complex. Tissue Eng. A. 20, 140206062754003. Available at: http://online.liebertpub.com/doi/abs/10.1089/ten.TEA.2013.0386?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%253dpubmed.

LeGeros, R., 1991. Calcium Phosphate in Oral Biology and Medicine. Karger, New York, USA.

Liu, M., et al., 2013. Tissue engineering stratified scaffolds for articular cartilage and subchondral bone defects repair. Orthopedics. 36 (11), 868−873. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24200433.

Loh, Q.L., Choong, C., 2013. Three-dimensional scaffolds for tissue engineering applications: role of porosity and pore size. Tissue Eng. B: Rev. 19 (6), 485−502. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid= 3826579&tool=pmcentrez&rendertype=abstract.

MacNeil, R.L., et al., 1998. Isolation of murine cementoblasts: unique cells or uniquely-positioned osteoblasts? Eur. J. Oral Sci. 106 (Suppl), 350−356. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9541247.

Marchesan, J.T., et al., 2011. Implications of cultured periodontal ligament cells for the clinical and experimental setting: a review. Arch. Oral Biol. 56 (10), 933−943. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21470594.

Matias, M.A., et al., 2003. Immunohistochemical localisation of extracellular matrix proteins in the periodontium during cementogenesis in the rat molar. Arch. Oral Biol. 48 (10), 709−716. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12971948.

Matthews, B.G., et al., 2016. Gene-expression analysis of cementoblasts and osteoblasts. J. Periodontal. Res. 51 (3), 304−312. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26215316.

McCulloch, C.A., Lekic, P., McKee, M.D., 2000. Role of physical forces in regulating the form and function of the periodontal ligament. Periodontology 2000(24), 56−72. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11276873.

Miller, A., Parker, S.B., 1984. Collagen: the organic matrix of bone [and discussion]. Philos. Trans. R. Soc. B: Biol. Sci. 304 (1121), 455−477. Available at: http://rstb.royalsocietypublishing.org/cgi/doi/10.1098/rstb.1984.0040.

Moreau, J.L., et al., 2007. Tissue engineering solutions for cleft palates. J. Oral Maxillofac. Surg. 65 (12), 2503−2511. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0278239107014887.

Mossey, P., Castilla, E., 2001. Global registry and database on craniofacial anomalies. In: Mossey, P. (Ed.), World Health Organisation Registry Meeting on Craniofacial Anomalies. WHO Publications, Bauru, Brazil.

Nanci, A., 2013. Ten Cate's Oral Histology. Elsevier, Amsterdam.

Nanci, A., Bosshardt, D.D., 2006. Structure of periodontal tissues in health and disease. Periodontology 2000(40), 11−28.

Newman, M., et al., 2012. Anatomy of the Periodontium. Elsevier Inc, Amsterdam.

Nijweide, P.J., Burger, E.H., Feyen, J.H., 1986. Cells of bone: proliferation, differentiation, and hormonal regulation. Physiol. Rev. 66 (4), 855−886. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3532144.

Park, C.H., et al., 2010. Biomimetic hybrid scaffolds for engineering human tooth-ligament interfaces. Biomaterials. 31 (23), 5945−5952. Available at: http://dx.doi.org/10.1016/j.biomaterials.2010.04.027.

Park, C.H., et al., 2014. Image-based, fiber guiding scaffolds: a platform for regenerating tissue interfaces. Tissue Eng. C: Methods. 20 (7), 1−36. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24188695.

Park, C.H., et al., 2014. Spatiotemporally controlled microchannels of periodontal mimic scaffolds. J. Dent. Res. 93 (12), 1304−1312. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25216511.

Park, C.H., et al., 2012. Tissue engineering bone-ligament complexes using fiber-guiding scaffolds. Biomaterials. 33 (1), 137−145. Available at: http://dx.doi.org/10.1016/j.biomaterials.2011.09.057.

Pihlstrom, B.L., Michalowicz, B.S., Johnson, N.W., 2005. Periodontal diseases. Lancet (London, England). 366 (9499), 1809−1820. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16298220.

Rams, T.E., Listgarten, M.A., Slots, J., 1994. Utility of radiographic crestal lamina dura for predicting periodontitis disease-activity. J. Clin. Periodontol. 21 (9), 571−576. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7806671.

Reddy, S., 2005. Biology of periodontal tissues. Essentials of Clinical Periodontology and Periodontics. Jaypee Brothers Medical Publishers, New Delhi, pp. 8−29., Chapter 2.

Structural biology. In: Rateitschak-Pluss, E.M., Wolf, H.F., Rateitschak, K.H., Hassell, T.M. (Eds.), Color Atlas of Dental Medicine, Periodontology. Thieme Publishing Group, New York.

Requicha, J.F., et al., 2014. A tissue engineering approach for periodontal regeneration based on a biodegradable double-layer scaffold and adipose-derived stem cells. Tissue Eng. A. 20 (17−18), 2483−2492. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24575867.

Requicha, J.F., et al., 2013. Design and characterization of a biodegradable double-layer scaffold aimed at periodontal tissue-engineering applications. J. Tissue Eng. Regenerative Med. 10 (5), 392−403. Available at: http://doi.wiley.com/10.1002/term.1816.

Saffar, J.L., Lasfargues, J.J., Cherruau, M., 1997. Alveolar bone and the alveolar process: the socket that is never stable. Periodontology 2000(13), 76−90. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9567924.

Schroeder, H.E., 1986a. Periodontal ligament: principal and other connective tissue fibers. In: Oksche, A., Vollrath, L. (Eds.), Handbook of Microscopic Anatomy. Springer, Berlin Heidelberg, pp. 196−208.

Schroeder, H.E., 1986b. Structure of alveolar process and bone. Handbook of Microscopic Anatomy Volume V/5: The Periodontium. Springer, Berlin, p. 152.

Schroeder, H.E., Listgarten, M.A., 1997. The gingival tissues: the architecture of periodontal protection. Periodontology 2000(13), 91−120. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9567925.

Schroeder, H.E., Listgarten, M.A., 2003. The junctional epithelium: from strength to defense. J. Dent. Res. 82 (3), 158−161. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12598541.

Seo, S.-J., et al., 2014. Strategies for osteochondral repair: focus on scaffolds. J. Tissue Eng. 5 (0), 2041731414541850. Available at: http://tej.sagepub.com/content/5/2041731414541850.full.

Shimono, M., Ishikawa, T., Enokiya, Y., et al., 2003. Biological characteristics of the junctional epithelium. J. Electron Microsc. 52 (6), 627−639. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14756251.

Shimono, M., Ishikawa, T., Ishikawa, H., et al., 2003. Regulatory mechanisms of periodontal regeneration. Microsc. Res. Tech. 60 (5), 491−502. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12619125.

Skoglund, A., Persson, G., 1985. A follow-up study of apicoectomized teeth with total loss of the buccal bone plate. Oral Surg. Oral Med. Oral Pathol. 59 (1), 78–81.

Solanki, G., 2012. A general overview of gingiva. Int. J. Biomed. Res. 3 (2), Available at: http://ssjournals.com/index.php/ijbr/article/view/709.

Sommerfeldt, D.W., Rubin, C.T., 2001. Biology of bone and how it orchestrates the form and function of the skeleton. Eur. Spine J. 10 (Suppl 2), S86–S95. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11716022.

Sundelacruz, S., Kaplan, D.L., 2009. Stem cell- and scaffold-based tissue engineering approaches to osteochondral regenerative medicine. Semin. Cell Dev. Biol. 20 (6), 646–655. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19508851.

Thavornyutikarn, B., et al., 2014. Bone tissue engineering scaffolding: computer-aided scaffolding techniques. Prog Biomater. 3, 61–102. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26798575.

Vaquette, C., et al., 2012. A biphasic scaffold design combined with cell sheet technology for simultaneous regeneration of alveolar bone/periodontal ligament complex. Biomaterials. 33 (22), 5560–5573. Available at: http://dx.doi.org/10.1016/j.biomaterials.2012.04.038.

White, S., Pharoah, M., 2009. Oral Radiology: Principles and Interpretation. Mosby Elsevier, Amsterdam, Netherlands.

Wills, D.J., Picton, D.C., Davies, W.I., 1972. An investigation of the viscoelastic properties of the periodontium in monkeys. J. Periodontal Res. 7 (1), 42–51. Available at: http://www.ncbi.nlm.nih.gov/pubmed/4272031.

Winning, T.A., Townsend, G.C., 2000. Oral mucosal embryology and histology. Clin. Dermatol. 18 (5), 499–511. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11134845.

Xiong, J., Gronthos, S., Bartold, P.M., 2013. Role of the epithelial cell rests of Malassez in the development, maintenance and regeneration of periodontal ligament tissues. Periodontology 2000. 63 (1), 217–233. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23931062.

Young, M.F., et al., 1992. Structure, expression, and regulation of the major noncollagenous matrix proteins of bone. Clin. Orthopaed. Related Res. 281, 275–294. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1499220.

This page intentionally left blank

Dental pulp tissue engineering and regenerative endodontic therapy

18

Priyadarshni Bindal[1], *Noor H. Abu Kasim*[1], *Thamil Selvee Ramasamy*[1], *Ali Dabbagh*[1], *Keyvan Moharamzadeh*[2] *and Wen L. Chai*[1]
[1]University of Malaya, Kuala Lumpur, Malaysia,
[2]University of Sheffield, Sheffield, United Kingdom

18.1 Introduction

The health and vitality of the tooth pulp significantly affect the preservation of tooth homeostasis, proprioception, and hence its longevity (Forsberg and Tedestam, 1989; Machado et al., 2012). The vital pulp not only acts as biosensor to perceive pathogenic stimuli and delivers nutrition, it also plays an essential role in the tooth and root development in immature teeth.

Trauma and bacterial infection are two primary factors that affect the pulp vitality. In general the pulp infections are mostly irreversible due to the anatomical location of the pulp and the restrained blood supply, which could result in inadequate availability of the immune system to neutralize the bacterial invasion. In such cases, conventional root canal therapy (RCT) is often employed as an established endodontic procedure to remove the contaminated pulp, disinfect, and obturate the root canal system. However in spite of high success rate, this treatment leaves the tooth in physiologically nonvital state (Ng et al., 2011). In terms of aesthetics, RCT also results in crown discoloration, mostly due to the staining effect of root filling materials (Lenherr et al., 2012). Previous studies indicate that although RCT prolongs the tooth survival, the treated teeth are still compromised in comparison to the teeth containing vital tissue. Moreover, over time, the root-treated teeth which lack sufficient blood supply and the proprioception, become brittle and more prone to fracture (Caplan et al., 2005).

In order to address the above limitations of RCT treatment, dental pulp regeneration by pulp therapies and regenerative endodontic procedures (REPs) has recently received increasing attention from the researchers in the field worldwide. The aim is to retain the pulp vitality, henceforth harnessing the self-repair capacity of dental pulp (Colombo et al., 2014). In REPs, the existing pulp tissue is vascularized in order to prolong the root development of immature teeth and maintain the vitality in the mature teeth (Andreasen et al., 2013). Dental pulp is the sole vascularized structure of the teeth, embedded in other highly mineralized compartments. This chapter aims to highlight the current advancements of the tooth pulp therapy and requirements for dental pulp regeneration, with focus on REPs, materials and

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00018-9
© 2017 Elsevier Ltd. All rights reserved.

clinical protocols. There are many in vitro and in vivo studies that show the potential of pulp tissue regeneration. However, there is a limited insight into the clinical perspective of dental pulp regeneration. This review can further aid clinicians to establish an appropriate treatment protocol for a given clinical scenarios well as researchers to address the limitations in present day pulp therapy protocols to enhance the treatment efficiency.

18.2 Pulp therapy

Pulp therapy involves partial or complete removal of the inflamed and infected pulp tissue caused by bacterial invasion or traumatic injury. The pulp therapies are generally categorized as vital and nonvital pulp therapies (NPTs).

18.2.1 Vital pulp therapy

Vital pulp therapy (VPT) aims to retain the compromised pulp that has not been destroyed due to caries, restorative procedures, or trauma (Ghoddusi et al., 2013). This approach could minimize the chances of infection and fracture in immature teeth with wide open apices and incompletely formed roots with fragile walls (Forsberg and Tedestam, 1989). In the mature teeth, VPT could prevent or treat the apical periodontitis caused by trauma or infection, promote healing of the inherent remnant pulp, and regenerate dental pulp, which is the best restorative approach for necrotic pulp canal (Trope, 2008).

Various VPT approaches have been clinically undertaken for both immature and mature teeth, based on the severity of pulp damage. Pulp capping (direct or indirect) and pulpotomy are typical VPT techniques employed to preserve the vitality of residual pulp and stimulate the root completion process for closing the root apex in young permanent teeth (Fig. 18.1). However, these therapies could not be undertaken in patients with associated periapical pathologies. Moreover, pulp capping with only calcium hydroxide ($Ca(OH)_2$) leads to formation of voids and tunnel effect which generate increased potential pathways for bacterial infiltration (Ng et al., 2011).

18.2.2 Nonvital pulp therapy

In NPT, the pulp tissue is completely removed. In an infected tooth, pulpectomy or root canal treatment are usually carried out to completely remove the pulp, followed by cleansing and root canal sealing. However, tooth discoloration and increased risk of tooth fracture are the main drawbacks of both apexification and pulpectomy approaches (Lin et al., 1992; Fuss et al., 2001).

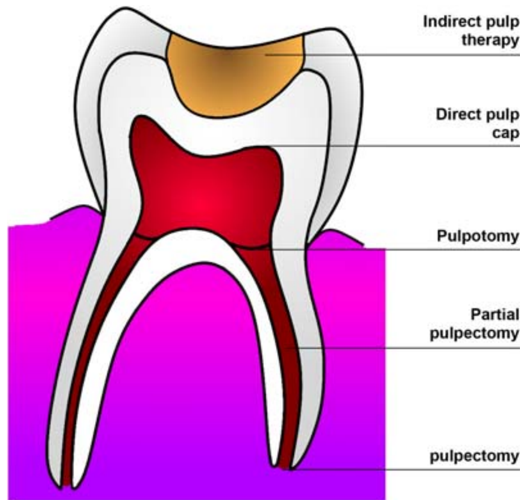


Figure 18.1 The extent of tooth tissue/pulp tissue removal in vital and nonvital pulp therapies. In indirect pulp therapy the infected dentin overlying the pulp is removed and coated with a $Ca(OH)_2$ layer to stimulate formation of secondary dentin and protect the underlying pulp. In direct pulp therapy the exposed vital pulp tissue is directly covered with a $Ca(OH)_2$ layer to stimulate generation of a dentinal bridge. Pulpotomy is performed by removing the infected pulp tissue to retain vitality of the remaining pulp tissue by using a $Ca(OH)_2$ dressing. In partial pulpectomy the pulp structure, except the pulp tissue in the roots, is removed, while pulpectomy involves complete pulp removal, including the pulp tissue in the roots.

18.3 Regenerative endodontic procedures

REPs are the biological methods for replacement of the pulp-dentin (Murray et al., 2007). Regeneration of dental pulp is accomplished by tissue engineering approaches which incorporate both biology and engineering to design a scaffold that can act as an efficient extracellular matrix, required to promote new tissue formation (Hargreaves et al., 2013). Several in vitro and in vivo attempts have been reported towards successful dental pulp regeneration, which mainly include root canal revascularization (blood clot) (Cotti et al., 2008; Garcia-Godoy and Murray, 2012; Chen et al., 2012; Ding et al., 2009), stem cell therapy (Nakashima and Iohara, 2014), gene therapy (Sonoyama et al., 2006), biodegradable scaffold implantation (Kuo et al., 2008), injectable scaffolds (Rosa et al., 2013), and 3D cell printing (Dusseiller et al., 2005; Jakab et al., 2008).

A continuous and sufficient blood supply from the remnant pulp into the canal is an essential factor in REP approaches for enhanced tooth completion rate. However, when the pulp is not present, scaffolds need to be placed to support the supplied cells for pulp regeneration and producing odontoblasts to lay reparative dentine. The scaffolds can be necrotic and uninfected pulp tissue in the tooth, or a

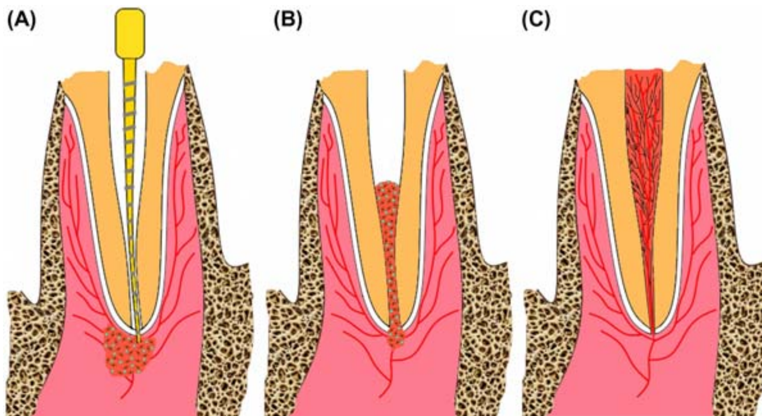


Figure 18.2 Biological basis of regenerative therapy. (A) Blood clot is initiated from the periapical area, (B) blood clot and pluripotent cell population enter the root canal, (C) the growth of pulp-like connective tissue becomes evident inside the root canal.

biocompatible material. In such cases, regenerative therapy involving blood clot is anticipated as a promising therapy for pulp-free canals and those with periapical pathologies (Fig. 18.2). Numerous case reports have reported on successful root completion and tooth vitality after induction of blood clot in the root canal (Cotti et al., 2008; Iwaya et al., 2001; Chueh and Huang, 2006; Jung et al., 2008). A stable blood clot not only serves as a scaffold for supporting proliferation of stem cells inside the canal, but also contains necessary factors for cell growth and differentiation (Nosrat et al., 2012). REPs by blood clots could be considered as a technically simple approach which utilizes the currently available medicaments and instruments without involving expensive biotechnological procedures. Moreover, tissue regeneration by the patient's own blood minimizes the risk of pathogen transmission and immune response compared to the pulp replaced by synthetic tissue engineering (Murray et al., 2007).

The blood clot was initially employed in endodontic therapy in 1961 (Östby, 1961). Several animal trials and human case reports (Table 18.1) were carried out to investigate the clinical potential of this approach. For instance the blood clots were successfully utilized for apical closure of the pulpless immature teeth of monkeys (Ham et al., 1972). It was also reported that induction of intracanal bleeding could improve the REP outcome in dogs teeth, confirmed by both radiographic and histologic findings (Thibodeau et al., 2007). In 2001, metronidazole (MTZ) and ciprofloxacin (CIPX) as antimicrobial compounds were demonstrated without mechanical instrumentation for continued development of the immature mandibular second molar with associated sinus tract (Iwaya et al., 2001). In 2004 a technique of revascularization in immature permanent mandibular second premolar was described using the triple antibiotic paste (comprised of CIPX, MTZ, and minocycline (MIN)), intentional apical irritation, and a perfect coronal seal (Banchs and Trope, 2004).

Table 18.1 Various case studies on regenerative endodontic therapy in open and closed apices

Ref.	Tooth no.	Age	Apical foramen	Findings		Procedural			Outcome	
				Radiological	Clinical	Scaffold	Irrigation	Intracanal medication	Root apex closure	Alleviation of symptoms
[a]	35	13	Open	Periapical lesion	Swelling, sinus tract	BC	5% NaOCl, 3% H_2O_2	CIPX, MTZ, $Ca(OH)_2$	Apical closure in 30 months	Sinus tract healed in 5 months
[b]	35	11	Open	Periapical lesion	ASX	BC	–	CIPX, MTZ, MIN	Yes	N/A
[c]	20	10	Open	Fractured central cus (dens evaginatus)	ASX	BC	2.5% NaOCl	$Ca(OH)_2$	Root apex maturation at 35 months	N/A
	28, 29	10	Open	Acute periradicular abscess	Pain, swelling	BC	1.5% NaOCl	$Ca(OH)_2$	Complete maturation of the root apex and healing of periradicular bone in 7 months	ASX at 7 months
	20	10	Open	Periradicular periodontitis	ASX	BC	2.5% NaOCl	$Ca(OH)_2$	Complete calcification of apical half of root	ASX
	29	9	Open	Periapical lesion	Pain	BC	2.5% NaOCl	$Ca(OH)_2$	Yes, 3 years	ASX
	20, 29	6–14	Open	Pulp necrosis, acute apical abscess	Pain, swelling	BC	2.5% NaOCl	$Ca(OH)_2$, IRM	Apical bone formation in 1–9 months	
[d]	21	9	Open	Periapical abscess	Pain, swelling	BC	1.25% NaOCl	CIPX, MTZ, CEC	Yes, 6 months	ASX at 11 weeks recall
[e]	29	10		Periapical abscess	Pain, sinus tract	BC	5% NaOCl	CIPX, MTZ, MIN	Yes	ASX
	29	10	Open	Periapical abscess, sinus tract	ASX	BC	5.25% NaOCl	CIPX, MTZ, MIN	Yes	ASX at 2-year recall

(*Continued*)

Table 18.1 (Continued)

Ref.	Tooth no.	Age	Apical foramen	Findings		Procedural			Outcome	
				Radiological	Clinical	Scaffold	Irrigation	Intracanal medication	Root apex closure	Alleviation of symptoms
	20	10	Open	–	Pain on percussion	BC	5% NaOCl	CIPX, MTZ, MIN	Yes	ASX at 10-month recall
	20	13	Open	–	ASX	BC	5.25% NaOCl	CIPX, MTZ, MIN	–	ASX at 2-year recall
	20	10	Open	–	ASX	BC	2.5% NaOCl	CIPX, MTZ, MIN	Yes	ASX at 2-year recall
	20	9	Open		ASX	BC	2.5% NaOCl	CIPX, MTZ, MIN	Yes	ASX at 2-year recall
	29	14	Open	–	ASX	BC	2.5% NaOCl	$Ca(OH)_2$	Yes, with completely healed sinus tract	ASX at 1- year follow up
	4	10	Open	–	ASX	BC	2.5% NaOCl	CIPX, MTZ, MIN	Yes	ASX 17-month recall
f	8, 9	9	Open	Complete crown fracture	ASX	BC	5.25% NaOCl	$Ca(OH)_2$	Yes	ASX 30-month recall, root fracture healed
g	20, 29	11	Open	Occlusal tubercle consistent with dens evaginatus	ASX	BC	6% NaOCl, saline, 2.0% CHX	CIPX, MTZ, MIN	Yes	ASX at 18-month recall
h	29	12	Open	Chronic periapical abscess	ASX	BC	6% NaOCl, 2% CHX	MTA	Apical diameter reduced in 6 weeks	
i	8	9	Closed	Acute apical abscess	Swelling	BC	1.25% NaOCl	CIPX, MTZ, CEC	Yes	ASX at 6-month recall
j	Premolars	8–11	Open	Acute and chronic Apical periodontitis	Fractured dens Invaginatus	BC	5.25% NaOCl	CIPX, MTZ, MIN	–	ASX for acute at 1-week recall

k	20	12	Open	Chronic periapical abscess	ASX	BC	1% NaOCl	CIPX, MTZ, AMX	Apical closure at 9 months recall	
l	8	7	Closed	Symptomatic apical periodontitis	Fractured, sensitive to percussion	BC	3% NaOCl	CIPX, MTZ, MIN	–	ASX at 6 weeks recall
m	8, 9	13	Closed	Pulpal necrosis	ASX apical periodontitis	BC	5.25% NaOCl, saline, 0.12% CHX	CIPX, MTZ, MIN	–	ASX at 3 weeks recall, sinus tract healed after 3 weeks
	20, 29	11	Closed	Pulpal necrosis, ASX apical periodontitis	ASX	BC	5.25% NaOCl, saline, 0.12% CHX	CIPX, MTZ, MIN	–	ASX at 5 weeks recall, sinus tract healed 30 days later
	8, 9	6	Closed	Pulpal necrosis, ASX apical periodontitis		BC	5.25% NaOCl, saline, 0.12% CHX	CIPX, MTZ, MIN	–	ASX at 6-month recall
	20, 29	11	Open	Apical periodontitis		BC with CollaPlug	5.25% NaOCl, saline, 0.12% CHX	CIPX, MTZ, MIN	70% root development and closure at 1-year recall	ASX at 1-year recall
n	Mandibular central incisors	7	Open	Apical periodontitis	Gingival swelling with mobilities	BC	2.5% NaOCl	AMX (600 mg/ day for 3 days)	Apical closure, thickening of root wall in 30 months	Swelling and sinus resolved after 15 days
o	Permanent molars	8–11	Open	Pulp necrosis	Mild sensitivity to palpation	BC	2.5% NaOCl.	Cavit	–	ASX in 3-month recall, Resolution of periradicular radiolucencies

(*Continued*)

Table 18.1 (Continued)

Ref.	Tooth no.	Age	Apical foramen	Findings		Procedural			Outcome	
				Radiological	Clinical	Scaffold	Irrigation	Intracanal medication	Root apex closure	Alleviation of symptoms
[p]	4	11	Open	Necrotic pulp, symptomatic apical periodontitis	Sensitive to both percussion and palpation	BC and PRP plug	2.5% NaOCl	CIPX, MTZ, MIN	Resolution of the periapical lesion, root development, continued apical closure	ASX at 5.5-month recall
		11	Open	Necrotic pulp with apical periodontitis	Replanted after accidental extraction	BC with PRP	5.25% NaOCl	CIPX, MTZ, MIN, Cavit	Root end apical closure	Resolution of symptoms at 5.5-month recall
[q]	20	14	Open	Pulp necrosis, symptomatic apical periodontitis	Sensitive to percussion and palpation	BC	2.5% NaOCl	CIPX, MTZ, MIN	Apical closure after 6 months	ASX at 6-month recall
	20	8	Open	–	Mild pain and sensitivity	BC	2.5% NaOCl	CIPX, MTZ, MIN	Resolution of radiolucency but no gain in root thickness and length	ASX at 8-month recall
[r]	30	9	Open	Pulpal necrosis, symptomatic apical periodontitis	Localized swelling and sensitivity to percussion and palpation	BC	5.25% NaOCl	CIPX, MTZ, MIN	Apical closure	ASX at 3-month recall
	30	8	Open	Pulpal necrosis, chronic apical abscess, sensitivity, to palpation	Visible swelling draining sinus tract	BC	5.25% NaOCl	CIPX, MTZ, MIN	Apical closure after 15 months	ASX at 6, 12, and 15-month recall

	Anterior maxillary teeth	14	Open	Necrotic immature teeth	Swelling and pain in all teeth	BC	5.25% NaOCl	CIPX, MTZ, MIN	60–70% root closure at 6-month recall	ASX at 2-month recall
[s]	Premolars	8–13	Open	Infected necrotic pulps, apical periodontitis abscess	Swelling and sinus tract with periapical lesion	BC	5.25% NaOCl	$Ca(OH)_2$, saline	Increased wall thickness, continued root development after 19.6 months	ASX at 6, 8 14, 20-month recall
[t]	Anterior teeth (upper and lower)	15–28	Open	Necrotic pulp	Periapical lesions	PRP on a collagen sponge	5.25% NaOCl	IRM	Apical closure, dentinal wall thickening	ASX at 6-month recall, periapical healing,
[u]	3–29	7–16	Open	Necrotic pulp, immature permanent teeth	Chronic or acute odontogenic infections	BC	5% NaOCl, saline	CIPX, MTZ, MIN, PG, MG	50% root formation and root maturation at 6 months	ASX at 6-month recall

Abbreviations. *AMX*, amoxicillin; *ASX*, asymptomatic; *BC*, blood clot; *CEC*, cefaclor; *CHX*, chlorhexidine; *CIPX*, ciprofloxacin; *IRM*, Intermediate Restorative Material; *MG*, macrogol; *MIN*, minocycline; *MTZ*, metronidazole; *PG*, propylene glyeol.
[a]Iwaya et al. (2001).
[b]Banchs and Trope (2004).
[c]Chueh and Huang (2006).
[d]Thibodeau et al. (2007).
[e]Jung et al. (2008).
[f]Cotti et al. (2008).
[g]Reynolds et al. (2009).
[h]Shin et al. (2009).
[i]Thibodeau (2009).
[j]Ding et al. (2009).
[k]Thomson and Kahler (2010).
[l]Kim et al. (2010a).
[m]Petrino et al. (2010).
[n]Iwaya et al. (2011).
[o]Cehreli et al. (2011).
[p]Torabinejad and Turman (2011).
[q]Jung et al. (2012).
[r]Nosrat et al. (2012).
[s]Chen et al. (2012).
[t]Jadhav et al. (2012).
[u]Dabbagh et al. (2012).

More recently, platelet rich plasma (PRP) and platelet rich fibrin (PRF) have also been administrated with blood clots to improve the revascularization outcome (Jadhav et al., 2012). PRP and PRF constitute very rich sources of key growth factors, which encourage proliferation and differentiation of progenitor/stem cells into the canal cavity (Huang et al., 2010a; Ding et al., 2009). Several growth factors including the transforming growth factor-1 and the vascular endothelial growth factors released from the platelets could stimulate stem cells and enhance their regenerative potential, stimulate collagen synthesis, control local inflammation, thus improving tissue healing. In a study on histological difference of the tissue generated in the canal after using PRP and blood clot in beagle dogs, significant pulp-like tissue formations and disappearance of periapical pathology with apical closure was observed in both groups. Moreover, in cases with insufficient bleeding from periapical tissues (Zhang et al., 2014), PRP, or PRF may be used inside the root canal rather than creating intracanal bleeding (Torabinejad and Parirokh, 2010; Torabinejad and Faras, 2012; Keswani and Pandey, 2013).

18.3.1 Regenerative endodontic procedure in different apical cases

In conventional endodontics, chemo-mechanical debridement of pulp cavity for removal of the bacterial infection and necrotic tissue ideally terminates at the apical foramen. A narrow but patent apical constriction is present in completely formed mature teeth. In immature teeth, the incomplete root formation results in an open apex, which is also observed in a number of pathological conditions (Nelson, 2014). Although the REPs mentioned in the literature have been employed in teeth with both open and closed apices; however, the procedure is mostly employed on young adult teeth with incomplete root formation. It is believed that apical papilla of young permanent tooth contains population of highly pluripotent stem cells that could easily propagate inside the root canal for pulp regeneration purpose.

18.3.1.1 Open apices

The root apex patency largely determines the success of regenerative therapies since the apical foramen is the sole route for restoration of blood supply and entrance of cells into the canal via blood clot. Therefore formation of pulp-like tissue inside the root canal cavity following the transplantation, replantation, or REP can be performed solely when apical foramen is patent (Laureys et al., 2013).

In particular the patency of apical foramen is deemed critical for tissue ingrowth in REP therapy of immature permanent teeth. Traumatized immature permanent teeth possessing open apices with sufficient patency to allow entry of blood clot into the canal are considered ideal for regenerative endodontic therapy (Laureys et al., 2001). In general, wider apical foramen and shorter teeth exhibit enhanced response to regenerative endodontic therapy compared to the teeth with narrow apical openings (Kling et al., 1986). However, the apical foramens with diameters above 1.1 mm have been shown appropriate for enhanced revascularization after replantation (Iwaya et al., 2001; Kling et al., 1986).

Revascularization in root canals with open apices has clinically shown potential to deliver mesenchymal stem cells (MSCs) inside the root canal of immature necrotic teeth following the REP therapy (Lovelace et al., 2011). This results in formation of a new tissue within the root canal via host cell homing, which in turn results in Kim et al. (2010b). Experimental investigations have illustrated that the apical part pulp could retain its vitality and coronally proliferate after reimplantation in presence of adequate extracellular matrix (ECM) and cells, leading to partial replacement of the necrotized region (Certosimo and Archer, 1995). Growing evidences also indicate that the stem cells of apical Papilla (SCAP) (Bakopoulou et al., 2011) as well as the inflamed periapical progenitor cells (Hargreaves et al., 2013) can exist in nonvital teeth with apical periodontitis. The cells from Hertwig's epithelial root sheath (HERS), being unaffected by any infection could have induced healing in these teeth.

18.3.1.2 Closed apices

The regenerative potency of dental pulp, especially in mature teeth, is extremely restricted, mostly because of their poor blood supply which results in poor self-healing capacity and regrowth (Sun et al., 2011). Due to this limitation, REP therapy has not been widely employed in teeth with mature apices.

In the case of closed apices a modified REP protocol is mostly adopted which involves preparation of the cavity access, initial canal cleansing, and shaping, followed by agitating the periapical tissue sufficiently by widening the root apex to facilitate uninterrupted flow of blood and periapical MSCs into the root canal. In another approach, instead of agitating the apical area to induce the blood clot formation in the canal, PRP was introduced into root canal (Torabinejad and Turman, 2011). In a case study on REP treatment of the maxillary central incisors with mature apices and apical periodontitis, the apical foramen was widened to 0.6 mm and a sterile collagen scaffold was inserted in the root canal. Bleeding in the canal was induced from the periapical region and the canal was then sealed using mineral trioxide aggregate (MTA). After 2 weeks the apical radiolucency was resolved and the clinical symptoms were regressed. At 6-month recall the tooth responded favorably to electric pulp test (Paryani and Kim, 2013).

18.3.1.3 Avulsed teeth

Traditional treatment protocol for avulsed teeth involves root canal treatment of the tooth post reimplantation. Attempts for regenerating dental pulp have also been made in replanted avulsed teeth. A successful tissue regeneration significantly relies on the predominance of the new tissue formation against the bacteria present in the pulp space (Kling et al., 1986). In majority of the cases the tooth crown is sound and safeguards the canal from bacterial diffusion inside the pulp space, favoring the new tissue formation over the bacterial infection (Banchs and Trope, 2004). There is little evidence supporting utilization of the endodontic revascularization on avulsed and replanted teeth.

Torabinejd and Turman (2011) reported on REP on an accidently extracted maxillary second premolar which had an open apex. PRP was used for pulp regeneration purpose. Clinical examination after 5.5 months revealed that tooth was symptom free the periapical lesion was resolved and there was apical closure and continued root development (Torabinejad and Turman, 2011). This indicated that the use of autogenous and biocompatible scaffolds with sufficient apical foramen patency can facilitate the outcome of regenerative therapies in an avulsed tooth.

18.3.2 Key components of regenerative endodontic procedures

The REP procedure is mainly based on the principles of tissue engineering which utilize appropriate sources of three key components including stem cells/progenitor, scaffolds, as well as the growth factors in order to govern the development of target tissue (Hargreaves et al., 2013).

18.3.2.1 Cells

Pulp contains pluripotent cells capable of division and differentiation into specialized lineages in response to morphogens. The pluripotent cells of pulp, dental papilla (Huang et al., 2010b; Seo et al., 2004), and periodontal ligament (Seo et al., 2004) are convenient for harvesting under appropriate regulations. Owing to the autogenous nature of these cells, introduction of foreign pathogens during regeneration process could be avoided (Murray et al., 2007). The main cell source during REP originates from the MSC from the periapical region. The concentration of MSC markers (CD73 and CD105) in blood clot is approximately 400−600 times higher than the circulating systemic blood, confirming the presence of stem cells in the blood clot which enter from the periapical area (Lovelace et al., 2011). The formation of pulp-like tissue has been shown in in vivo studies when MSCs of dental origin such as dental pulp stem cells (DPSCs) (Prescott et al., 2008), stem cells from human exfoliated deciduous teeth (SHED) (Cordeiro et al., 2008), and stem cells from apical papilla (SCAP) (Sonoyama et al., 2006; Gronthos et al., 2000) were introduced (Miura et al., 2003). However, this may not applied to the stem cells which originate from bone marrow due to their inclination to differentiate into odontoblasts (Huang, 2011).

18.3.2.2 Scaffolds

Scaffolds are 3D structures which promote optimal cell growth and differentiation (Abdal-hay et al., 2013). ECM molecules responsible for stem cells differentiation are aided by a suitable scaffold, acting as a platform for cell localization and migration (Yuan et al., 2011). An ideal scaffold must allow facilitated cell attachment, migration, proliferation, and 3D arrangement in order to regenerate the structural and functional characteristics of the target tissue with adequate mechanical integrity. Biocompatibility is the most crucial characteristic of the scaffolds required to prevent adverse tissue reactions (Galler et al., 2011a).

Both synthetic and natural biomaterials with the correct surface morphology and the mechanical integrity have been employed to create scaffolds in REP therapies. Synthetic scaffold are traditionally fabricated using rapid prototyping or 3D printers with different degree of success (Zhang et al., 2013), using polylactic acid or polyglycolic acid (Young et al., 2002; Bäumchen et al., 2009) which have been shown to result in generation of pulp-like tissue after implantation into subcutaneous areas in immuno-compromised mice (Buurma et al., 1999). Recently with the advancement of nanotechnology, a number of nanofibrous polymers for partial or complete mimicking of ECMs have been introduced which provide large surface area and interconnected pores using fibers with nanoscale dimension (Tatullo et al., 2015; Park et al., 2010). These scaffolds have been shown to enhance cell proliferation, retain cell phenotype, guide the growth and differentiation of stem cells, and trigger pathways for cell-signaling with chemical and physical stimuli (Gupte and Ma, 2012). The macroporous polymer scaffolds fabricated by polymer casting technique have also been utilized for pulp tissue engineering (Sakai et al., 2011). Self-assembling nanofibrous scaffolds are useful for fabrication of customized matrices with controlled mechanical integrity (Galler et al., 2011a), cell encapsulation, drug delivery systems for disinfection of root canal (Bottino et al., 2013; Palasuk et al., 2014), and controlled delivery of bioactive agents such as antimicrobials and angiogenic factors (Cavalcanti et al., 2013; Coyac et al., 2013). Utilization of injectable hydrogel polymers provides sufficient support for intracanal delivery of stem cells and biomolecules (Rosa et al., 2013; Cavalcanti et al., 2013), however it lacks mechanical integrity. A promising self-assembling peptide hydrogel-based nanofibrous scaffold has been developed with the commercial name Puramatrix (Rosa et al., 2013). A combination of SHED stem cells and Puramatrix was administered inside the full-length root canals in order to form a pulp-like tissue (Rosa et al., 2013). The macroporous polymer scaffolds fabricated by polymer casting have also been utilized for pulp tissue engineering (Sakai et al., 2011). Although these scaffolds support cell attachment and proliferation and guide cell differentiation, their high intrinsic stiffness raised clinical concerns in terms of scaffold attachment to dentin walls along the root canal length, a key feature in successful pulp regeneration.

Natural scaffolds such as matrices derived from biological sources (collagen, chitosan, silk, PRP, and PRF) (Vacanti et al., 1998; Bansal et al., 2015) have also shown potential to be used as scaffolds in REP therapies. In particular, autogenous scaffolds involving blood clot from intracanal bleeding, PRP/PRF, and collagen have shown positive effect on the outcome of REP therapy (Narayana et al., 2012). A sufficiently vascularized pulp tissue was regenerated with new dentine deposition in pulpotomized pulp-chamber spaces of dog teeth filled with collagen scaffold (Iohara et al., 2009). A human endodontic regenerative study showed that using collagen scaffold with blood clot in the root canal improved the revascularization outcome (Garcia-Godoy and Murray, 2012). A recent study reported pulp-like tissue formation by utilizing silk fibroin scaffold loaded with basic fibroblast growth factor and seeded with DPSCs (Yang et al., 2015). A combination of stem cells and inductive scaffolds could be employed to enhance the efficacy of dentin-pulp complex regeneration (Galler et al., 2011a).

18.3.2.3 Growth factors

Growth factors are essential components of REP therapies required for stimulation of cellular growth, proliferation, healing, and differentiation. These components are generally composed of proteins or steroids which serve as signaling molecules between the cells, and enhance their differentiation and maturation (Dorland, 2011). For instance, insulin-like and fibroblast growth factors stimulate blood vessel differentiation (angiogenesis), whereas bone morphogenetic proteins promote differentiation of bone cells. Blood clot is believed to contain various growth factors and stem cells from periapical area (Huang et al., 2006).

18.3.3 Clinical protocols of regenerative endodontics procedure

REP therapies are comprised of a number of procedural protocols that play an imperative role in determining the clinical prognosis. Three major procedural components involve irrigation, intracanal medicament, and intracanal barrier.

18.3.3.1 Irrigation

Irrigation is known as a potential approach to influence the survival of the adjacent cells to the root walls and the residing cells in the periapical tissues, via both direct and indirect approaches. Most preferred irrigant in standard root canal procedures is sodium hypochlorite (NaOCl) (Kontakiotis et al., 2014) with potent antimicrobial action at the concentrations above 3%. However, due to its potential toxicity at these concentrations, NaOCl concentrations below 1.5% is recommended by American Association of Endodntists (AAE) (American Association of Endodontists (AAE), 2013). In some case reports, NaOCl was combined with chlorhexidine (CHX) at the first REP appointment and the canal was irrigated with saline between two irrigation steps by NaOCl and CHX to enhance the efficacy of canal disinfection (Petrino et al., 2010; Kuruvilla and Kamath, 1998; Weber et al., 2003). Investigation of different irrigant regimens has shown that the choice of irrigant would significantly affect the survivability of the cells in REP therapy (Trevino et al., 2011). Application of ethylenediamine tetraacetic acid (EDTA) in irrigation protocol could be harmless, while 2% CHX is found detrimental to the SCAP (Trevino et al., 2011). 17% EDTA supports the SCAP survival and adoption to the dentinal walls of the root canal, as well as enhanced proliferation of growth factors from demineralized dentin, resulting in improved differentiation of pulp-originated stem cells to the odontoblast-like cells (Galler et al., 2011b). The final irrigation by 17% EDTA could partially converse the detrimental influences of high-concentration NaOCl solutions on the SCAPs survival and differentiation (Martin et al., 2014). Therefore this EDTA concentration is recommended as a final rinse in REP therapies, due to its capability to optimize the environmental conditions for regeneration of pulp tissue (Trevino et al., 2011).

18.3.3.2 Intracanal medication

Root canal disinfection is an integral part of any radical or regenerative pulp therapy. Disinfection of the root canal spaces in the immature teeth is mostly challenging and thus, more effective antimicrobial regimens are required to create a conducive environment (Bansal et al., 2015). There is a continuous debate between the use of calcium hydroxide ($Ca(OH)_2$) dressing and triple antibiotic paste. $Ca(OH)_2$ is the preferred intracanal medication in apexification and pulpotomy procedures (Mohammadi and Dummer, 2011). However, the high pH levels obtained by this material is assumed to reduce the viability and regenerative capacity of the apical vital cells (Spångberg, 1968). Some evidences indicate that insertion of $Ca(OH)_2$ in the root canal of human tooth may prevent revascularization coronal to the medicament location (Schroder and Granath, 1971). On the other hand, $Ca(OH)_2$ could stimulate the HERS, leading to enhanced development of proper root morphology (Saad, 1988). A recent study on human dentine disks also reported that $Ca(OH)_2$ promoted the proliferation of SCAPs (Althumairy et al., 2014). As a result, the current agreement for utilizing $Ca(OH)_2$ is limited to the coronal part of the root canal for effective canal disinfection, while avoiding any detrimental effect on viability of cells in the periapical region (Hargreaves et al., 2013).

Based on the clinical considerations, application of either $Ca(OH)_2$ or antibiotic combination pastes are recommended by the AAE. Nonetheless, triple antibiotic paste is the most preferred intracanal medicament for REP therapy (Althumairy et al., 2014), which has been shown to provide improved disinfection in cases of revascularization (Sato et al., 1993). The ability of triple antibiotic pastes to eradicate bacteria in dentinal tubules (Sato et al., 1996; Hoshino et al., 1996) is probably the main reason for their preferred use in REP therapies. Although various antibiotic combinations have been utilized to manage the root canal infection (Banchs and Trope, 2004; Iwaya et al., 2001), triple antibiotic combination paste comprised of CIPX, MTZ, and MIN is considered as the mostly preferred intracanal medication for REP therapy. According to the microbiological studies, this paste is able to maximally eliminate the colonizing bacteria in dentinal tubules (Sato et al., 1996). However, some in vitro studies perceived damaging effects of antibiotic pastes on SCAP survival at concentrations higher than 1 mg/ml (Ruparel et al., 2012; Althumairy et al., 2014). Moreover, these pastes increase the tooth vulnerability to discoloration, probably due to the presence of MIN. In order to overcome this limitation, MIN is either removed from the antibiotic paste (double antibiotic paste) or substituted with cefaclor (Kim et al., 2010a). The apical negative pressure irrigation system, EndoVac in lieu of intracanal antibiotics placement is also suggested (Cohenca et al., 2010).

18.3.3.3 Intracanal barrier

Intracanal barrier is a physical and chemical obstacle which prevents canal reinfection and minimizes the coronal leakage (Cohenca, 2014). The majority of cases undergoing REP therapies have employed MTA as the coronal barrier because of

its sealing capability (Camilleri et al., 2004), biocompatibility (Tselnik et al., 2004), high setting ability in presence of moisture (Camilleri, 2007), and tissue-conductive properties (Torabinejad and Parirokh, 2010). The coronal edge of MTA should be placed 1−2 mm apical to the cemento-enamel junction (versus 3−4 mm) to allow improved root development (Banchs and Trope, 2004). Collagen sponge has also been used to promote tissue ingrowth, mostly when insufficient intracanal bleeding is observed (Jung et al., 2012). In some cases, MTA could be placed over a collagen matrix to avoid apical displacement of the material (Petrino et al., 2010). However, the influence of the collagen matrix on the REP outcome is not well-defined. MTA could also be replaced by ready-to-use bioceramic materials which present comparable degrees of biocompatibility and bioactivity with that of MTA (Ma et al., 2011; De-Deus et al., 2009). Calcium enriched mixture have also been suggested as alternatives to MTA, due to its potential to promote differentiation into dentin-like hard tissue.

18.4 Conclusion

Although regenerative endodontics is shown as a promising development in endodontics, this therapy has not been well-established in clinical setting mostly due to the insufficient supportive findings for encouraging the clinicians to employ this approach. The present literature on this technique is mostly available as case series without undertaking randomized controlled trials. Moreover, some of these case reports have reported inconsistent post-treatment findings such as ingrowth of granulation, fibrous tissues instead of pulp, as well as ingrowth of cementum and bone. The major differences among pre-, intra-, and postoperative outcomes such as empty canal, no pulp-like tissue on histological analysis as well as the lack of sufficient evidences for the consistent outcome, inhibit the development of a standardized protocol.

The high variability of clinical protocols in REPs necessitates standardization of the clinical protocols to accurately anticipate the outcome of these therapies. Patency of the apical foramen has to be established and maintained prior to considering regenerative endodontic treatment. In spite of lacking any consensus on the optimal size of the apical foramen desirable for successful pulp regeneration, it is certainly mandatory to have an uninterrupted entry of blood clot in the prepared or unprepared canals for promising accomplishment of regenerative endodontic therapy.

Methods of in situ tissue regeneration relying on endogenous cell homing, functional stimulation, and local tissue responses hold great promise. In contrast to the clinical REPs, in situ tissue regeneration of dental pulp follows the classical tissue engineering techniques including the utilization of exogenous scaffolds and growth factors. On the hindsight, modifying current in situ regenerative endodontic protocols by incorporating classical tissue engineering approaches may result in more successful therapies.

Acknowledgments

This study was financially supported by (UM.C/625/1/HIR/MOHE/DENT/05). The authors wish to thank Mr. Nareshwaran Gnanasegaran for providing insight and expertise that greatly assisted in improving the quality of this work. The authors report no conflicts of interest.

References

Abdal-Hay, A., Lim, J., Shamshi Hassan, M., Lim, J.K., 2013. Ultrathin conformal coating of apatite nanostructures onto electrospun nylon 6 nanofibers: mimicking the extracellular matrix. Chem. Eng. J. 228, 708–716.

Althumairy, R.I., Teixeira, F.B., Diogenes, A., 2014. Effect of dentin conditioning with intracanal medicaments on survival of stem cells of apical papilla. J. Endodont. 40, 521–525.

American Association of Endodontists (AAE), 2013. Clinical Considerations for Regenerative Procedures. American Association of Endodontists (AAE), Chicago, IL.

Andreasen, J.O., Andreasen, F.M., Andersson, L. (Eds.), 2013. Textbook and Color Atlas of Traumatic Injuries to the Teeth. John Wiley & Sons, Hoboken, NJ.

Bakopoulou, A., Leyhausen, G., Volk, J., Tsiftsoglou, A., Garefis, P., Koidis, P., et al., 2011. Comparative analysis of in vitro osteo/odontogenic differentiation potential of human dental pulp stem cells (DPSCs) and stem cells from the apical papilla (SCAP). Arch. Oral Biol. 56, 709–721.

Banchs, F., Trope, M., 2004. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? J. Endodont. 30, 196–200.

Bansal, R., Jain, A., Mittal, S., 2015. Current overview on challenges in regenerative endodontics. J. Conserv. Dent. 18, 1–6.

Bäumchen, F., Smeets, R., Koch, D., Gräber, H.G., 2009. The impact of defined polyglycolide scaffold structure on the proliferation of gingival fibroblasts in vitro: a pilot study. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol. 108, 505–513.

Bottino, M.C., Kamocki, K., Yassen, G.H., Platt, J.A., Vail, M.M., Ehrlich, Y., et al., 2013. Bioactive nanofibrous scaffolds for regenerative endodontics. J. Dent. Res. 92, 963–969.

Buurma, B., Gu, K., Rutherford, R.B., 1999. Transplantation of human pulpal and gingival fibroblasts attached to synthetic scaffolds. Eur. J. Oral Sci. 107, 282–289.

Camilleri, J., 2007. Hydration mechanisms of mineral trioxide aggregate. Int. Endodont. J. 40, 462–470.

Camilleri, J., Montesin, F.E., Papaioannou, S., Mcdonald, F., Pitt Ford, T.R., 2004. Biocompatibility of two commercial forms of mineral trioxide aggregate. Int. Endodont. J. 37, 699–704.

Caplan, D.J., Cai, J., Yin, G., White, B.A., 2005. Root canal filled versus non-root canal filled teeth: a retrospective comparison of survival times. J. Public Health Dent. 65, 90–96.

Cavalcanti, B.N., Zeitlin, B.D., Nor, J.E., 2013. A hydrogel scaffold that maintains viability and supports differentiation of dental pulp stem cells. Dent. Mater. 29, 97–102.

Cehreli, Z.C., Isbitiren, B., Sara, S., Erbas, G., 2011. Regenerative endodontic treatment (revascularization) of immature necrotic molars medicated with calcium hydroxide: a case series. J. Endodont. 37, 1327–1330.

Certosimo, A., Archer, R., 1995. A clinical evaluation of the electric pulp tester as an indicator of local anesthesia. Oper. Dent. 21, 25–30.

Chen, M.Y., Chen, K.L., Chen, C.A., Tayebaty, F., Rosenberg, P.A., Lin, L.M., 2012. Responses of immature permanent teeth with infected necrotic pulp tissue and apical periodontitis/abscess to revascularization procedures. Int. Endodont. J. 45, 294–305.

Chueh, L.H., Huang, G.T., 2006. Immature teeth with periradicular periodontitis or abscess undergoing apexogenesis: a paradigm shift. J. Endodont. 32, 1205–1213.

Cohenca, N. (Ed.), 2014. Disinfection of Root Canal Systems: The Treatment of Apical Periodontitis. John Wiley & Sons, Ames, IA.

Cohenca, N., Heilborn, C., Johnson, J.D., Flores, D.S.H., Ito, I.Y., Da Silva, L.A.B., 2010. Apical negative pressure irrigation versus conventional irrigation plus triantibiotic intracanal dressing on root canal disinfection in dog teeth. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol. 109, e42–e46.

Colombo, J.S., Moore, A.N., Hartgerink, J.D., D'souza, R.N., 2014. Scaffolds to control inflammation and facilitate dental pulp regeneration. J. Endodont. 40, S6–S12.

Cordeiro, M.M., Dong, Z., Kaneko, T., Zhang, Z., Miyazawa, M., Shi, S., et al., 2008. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J. Endodont. 34, 962–969.

Cotti, E., Mereu, M., Lusso, D., 2008. Regenerative treatment of an immature, traumatized tooth with apical periodontitis: report of a case. J. Endodont. 34, 611–616.

Coyac, B.R., Chicatun, F., Hoac, B., Nelea, V., Chaussain, C., Nazhat, S.N., et al., 2013. Mineralization of dense collagen hydrogel scaffolds by human pulp cells. J. Dent. Res. 92, 648–654.

Dabbagh, B., Alvaro, E., Vu, D.-D., Rizkallah, J., Schwartz, S., 2012. Clinical complications in the revascularization of immature necrotic permanent teeth. Pediatr. Dent. 34, 414–417.

De-Deus, G., Canabarro, A., Alves, G., Linhares, A., Senne, M.I., Granjeiro, J.M., 2009. Optimal cytocompatibility of a bioceramic nanoparticulate cement in primary human mesenchymal cells. J. Endodont. 35, 1387–1390.

Ding, R.Y., Cheung, G.S.-P., Chen, J., Yin, X.Z., Wang, Q.Q., Zhang, C.F., 2009. Pulp revascularization of immature teeth with apical periodontitis: a clinical study. J. Endodont. 35, 745–749.

Dorland, W.A.N., 2011. Dorland's Illustrated Medical Dictionary. Elsevier Health Sciences, Philadelphia, PA.

Dusseiller, M.R., Schlaepfer, D., Koch, M., Kroschewski, R., Textor, M., 2005. An inverted microcontact printing method on topographically structured polystyrene chips for arrayed micro-3-D culturing of single cells. Biomaterials. 26, 5917–5925.

Forsberg, C., Tedestam, G., 1989. Traumatic injuries to teeth in Swedish children living in an urban area. Swed. Dent. J. 14, 115–122.

Fuss, Z., Lustig, J., Katz, A., Tamse, A., 2001. An evaluation of endodontically treated vertical root fractured teeth: impact of operative procedures. J. Endodont. 27, 46–48.

Galler, K.M., D'souza, R.N., Federlin, M., Cavender, A.C., Hartgerink, J.D., Hecker, S., et al., 2011b. Dentin conditioning codetermines cell fate in regenerative endodontics. J. Endodont. 37, 1536–1541.

Galler, K., D'souza, R., Hartgerink, J., Schmalz, G., 2011a. Scaffolds for dental pulp tissue engineering. Adv. Dent. Res. 23, 333–339.

Garcia-Godoy, F., Murray, P.E., 2012. Recommendations for using regenerative endodontic procedures in permanent immature traumatized teeth. Dent. Traumatol. 28, 33–41.

Ghoddusi, J., Forghani, M., Parisai, I., 2013. New approaches in vital pulp therapy in permanent teeth. Int. Endodont. J. 9, 15–22.

Gronthos, S., Mankani, M., Brahim, J., Robey, P.G., Shi, S., 2000. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc. Natl. Acad. Sci. U.S.A. 97, 13625−13630.

Gupte, M.J., Ma, P.X., 2012. Nanofibrous scaffolds for dental and craniofacial applications. J. Dent. Res. 91, 227−234.

Ham, J.W., Patterson, S.S., Mitchell, D.F., 1972. Induced apical closure of immature pulpless teeth in monkeys. Oral Surg. Oral Med. Oral Pathol. 33, 438−449.

Hargreaves, K.M., Diogenes, A., Teixeira, F.B., 2013. Treatment options: biological basis of regenerative endodontic procedures. J. Endodont. 39, S30−S43.

Hoshino, E., Kurihara-Ando, N., Sato, I., Uematsu, H., Sato, M., Kota, K., et al., 1996. In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. Int. Endodont. J. 29, 125−130.

Huang, F.-M., Yang, S.-F., Zhao, J.-H., Chang, Y.-C., 2010a. Platelet-rich fibrin increases proliferation and differentiation of human dental pulp cells. J. Endodont. 36, 1628−1632.

Huang, G.T., Yamaza, T., Shea, L.D., Djouad, F., Kuhn, N.Z., Tuan, R.S., et al., 2010b. Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. Tissue Eng. A. 16, 605−615.

Huang, G.T.-J., 2011. Dental pulp and dentin tissue engineering and regeneration−advancement and challenge. Front. Biosci. (Elite Edition). 3, 788−800.

Huang, G.T.-J., Shagramanova, K., Chan, S.W., 2006. Formation of odontoblast-like cells from cultured human dental pulp cells on dentin in vitro. J. Endodont. 32, 1066−1073.

Iohara, K., Zheng, L., Ito, M., Ishizaka, R., Nakamura, H., Into, T., et al., 2009. Regeneration of dental pulp after pulpotomy by transplantation of CD31(-)/CD146(-) side population cells from a canine tooth. Regen. Med. 4, 377−385.

Iwaya, S.I., Ikawa, M., Kubota, M., 2001. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dent. Traumatol. 17, 185−187.

Iwaya, S.-I., Ikawa, M., Kubota, M., 2011. Revascularization of an immature permanent tooth with periradicular abscess after luxation. Dent. Traumatol. 27, 55−58.

Jadhav, G., Shah, N., Logani, A., 2012. Revascularization with and without platelet-rich plasma in nonvital, immature, anterior teeth: a pilot clinical study. J. Endodont. 38, 1581−1587.

Jakab, K., Norotte, C., Damon, B., Marga, F., Neagu, A., Besch-Williford, C.L., et al., 2008. Tissue engineering by self-assembly of cells printed into topologically defined structures. Tissue Eng. A. 14, 413−421.

Jung, I.Y., Lee, S.J., Hargreaves, K.M., 2008. Biologically based treatment of immature permanent teeth with pulpal necrosis: a case series. J. Endodont. 34, 876−887.

Jung, I.Y., Lee, S.J., Hargreaves, K.M., 2012. Biologically based treatment of immature permanent teeth with pulpal necrosis: a case series. Texas Dent. J. 129, 601−616.

Keswani, D., Pandey, R., 2013. Revascularization of an immature tooth with a necrotic pulp using platelet-rich fibrin: a case report. Int. Endodont. J. 46, 1096−1104.

Kim, J.Y., Xin, X., Moioli, E.K., Chung, J., Lee, C.H., Chen, M., et al., 2010b. Regeneration of dental-pulp-like tissue by chemotaxis-induced cell homing. Tissue Eng. A. 16, 3023−3031.

Kim, J.-H., Kim, Y., Shin, S.-J., Park, J.-W., Jung, I.-Y., 2010a. Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: a case report. J. Endodont. 36, 1086−1091.

Kling, M., Cvek, M., Mejare, I., 1986. Rate and predictability of pulp revascularization in therapeutically reimplanted permanent incisors. Endodont. Dent. Traumatol. 2, 83−89.

Kontakiotis, E.G., Filippatos, C.G., Agrafioti, A., 2014. Levels of evidence for the outcome of regenerative endodontic therapy. J. Endodont. 40, 1045−1053.

Kuo, T.F., Huang, A.T., Chang, H.H., Lin, F.H., Chen, S.T., Chen, R.S., et al., 2008. Regeneration of dentin-pulp complex with cementum and periodontal ligament formation using dental bud cells in gelatin-chondroitin-hyaluronan tri-copolymer scaffold in swine. J. Biomed. Mater. Res. A. 86, 1062–1068.

Kuruvilla, J.R., Kamath, M.P., 1998. Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined, as endodontic irrigants. J. Endodont. 24, 472–476.

Laureys, W.G., Cuvelier, C.A., Dermaut, L.R., DE Pauw, G.A., 2013. The critical apical diameter to obtain regeneration of the pulp tissue after tooth transplantation, replantation, or regenerative endodontic treatment. J. Endodont. 39, 759–763.

Laureys, W., Beele, H., Cornelissen, R., Dermaut, L., 2001. Revascularization after cryopreservation and autotransplantation of immature and mature apicoectomized teeth. Am. J. Orthodont. Dentofac. Orthoped. 119, 346–352.

Lenherr, P., Allgayer, N., Weiger, R., Filippi, A., Attin, T., Krastl, G., 2012. Tooth discoloration induced by endodontic materials: a laboratory study. Int. Endodont. J. 45, 942–949.

Lin, L.M., Skribner, J.E., Gaengler, P., 1992. Factors associated with endodontic treatment failures. J. Endodont. 18, 625–627.

Lovelace, T.W., Henry, M.A., Hargreaves, K.M., Diogenes, A., 2011. Evaluation of the delivery of mesenchymal stem cells into the root canal space of necrotic immature teeth after clinical regenerative endodontic procedure. J. Endodont. 37, 133–138.

Ma, J., Shen, Y., Stojicic, S., Haapasalo, M., 2011. Biocompatibility of two novel root repair materials. J. Endodont. 37, 793–798.

Machado, E., Fernandes, M.H., DE Sousa Gomes, P., 2012. Dental stem cells for craniofacial tissue engineering. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 113, 728–733.

Martin, D.E., DE Almeida, J.F., Henry, M.A., Khaing, Z.Z., Schmidt, C.E., Teixeira, F.B., et al., 2014. Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. J. Endodont. 40, 51–55.

Miura, M., Gronthos, S., Zhao, M., Lu, B., Fisher, L.W., Robey, P.G., et al., 2003. SHED: stem cells from human exfoliated deciduous teeth. Proc. Natl. Acad Sci. U.S.A. 100, 5807–5812.

Mohammadi, Z., Dummer, P.M.H., 2011. Properties and applications of calcium hydroxide in endodontics and dental traumatology. Int. Endodont. J. 44, 697–730.

Murray, P.E., Garcia-Godoy, F., Hargreaves, K.M., 2007. Regenerative endodontics: a review of current status and a call for action. J. Endodont. 33, 377–390.

Nakashima, M., Iohara, K., 2014. Mobilized dental pulp stem cells for pulp regeneration: initiation of clinical trial. J. Endodont. 40, S26–S32.

Narayana, P., Hartwell, G.R., Wallace, R., Nair, U.P., 2012. Endodontic clinical management of a dens invaginatus case by using a unique treatment approach: a case report. J. Endodont. 38, 1145–1148.

Nelson, S.J., 2014. Wheeler's Dental Anatomy, Physiology and Occlusion. Saunders Elsevier, St. Louis, MO.

Ng, Y.L., Mann, V., Gulabivala, K., 2011. A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: part 1: periapical health. Int. Endodont. J. 44, 583–609.

Nosrat, A., Homayounfar, N., Oloomi, K., 2012. Drawbacks and unfavorable outcomes of regenerative endodontic treatments of necrotic immature teeth: a literature review and report of a case. J. Endodont. 38, 1428–1434.

Östby, B.N., 1961. The role of the blood clot in endodontic therapy. An experimental histologic study. Acta Odontol. Scand. 19, 323–353.

Palasuk, J., Kamocki, K., Hippenmeyer, L., Platt, J.A., Spolnik, K.J., Gregory, R.L., et al., 2014. Bimix antimicrobial scaffolds for regenerative endodontics. J. Endodont. 40, 1879–1884.

Park, C.H., Rios, H.F., Jin, Q., Bland, M.E., Flanagan, C.L., Hollister, S.J., et al., 2010. Biomimetic hybrid scaffolds for engineering human tooth-ligament interfaces. Biomaterials. 31, 5945–5952.

Paryani, K., Kim, S.G., 2013. Regenerative endodontic treatment of permanent teeth after completion of root development: a report of 2 cases. J. Endodont. 39, 929–934.

Petrino, J.A., Boda, K.K., Shambarger, S., Bowles, W.R., Mcclanahan, S.B., 2010. Challenges in regenerative endodontics: a case series. J. Endodont. 36, 536–541.

Prescott, R.S., Alsanea, R., Fayad, M.I., Johnson, B.R., Wenckus, C.S., Hao, J., et al., 2008. In vivo generation of dental pulp-like tissue by using dental pulp stem cells, a collagen scaffold, and dentin matrix protein 1 after subcutaneous transplantation in mice. J. Endodont. 34, 421–426.

Reynolds, K., Johnson, J.D., Cohenca, N., 2009. Pulp revascularization of necrotic bilateral bicuspids using a modified novel technique to eliminate potential coronal discolouration: a case report. Int. Endodont. J. 42, 84–92.

Rosa, V., Zhang, Z., Grande, R., Nör, J., 2013. Dental pulp tissue engineering in full-length human root canals. J. Dent. Res. 92, 970–975.

Ruparel, N.B., Teixeira, F.B., Ferraz, C.C., Diogenes, A., 2012. Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. J. Endodont. 38, 1372–1375.

Saad, A.Y., 1988. Calcium hydroxide and apexogenesis. Oral Surg. Oral Med. Oral Pathol. 66, 499–501.

Sakai, V.T., Cordeiro, M.M., Dong, Z., Zhang, Z., Zeitlin, B.D., Nor, J.E., 2011. Tooth slice/scaffold model of dental pulp tissue engineering. Adv. Dent. Res. 23, 325–332.

Sato, I., Ando-Kurihara, N., Kota, K., Iwaku, M., Hoshino, E., 1996. Sterilization of infected root-canal dentine by topical application of a mixture of ciprofloxacin, metronidazole and minocycline in situ. Int. Endodont. J. 29, 118–124.

Sato, T., Hoshino, E., Uematsu, H., Noda, T., 1993. In vitro antimicrobial susceptibility to combinations of drugs of bacteria from carious and endodontic lesions of human deciduous teeth. Oral Microbiol. Immunol. 8, 172–176.

Schroder, U., Granath, L.E., 1971. Early reaction of intact human teeth to calcium hydroxide following experimental pulpotomy and its significance to the development of hard tissue barrier. Odontol. Revy. 22, 379–395.

Seo, B.-M., Miura, M., Gronthos, S., Bartold, P.M., Batouli, S., Brahim, J., et al., 2004. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet. 364, 149–155.

Shin, S.Y., Albert, J.S., Mortman, R.E., 2009. One step pulp revascularization treatment of an immature permanent tooth with chronic apical abscess: a case report. Int. Endodont. J. 42, 1118–1126.

Sonoyama, W., Liu, Y., Fang, D., Yamaza, T., Seo, B.-M., Zhang, C., et al., 2006. Mesenchymal stem cell-mediated functional tooth regeneration in swine. PloS One. 1, e79.

Spångberg, L., 1968. Biological effects of root canal filling materials. 4. Effect in vitro of solubilised root canal filling materials on HeLa cells. Odontol. Revy. 20, 289–299.

Sun, H.H., Jin, T., Yu, Q., Chen, F.M., 2011. Biological approaches toward dental pulp regeneration by tissue engineering. J. Tissue Eng. Regen. Med. 5, e1–e16.

Tatullo, M., Marrelli, M., Shakesheff, K.M., White, L.J., 2015. Dental pulp stem cells: function, isolation and applications in regenerative medicine. J. Tissue Eng. Regen. Med. 9, 1205–1216.

Thibodeau, B., 2009. Case report: pulp revascularization of a necrotic, infected, immature, permanent tooth. Pediatr. Dent. 31, 145−148.

Thibodeau, B., Teixeira, F., Yamauchi, M., Caplan, D.J., Trope, M., 2007. Pulp revascularization of immature dog teeth with apical periodontitis. J. Endodont. 33, 680−689.

Thomson, A., Kahler, B., 2010. Regenerative endodontics—biologically-based treatment for immature permanent teeth: a case report and review of the literature. Aust. Dent. J. 55, 446−452.

Torabinejad, M., Faras, H., 2012. A clinical and histological report of a tooth with an open apex treated with regenerative endodontics using platelet-rich plasma. J. Endodont. 38, 864−868.

Torabinejad, M., Parirokh, M., 2010. Mineral trioxide aggregate: a comprehensive literature review-part II: leakage and biocompatibility investigations. J. Endodont. 36, 190−202.

Torabinejad, M., Turman, M., 2011. Revitalization of tooth with necrotic pulp and open apex by using platelet-rich plasma: a case report. J. Endodont. 37, 265−268.

Trevino, E.G., Patwardhan, A.N., Henry, M.A., Perry, G., Dybdal-Hargreaves, N., Hargreaves, K.M., et al., 2011. Effect of irrigants on the survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips. J. Endodont. 37, 1109−1115.

Trope, M., 2008. Regenerative potential of dental pulp. J. Endodont. 34, S13−S17.

Tselnik, M., Baumgartner, J.C., Marshall, J.G., 2004. Bacterial leakage with mineral trioxide aggregate or a resin-modified glass ionomer used as a coronal barrier. J. Endodont. 30, 782−784.

Vacanti, J.P., Langer, R., Upton, J., Marler, J.J., 1998. Transplantation of cells in matrices for tissue regeneration. Adv. Drug Deliv. Rev. 33, 165−182.

Weber, C.D., Mcclanahan, S.B., Miller, G.A., Diener-West, M., Johnson, J.D., 2003. The effect of passive ultrasonic activation of 2% chlorhexidine or 5.25% sodium hypochlorite irrigant on residual antimicrobial activity in root canals. J. Endodont. 29, 562−564.

Yang, J.W., Zhang, Y.F., Sun, Z.Y., Song, G.T., Chen, Z., 2015. Dental pulp tissue engineering with bFGF-incorporated silk fibroin scaffolds. J. Biomater. Appl. 30, 221−229.

Young, C., Terada, S., Vacanti, J., Honda, M., Bartlett, J., Yelick, P., 2002. Tissue engineering of complex tooth structures on biodegradable polymer scaffolds. J. Dent. Res. 81, 695−700.

Yuan, Z., Nie, H., Wang, S., Lee, C.H., Li, A., Fu, S.Y., et al., 2011. Biomaterial selection for tooth regeneration. Tissue Eng. B: Rev. 17, 373−388.

Zhang, D.-D., Chen, X., Bao, Z.-F., Chen, M., Ding, Z.-J., Zhong, M., 2014. Histologic comparison between platelet-rich plasma and blood clot in regenerative endodontic treatment: an animal study. J. Endodont. 40, 1388−1393.

Zhang, L., Morsi, Y., Wang, Y., Li, Y., Ramakrishna, S., 2013. Review scaffold design and stem cells for tooth regeneration. Jpn. Dent. Sci. Rev. 49, 14−26.

Oral nerve tissue repair and regeneration

19

Hossein E. Jazayeri[1], *Farahnaz Fahimipour*[2], *Mohammadreza Tahriri*[2], *Luis Almeida*[2] and *Lobat Tayebi*[2]
[1]University of Pennsylvania School of Dental Medicine, Philadelphia, PA, United States, [2]Marquette University School of Dentistry, Milwaukee, WI, United States

19.1 Introduction

The nervous system is an extremely complex and integral part of the body that is responsible for sensory and motor functions, and injury to neuronal tissue is tremendously difficult to repair (Ghasemi-Mobarakeh et al., 2011). Part of this reason has to do with the fact that mature neurons are not capable of undergoing mitosis (Prabhakaran et al., 2009). Approximately 2.8% of trauma patients suffer from peripheral nerve injury that often leads to a debilitating lifestyle change (Ciardelli and Chiono, 2006). Strategies are mostly centered on ultimately regenerating axons to restore functionality because of their role as the regulator of neuronal electrical signaling (Schmidt et al., 1997). Tissue engineering in this medical discipline typically involves the design of a polymeric scaffold, natural or synthetic, and seeding the scaffold with neural cells to create a 3D tissue replacement that can be implanted in the site of injury (Yang et al., 2004). Bioactive signals for the maintenance of axonal function are typically carried out by neural tissue engineering, and nerve guidance channels are composed of biomaterials and have recently paved the way for a new era of regenerative medicine applications stemming from traditional nerve grafting procedures (Hadlock et al., 2000; Ellis and Chaudhuri, 2008; Li and Hoffman-Kim, 2008). In these guidance channels, like tissue engineering scaffolds for any particular tissue type, appropriate dimensions, adequate biodegradability, easily controlled, and mechanically durable must be present to facilitate safe and optimal regeneration (Jazayeri et al., 2017; Khoshroo et al., 2017a; Fahmy et al., 2016; Fahimipour et al., 2016; Khojasteh et al., 2016a; Khoshroo et al., 2017b; Yazdimamaghani et al., 2014a,b,c; Heidari et al., 2015; Tahmasbi Rad et al., 2014; Shabafrooz et al., 2014). Because of the natural electrical conduction of nerve cells the regenerative scaffold or guidance channel needs to be designed in a way that will not only induce the proliferation of immature neurons and neural cell precursors but also will be conducive to the electrical properties necessary for normal nerve tissue function, which is why external electrical stimulation have been simulated multiple times and reported to positively influence nerve growth, even for in vivo scenarios (McCaig and Zhao, 1997; Zhao et al., 1999; Wang et al., 2004; Khojasteh et al., 2016b).

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00019-0
© 2017 Elsevier Ltd. All rights reserved.

Nerve tissue engineering that is applicable to the oral cavity, dentistry, and oral and maxillofacial surgery involves the repair of the primary nerve responsible for sensory and motor function of the maxilla and mandible, the trigeminal nerve. Similarly, the facial nerve, which courses in the vicinity of the parotid gland and temporomandibular joint, two very critical structures that are very well understood and examined by dental professionals, innervates muscles that give rise to facial expressions and can be subject to severe neuralgia or injury that would ultimately cause unilateral or bilateral facial paralysis and loss of function (Jones, 2010). The subject of this chapter, will dive into the anatomy and microanatomy of neural tissue, the most commonly used tissue engineering approaches in the discipline, providing an overview of the scaffolds, cell types employed, and bioactive agents as well as the laboratory and clinical applications available in the literature.

19.2 Peripheral neuroanatomy applicable to dentistry

This section will pertain to the maxillary (V2) and mandibular (V3) divisions of the trigeminal nerve which innervate the upper and lower jaw, respectively. The maxillary division of the nerve passes through the foramen rotundum and courses into the superior portion of the pterygopalatine fossa, ultimately emerging of the infraorbital foramen to supply the middle section of the face and nose. Also, its branches that give rise to the alveolar nerves supply the innervation of the maxilla (Jones, 2010).

The mandibular division of the trigeminal, on the other end, exits the inferior cranium via the foramen ovale into the infratemporal fossa. At this point the lingual nerve separates immediately. The chorda tympani nerve also contributes fibers to it that are responsible for taste. The nerve dives along the lateral portion of the medial pterygoid and curves medially into the mouth and into the tongue to provide its nervous supply (Sicher, 1962). The inferior alveolar nerve branch wraps around the inferior aspect of the lateral pterygoid muscle and turns laterally to course through the mandibular foramen and exits via the mental foramen, providing innervation to the lower jaw and giving rise to small nervous branches that supply the mandibular teeth (Sicher, 1962). The cell body of the fifth cranial nerve sits in the trigeminal ganglion, and its axons reach out to the sensory receptors found in pulp, mucosa, periosteum, and temporomandibular joint disorders, providing sensory innervation to virtually the main structures associated with normal tooth and jaw function. The information provided by the sensory receptors will synapse in the trigeminal ganglion, pass on to the midbrain, and then terminate in the cortex. Each nerve trunk is surrounded by a dense connective tissue covering, called the epineurium, and each trunk is made up of nerve fascicles, surrounded by a connective tissue covering called the perineurium. Individual fascicles are composed of separate nerves joined together and wrapped in their own connective tissue covering called the endoneurium (Sicher, 1962).

19.3 Nerve tissue engineering principles

Polymers are the most widespread biomaterial in applications for neural scaffold development, and both natural polymers, such as chitosan, collagen, and gelatin, and synthetic polymers, such as polycaprolactone (PCL) and poly lactic-co-glycolic acid (PLGA), have been employed to promote nerve tissue engineering, but due to the fact that each of these aforementioned materials have their own individual limitations, typically a technique will be carried out to optimize material properties that are conducive to the most optimal repair (Subramanian et al., 2009). Some of these techniques include electrospinning or polymer blending, in addition to incorporating growth factors and inducing surface modification (Amado et al., 2008; Novikova et al., 2008; Crompton et al., 2007; Duan et al., 2007). In addition to the biomaterials used to induce peripheral nerve regeneration by way of nerve guidance channels, stem cell therapy has also given rise to successful axonal regeneration in animal models (Im, 2013). Both bone marrow-derived mesenchymal stem cells (BMSCs) and adipose-derived stem cells (ADSCs), as well as recently applied umbilical cord stromal cells can reconstruct and insulate axons in a similar fashion (Kitada, 2012). The potent ability of stem cells to differentiate and the potential for MSCs to become Schwann cells, the main cell type in the peripheral nervous system that is responsible for axonal regeneration and myelination has perpetuated their rising popularity in nerve repair applications (see Fig. 19.1) (Kitada, 2012).

19.3.1 Traditional tissue engineering techniques

19.3.1.1 Extrusion and rapid prototyping

Using a custom-designed extrusion tool the appropriate optimization of temperature, in addition to the application of pressure between the nozzle and piston, allows for the material to be dispensed on the platform. The resulting geometry of the scaffold can be assessed with scanning electron microscope (SEM) micrographs, especially the porosity of the scaffold, which is integral for its mechanical properties and bioactivity (Widmer et al., 1998). Extrusion bioprinters apply force to dispense a suspensory hydrogel solution in a continuous fashion to prevent fabrication from being interrupted, which is advantageous (Schlosshauer et al., 2003). Three-dimensional bioprinting, a form of rapid prototyping (RP) that involves custom extrusion, is the newest and most user-friendly technique in scaffold design with the one of the highest potentials in rapid, large-scale manufacture, and commercialization of bioengineered tissue substitutes (Jeong and Atala, 2015).

19.3.1.2 Electrospinning

The electrospinning method propagates the design of randomly organized nanofibrous compounds on the nanometric to micrometric scale with the induction of an electric voltage to place a surface charge on the solution; if the aforementioned voltage exceeds the material's critical value, the surface tension is dominated by

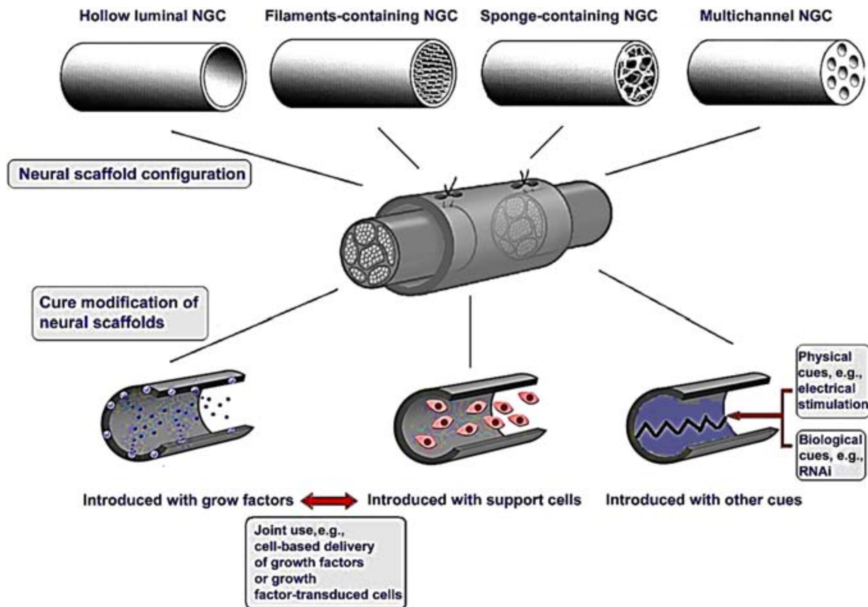


Figure 19.1 A schematic drawing showing construction of an ideal tissue-engineered nerve graft incorporating different biological and physical cues to a neural scaffold.
Source: Adapted from Gu, X., Ding, F., Williams, D.F., 2014. Neural tissue engineering options for peripheral nerve regeneration. Biomaterials 35, 6143−6156 with permission.

electrostatic forces to cause the release of a jet stream between the needle and collector (Chiono and Tonda-Turo, 2015) (see Fig. 19.2). The resemblance in structure between the scaffold fabricated by electrospinning and the native environment of the extracellular matrix makes this method favorable (Sill and von Recum, 2008). Furthermore, porous design and the exertion of manual control over porosity of the scaffold enhances cell-scaffold interactions. Xie et al. provide an abundance of applications for peripheral nerve regeneration that demonstrates the rising potential of reparative tissue engineering schemes in this capacity (Xie et al., 2010). The synthesis of electrospun nanofibers increases the potency for cell adhesion and the ingrowth that is critical for tissue replacement (Mo et al., 2004; Xu et al., 2004; Naghavi Alhosseini et al., 2015; Zamani et al., 2013). Not only do these tissue engineering scaffolds induce axonal growth in addition to subsequent myelination, which is critical for neural signaling and saltatory conduction, but they also have exhibited very comparable postimplantation effects to traditional autografting (Chew et al., 2007; Zhu et al., 2011).

19.3.1.3 Porogen leaching

Inner fillers can be synthesized by combining the primary solvent with a second component, known as a porogen, that is typically inorganic. Salt leaching, for

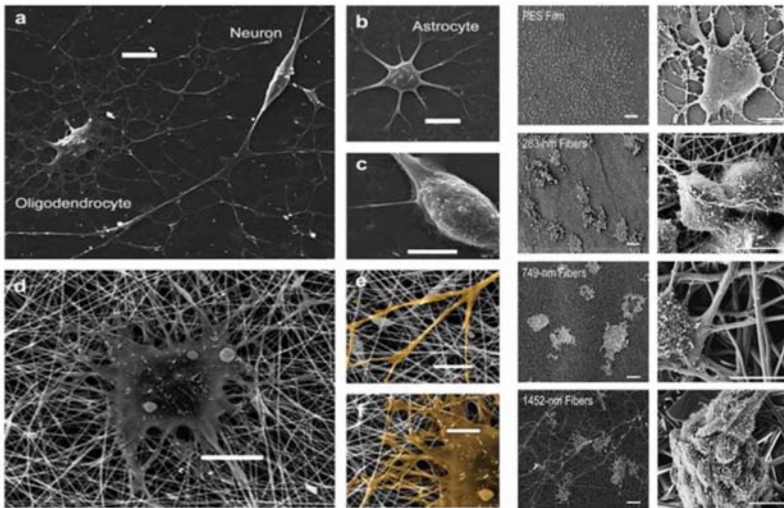


Figure 19.2 SEM images of neural stem/progenitor cells (NSCs) cultured on different substrates for 5 days. (a–c) Cells cultured on laminin-coated electrospun polyethersulfone (PES) fiber meshes; (d–f) SEM images of NSCs cultured in culture media with 20 ng/mL fibroblast growth factor (FGF)-2 for 5 days.
Source: Adapted from Christopherson, G.T., Song, H., Mao, H.-Q., 2009. The influence of fiber diameter of electrospun substrates on neural stem cell differentiation and proliferation. Biomaterials 30, 556–564 with permission.

example, can determine the morphology of the scaffold, but a significant disadvantage to this method is that the connectedness of pores is not ideal (Kokai et al., 2009; Azami et al., 2010). Blending the primary substrate with a porogen confers control over pore size by blend composition, and although the pores are not connected as seen in other methods, they do allow nutrient diffusion that will assist regeneration (Chiono et al., 2011). Owing to macromolecular chain stability being integral to leaching success, Chiono et al. recommend that glassy polymers be used instead of elastomeric polymers (Chiono and Tonda-Turo, 2015).

19.3.1.4 Freeze drying

Freeze drying is a simple technique that does not necessitate the addition of a porogen to construct porous scaffolds. Aqueous polymer solutions are formulated, frozen, then sublimated (Stokols and Tuszynski, 2004). Natural polymers are favorable in this technique because of their solubility in hydrophilic solvents and ability to fabricate various pore morphologies (Stokols and Tuszynski, 2004). Freeze drying has been successful in yielding porous scaffolds for peripheral nerve regeneration with interconnected porosity that ultimately give rise to adequate cell invasion and tissue replacement (Uebersax et al., 2007). In order to improve mechanical properties, crosslinking has been applied to freeze dried scaffolds in nerve tissue

repair (Tonda-Turo et al., 2011; Yang et al., 2004). The mechanism of crosslinking can reduce the rate of biodegradation and optimize shape and durability under physiological conditions (Kanungo et al., 2008).

19.3.1.5 Phase separation

The process of phase separation in scaffold design for peripheral nerve tissue engineering involves turning a uniform polymer solution into a two-phase system constituting a polymer-rich phase composing the scaffold matrix and polymer-poor phase composing the pores. Thermodynamic instability induction typically leads to phase separation techniques (Chiono et al., 2011). A few studies have demonstrated the use of freeze dried scaffolds for the repair of peripheral nerves, and it can be inferred that freeze drying may be increasingly popular for tissue engineering (Chiono et al., 2011; Hsu and Ni, 2009; Sun et al., 2012).

19.3.1.6 Injectable hydrogels

To physically support the lumen of nerve guidance channels, hydrogels can be added to scaffolds as a filler material within synthetic nerve conduits (Lin and Marra, 2012). Applying a hydrogel filler to the scaffold would facilitate an easier method to load bioactive agents, stem cells, and growth factors (Tonda-Turo et al., 2017). Injectable hydrogels do not only serve the purpose of regenerating damaged neural tissue; drug delivery systems have been fabricated with this approach, in addition to the loading of growth factors and other bioactive agents (Pfister et al., 2007). The high proportion of water in hydrogel makeup and structural resemblance to the soft tissue foundation of peripheral nerves make them viable candidates for encapsulation (Suri and Schmidt, 2010).

19.3.2 Nerve tissue engineering materials

19.3.2.1 Natural polymers

Natural polymers, such as collagen, are significantly advantageous in their compatibility to host tissues (see Fig. 19.3). A collagen-based scaffold, because of its biochemical similarity to existing macromolecules and proteins already present in the host, is unlikely to elicit an immune response and is known to be effectively biocompatible and biodegradable, qualities that are essential to the overall success of scaffolds in tissue engineering. The natural polymer is extractable from abundant sources, such as humans and bovines, among other mammals. Collagen molecules contain biochemical cues and glycoprotein receptors that enhance the interaction between cells and the material. Metabolic activity and permeability similarly have been reported to heighten with the application of collagen to scaffolds in peripheral nerve regeneration (Vasita and Katti, 2006; Parenteau-Bareil et al., 2010; Tierney et al., 2009).

Gelatin, which is the denatured form of collagen, can be applied to scaffolds for peripheral nerve regeneration. Its biocompatibility, lack of immunogenicity,

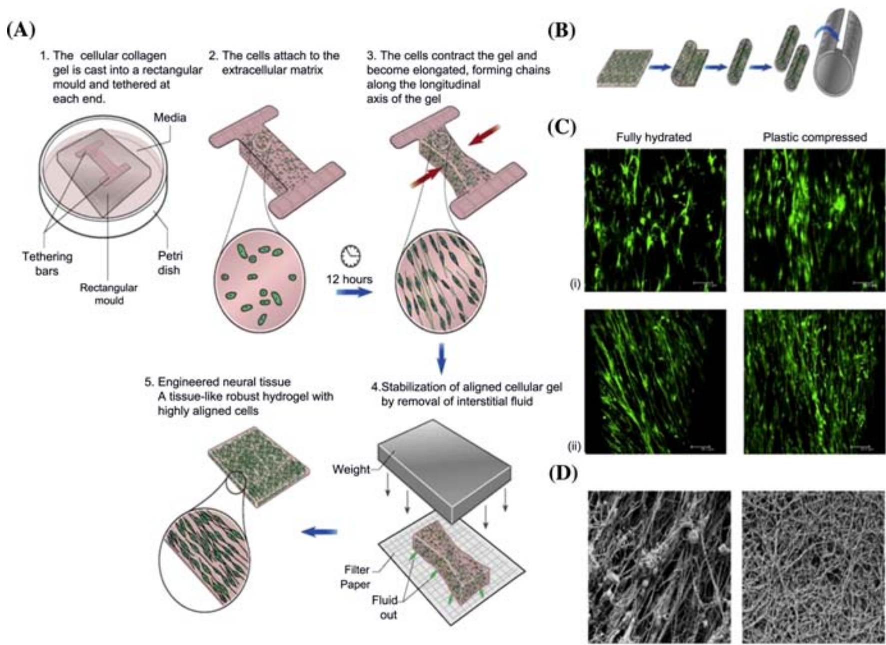


Figure 19.3 Stages in the fabrication of engineered neural tissue (EngNT) constructs (A). A EngNT rods were made by rolling the flat sheets, as an method to delivery within a repair device. (B) Alignment of neuronal cells and collagen fibrils within EngNT. Confocal micrographs show highly aligned elongated neuronal cells (C). SEM shown the alignment of collagen fibrils in EngNT in contrast to the random orientation of fibrils in equivalent acellular plastic compressed collagen material (ii) (D).
Source: Adapted from Georgiou, M., Bunting, S.C., Davies, H.A., Loughlin, A.J., Golding, J.P., Phillips, J.B., 2013. Engineered neural tissue for peripheral nerve repair. Biomaterials 34, 7335−7343 with permission.

resemblance to the native extracellular matrix, and low cost make the material favorable (Yazdimamaghani et al., 2015, 2014c,d; Emami et al., 2010; Raz et al., 2014). In fact, their addition to polycaprolactone scaffolds, markedly improved not only the mechanical properties for nerve repair but also the differentiation of neural precursor cells and neurite outgrowth (Ghasemi-Mobarakeh et al., 2008).

Chitosan, traditionally known as a potent wound healing agent, can be manipulated into a variety of forms for tissue engineering purposes, such as mesh, tubes, sponges, and films. The material promotes neural regeneration, popularly in the application of sciatic nerve injuries, as evidenced by the literature (Lin et al., 2008; Wang et al., 2008, 2010). Chitosan scaffolds have the proper mechanical stability, cell migration and adhesion capability, and proven contact guidance for axonal growth (Wang et al., 2009).

Hyaluronic acid (HA), despite its relatively low solubility in water, is another natural polymer that can be considered for neural tissue regeneration because of its

adhesive potential and lack of immunogenicity. However, due to the solubility factor, the application of HA necessitates an additional component that it can be crosslinked to for it to be effective in regeneration (Timnak et al., 2011; Yang et al., 2004). HA also can prevent perineural scarring, and it can be combined with a multitude of growth factors (Ozgenel, 2003). The biomimetic material, when combined in composite with other materials and stem cells, has shown to restore motor function, which is vital for the mandibular division of the trigeminal nerve that is responsible for the motor function of muscles of mastication (Park et al., 2010).

Fibrin, which is similar chitosan in its wound healing applications, can be yielded from pooled plasma. The clotting cascade gives rise to fibrin by way of a series of proteolytic cleavages that end in the covalent crosslinking of fibrin monomers for stabilization. This crosslinking can result in property variation, which gives the user more control to determine how the fabrication of a fibrin-based scaffold can be tailored for a specific therapeutic aim. Fibrin scaffolds are efficacious in their cell adhesion properties as well, allowing for neurite extension and nerve guidance channel formation that supports regeneration (Sakiyama-Elbert and Hubbell, 2000; Willerth and Sakiyama-Elbert, 2007).

19.3.2.2 Synthetic polymers

Synthetic polymers, such as poly L-lactic acid (PLLA), are extremely variable in their mechanical properties and degradation times. These materials, like natural polymers, can be combined to produce an optimal regenerative response with little to no immunogenic backfire. Neural stem cells can be seeded on PLLA scaffolds to yield neurite outgrowth. Although this polymer is not popular in peripheral nerve tissue engineering schemes because of the failure of mechanical properties as well as poor degradation rate, some studies have shown gradual progress in their use (Vasita and Katti, 2006; Tierney et al., 2009; Vindigni et al., 2009). Poly(ethylene glycol), or PEG, scaffolds can close off cell membranes if damage is caused to limit apoptosis. Crosslinking mechanisms can produce different scaffolds with diverse properties that are tailored for a specific application, including drug delivery. Modification of the properties of PEG can also enhance adhesion capabilities and cell infiltration, serving as a useful biomaterial for neural tissue engineering (Alcantar et al., 2000; Elbert and Hubbell, 2001; Nguyen and West, 2002; Groll et al., 2005). Poly(glycolic acid), or PGA, and poly(lactic acid), PLA, are additional examples of synergistic synthetic copolymers for this biomedical application. Postimplantation hydrolysis of the molecular bonds occurs in the biomaterials and their byproducts result in a decreased pH in the local environment. Changing the ratio of PLA and PGA in the formula will manipulate some of the scaffold characteristics, such as degradation rate (Athanasiou et al., 1996; Gunatillake and Adhikari, 2003). Nerve stem cell differentiation and neurite outgrowth have also been seen in a similar polymer, PLGA, which is widely popular for a vast number of different tissue engineering applications (Bini et al., 2006; Molander et al., 1982). PCL has been studied extensively for the regeneration of multiple tissue types. Functionalized nanofibrous PCL scaffolds have been reported in the literature

to support the growth and differentiation of nerve precursor cells. Similar to the aforementioned synthetic polymers, surface modification is necessary for this kind of scaffold because of its hydrophobic nature and molecular structure that is does not strongly promote cell adhesion (Ghasemi-Mobarakeh et al., 2010).

19.3.2.3 Conductive polymers

A mention of conductive polymers is necessary in this chapter as electrical stimulation of the repaired nerve is critical to neural signaling that is responsible for sensory and motor function. Polypyrrole (PPy) is a biomaterial that supports cell adhesion and is generally viewed as effective for tissue engineering purposes. A PPy/PLGA mesh was reported to support the differentiation of rat hippocampal neurons, making them viable for conductive peripheral nerve repair. Similarly, functionalized carbon nanotubes (CNTs) supported rat hippocampal neurons and demonstrated multidirectional neurite outgrowth. Both of these are ideal candidates for conductive nerve regeneration (Ghasemi-Mobarakeh et al., 2009; Ghasemi-Mobarakeh et al., 2011).

Some natural and synthetic polymeric biomaterials utilized for neural tissue engineering has been given in Table 19.1.

19.4 Applicable stem cells in oral and maxillofacial nerve repair

Many stem cells and growth factors have been applied to peripheral nerve regeneration, but this chapter will offer a brief insight into those that are relevant for their applications in the field of dentistry and oral and maxillofacial surgery, as reported in the literature. As mentioned above, BMSCs, dental pulp stem cells (DPSCs), and ADSCs are the major cell types that have been known to induce neural tissue engineering by engrafting myelinated axons.

DPSCs constitute a population of progenitor cells with self-renewal capacity, differentiation potential, and regenerative capabilities. It has been shown that DPSCs respond to inductive chemical as well as environmental cues to differentiate into mature neuronal cells both in vitro and in vivo (see Fig. 19.4) (Király et al., 2009). BMSCs can induce hematopoiesis and differentiate into different types of mesenchymal cells. Their role in replacing Schwann cells, the main cell type that is responsible for inducing axonal repair in the peripheral nervous system, is supremely valuable and has been effective in multiple experimental studies (Hu et al., 2013; Ding et al., 2010; Yang et al., 2011). ADSCs, although similar to BMSCs in phenotype and multilineage differentiation capability, are much more easily harvested. They can be extracted from adipose tissue by liposuction. ADSCs, moreover, repaired peripheral nerve defects and bridged the voids in multiple experimental studies and animal models (Rider et al., 2008; Zhang et al., 2010; Liu et al., 2011; Orbay et al., 2012).

Table 19.1 **Some natural and synthetic polymeric biomaterials used for neural tissue engineering**

	Fabrication method	Advantage	Disadvantage	Ref
Collagen	Electrospinning with poly (l-lactic acid)-co-poly-(3-caprolactone)	Biocompatibility, low antigenicity response, several isolation sources, adjustable crosslinking, biodegradation, and mechanical properties	Poor structural and mechanical stability upon water uptake	Prabhakaran et al. (2009)
Chitosan	Molding of hollow conduits	Nontoxicity, low cost, nonimmunogenicity, antibacterial activity, functionalization versatility, adjustable physicochemical features	Solubility in acidic media, timely biodegradation	Ao et al. (2011)
Hyaluronic acid	Hydrogel formation	Biocompatibility, biodegradability, low immunogenicity, wound healing properties	Low cellular adhesion	Pan et al. (2009)
PLGA	Microfluidic-assisted microfiber fabrication	Tunable biodegradability, nontoxicity, versatile forming features	Brittleness, acidic byproducts on degradation	Hwang et al. (2008)
PCL	Template synthesized nanowire fabrication	Biocompatibility, biodegradability, high mechanical properties, film forming properties, versatility for various fabrication techniques, slow biodegradation	Possibility of cytotoxic effects of using organic solvents	Bechara et al. (2010)
PLA	Porous conduits manufactured with combined solvent casting, extrusion, and particulate leaching techniques	Biodegradable, ultrafine continuous fibers, high surface-to-volume ratio, high porosity, varied distribution of pore size	Release of acidic products on degradation, poor processing ability, Possibility of cytotoxic effects of using organic solvents	Evans et al. (1999)

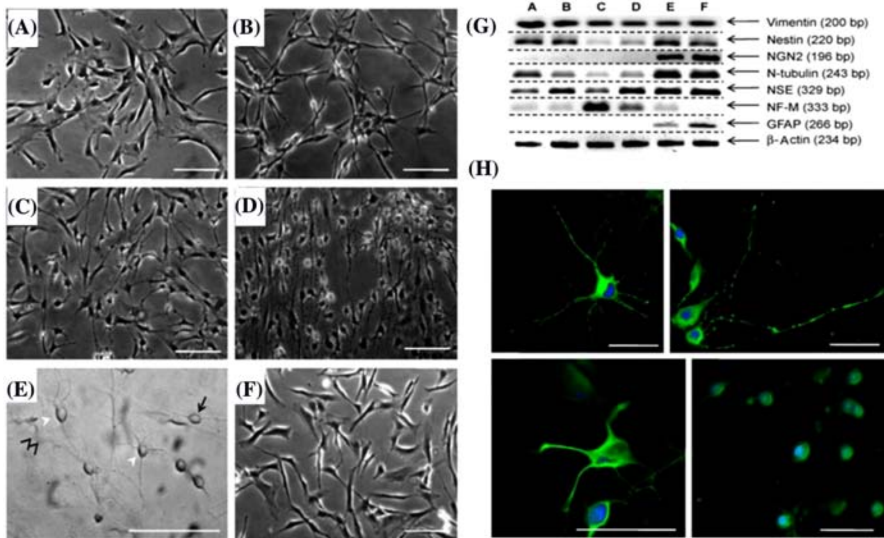


Figure 19.4 Morphological changes of DPSCs through neuronal differentiation. (A) Control DPSCs showed spindle-shaped morphology. (B) DPSCs cells formed clusters after 24 h of induction. (C) One day after the inductive medium removal, the aggregates spread, and the cells started to take a more spherical shape and developed extending processes. (D) Cells showed morphological features typical of neurons after 3 days of maturation. (E) Most of the cells displayed either multi- or bipolar forms after 10 days of differentiation. (F) Gene expression during DPSCs neural differentiation, evaluated by semiquantitative RT-PCR (G). DPSCs expressed neural markers after 10 days of differentiation. (H) Immunofluorescent staining of DPSCs for expression of neuronal markers by induction culture medium in 2D and 3D culture.
Source: Adapted from Király, M., Porcsalmy, B., Pataki, A., Kádár, K., Jelitai, M., Molnár, B., et al., 2009. Simultaneous PKC and cAMP activation induces differentiation of human dental pulp stem cells into functionally active neurons. Neurochem. Int. 55, 323–332 with permission.

19.5 Growth factors in oral and maxillofacial nerve repair

Thus far, the most common growth factors with neurotrophic actions and neurotrophins used in nerve repair are as follows: nerve growth factor, FGFs, vascular endothelial growth factor, ciliary neurotrophic factor, glial cell line-derived neurotrophic factor, neurotrophin-3, brain-derived neurotrophic factor, neurotrophin-3,4,5, mechano growth factor 1, leukemia inhibitory factor, and survival motor neuron-derived factor (Oliveira et al., 2013). Some of these growth factors/cytokines have been employed to provoke multimodal neurotrophic effects through diverse biological pathways, including nerve regeneration pathways (Kimura et al., 2016; Oliveira

et al., 2013). The sustained release of growth factors compared to rapid bolus release has been found to result in improved nerve regeneration (Xu et al., 2003). In view of this, various approaches to achieve sustained delivery of neurotrophic factors have been developed so far.

19.6 Clinical applications and future trends

Currently, very little literature exists on treating trigeminal neuropathy with tissue engineering methods in human beings (Rosén et al., 2016). However, mesenchymal stem cells are being expanded upon to pave the way for tissue regeneration to potentially become a standard in medicine. Biomaterial developments in nerve tissue engineering have been occurring for decades, and although the rise in the application of composite blends and various formulations may result in more potent scaffolds with optimal characteristics for repair, the advancement of innovative techniques, such as RP and 3D Printing, are becoming more relevant to the discussion of making improvements in regeneration and normalizing the discipline of tissue regeneration. While significant progress has been made in spinal nerve repair, we have yet to see a drastic rise in studies that have made marked strides in the repair of trigeminal nerve and other nerves relevant to the dental field. Current research focuses on in vitro and in vivo success in repairing neuropathies in the head and neck with the use of polymeric scaffolds, stem cells, and growth factors mentioned in this chapter, and with the rapid advent of 3D Printing in the medical field, we may see a transition to more frequent clinical trials with this approach (Stansbury and Idacavage, 2016).

19.7 Conclusion

This chapter reviews the progress made in peripheral nerve tissue engineering with a more specialized outlook on its applications relevant to the field of dentistry. Several techniques currently exist to produce efficacious scaffolds that are mechanically and biologically conducive to successful nerve repair. Natural and synthetic polymers, are the most widely popular biomaterials used for regenerating and tissue engineering of nerves. The appropriate stem cells and growth factors that can synergistically work with the biomaterial to repair nerves have shown great promise in this field as well. These advancements in neural tissue engineering demonstrate the rapidly growing application in translational research and provide scientists and clinicians with the hope and determination to make significant breakthroughs in patient care in the coming decades.

References

Alcantar, N.A., Aydil, E.S., Israelachvili, J.N., 2000. Polyethylene glycol-coated biocompatible surfaces. J. Biomed. Mater. Res. 51, 343–351.

Amado, S., Simoes, M.J., Armada Da Silva, P.A., Luis, A.L., Shirosaki, Y., Lopes, M.A., et al., 2008. Use of hybrid chitosan membranes and N1E-115 cells for promoting nerve regeneration in an axonotmesis rat model. Biomaterials. 29, 4409–4419.

Ao, Q., Fung, C.-K., Tsui, A.Y.-P., Cai, S., Zuo, H.-C., Chan, Y.-S., et al., 2011. The regeneration of transected sciatic nerves of adult rats using chitosan nerve conduits seeded with bone marrow stromal cell-derived Schwann cells. Biomaterials. 32, 787–796.

Athanasiou, K.A., Niederauer, G.G., Agrawal, C.M., 1996. Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. Biomaterials. 17, 93–102.

Azami, M., Moztarzadeh, F., Tahriri, M., 2010. Preparation, characterization and mechanical properties of controlled porous gelatin/hydroxyapatite nanocomposite through layer solvent casting combined with freeze-drying and lamination techniques. J. Porous Mater. 17, 313–320.

Bechara, S.L., Judson, A., Popat, K.C., 2010. Template synthesized poly (ε-caprolactone) nanowire surfaces for neural tissue engineering. Biomaterials. 31, 3492–3501.

Bini, T.B., Gao, S.J., Wang, S., Ramakrishna, S., 2006. Poly(l-lactide-co-glycolide) biodegradable microfibers and electrospun nanofibers for nerve tissue engineering: an in vitro study. J. Mater. Sci. 41, 6453–6459.

Chew, S.Y., Mi, R., Hoke, A., Leong, K.W., 2007. Aligned protein-polymer composite fibers enhance nerve regeneration: a potential tissue-engineering platform. Adv. Funct. Mater. 17, 1288–1296.

Chiono, V., Sartori, S., Rechichi, A., Tonda-Turo, C., Vozzi, G., Vozzi, F., et al., 2011. Poly (ester urethane) guides for peripheral nerve regeneration. Macromol. Biosci. 11, 245–256.

Chiono, V., Tonda-Turo, C., 2015. Trends in the design of nerve guidance channels in peripheral nerve tissue engineering. Prog. Neurobiol. 131, 87–104.

Christopherson, G.T., Song, H., Mao, H.-Q., 2009. The influence of fiber diameter of electrospun substrates on neural stem cell differentiation and proliferation. Biomaterials. 30, 556–564.

Ciardelli, G., Chiono, V., 2006. Materials for peripheral nerve regeneration. Macromol. Biosci. 6, 13–26.

Crompton, K.E., Goud, J.D., Bellamkonda, R.V., Gengenbach, T.R., Finkelstein, D.I., Horne, M.K., et al., 2007. Polylysine-functionalised thermoresponsive chitosan hydrogel for neural tissue engineering. Biomaterials. 28, 441–449.

Ding, F., Wu, J., Yang, Y., Hu, W., Zhu, Q., Tang, X., et al., 2010. Use of tissue-engineered nerve grafts consisting of a chitosan/poly(lactic-co-glycolic acid)-based scaffold included with bone marrow mesenchymal cells for bridging 50-mm dog sciatic nerve gaps. Tissue Eng. A. 16, 3779–3790.

Duan, X., Mclaughlin, C., Griffith, M., Sheardown, H., 2007. Biofunctionalization of collagen for improved biological response: scaffolds for corneal tissue engineering. Biomaterials. 28, 78–88.

Elbert, D.L., Hubbell, J.A., 2001. Conjugate addition reactions combined with free-radical cross-linking for the design of materials for tissue engineering. Biomacromolecules. 2, 430–441.

Ellis, M.J., Chaudhuri, J.B., 2008. Human bone derived cell culture on PLGA flat sheet membranes of different lactide:glycolide ratio. Biotechnol. Bioeng. 101, 369−377.

Emami, S.H., Abad, A.M.A., Bonakdar, S., Tahriri, M.R., Samadikuchaksaraei, A., Bahar, M.A., 2010. Preparation and evaluation of chitosan-gelatin composite scaffolds modified with chondroitin-6-sulphate. Int. J. Mater. Res. 101, 1281−1285.

Evans, G., Brandt, K., Widmer, M., Lu, L., Meszlenyi, R., Gupta, P., et al., 1999. In vivo evaluation of poly (L-lactic acid) porous conduits for peripheral nerve regeneration. Biomaterials. 20, 1109−1115.

Fahimipour, F., Kashi, T.J., Khoshroo, K., Dashtimoghadam, E., Rasoulianborujeji, M., Tayebi, L., 2016. 3D-printed β-TCP/collagen scaffolds for bone tissue engineering. Dent. Mater. 32, e57.

Fahmy, M.D., Jazayeri, H.E., Razavi, M., Masri, R., Tayebi, L., 2016. Three-dimensional bioprinting materials with potential application in preprosthetic surgery. J. Prosthodont. 25, 310−318.

Georgiou, M., Bunting, S.C., Davies, H.A., Loughlin, A.J., Golding, J.P., Phillips, J.B., 2013. Engineered neural tissue for peripheral nerve repair. Biomaterials. 34, 7335−7343.

Ghasemi-Mobarakeh, L., Prabhakaran, M.P., Morshed, M., Nasr-Esfahani, M.H., Ramakrishna, S., 2008. Electrospun poly(epsilon-caprolactone)/gelatin nanofibrous scaffolds for nerve tissue engineering. Biomaterials. 29, 4532−4539.

Ghasemi-Mobarakeh, L., Prabhakaran, M.P., Morshed, M., Nasr-Esfahani, M.H., Ramakrishna, S., 2009. Electrical stimulation of nerve cells using conductive nanofibrous scaffolds for nerve tissue engineering. Tissue Eng. A. 15, 3605−3619.

Ghasemi-Mobarakeh, L., Prabhakaran, M.P., Morshed, M., Nasr-Esfahani, M.H., Ramakrishna, S., 2010. Bio-functionalized PCL nanofibrous scaffolds for nerve tissue engineering. Mater. Sci. Eng. C-Mater. Biol. Appl. 30, 1129−1136.

Ghasemi-Mobarakeh, L., Prabhakaran, M.P., Morshed, M., Nasr-Esfahani, M.H., Baharvand, H., Kiani, S., et al., 2011. Application of conductive polymers, scaffolds and electrical stimulation for nerve tissue engineering. J. Tissue Eng. Regen. Med. 5, e17−e35.

Groll, J., Fiedler, J., Engelhard, E., Ameringer, T., Tugulu, S., Klok, H.A., et al., 2005. A novel star PEG-derived surface coating for specific cell adhesion. J. Biomed. Mater. Res. A. 74, 607−617.

Gu, X., Ding, F., Williams, D.F., 2014. Neural tissue engineering options for peripheral nerve regeneration. Biomaterials. 35, 6143−6156.

Gunatillake, P.A., Adhikari, R., 2003. Biodegradable synthetic polymers for tissue engineering. Eur. Cell Mater. 5, 1−16, discussion 16.

Hadlock, T., Sundback, C., Hunter, D., Cheney, M., Vacanti, J.P., 2000. A polymer foam conduit seeded with Schwann cells promotes guided peripheral nerve regeneration. Tissue Eng. 6, 119−127.

Heidari, F., Bahrololoom, M.E., Vashaee, D., Tayebi, L., 2015. In situ preparation of iron oxide nanoparticles in natural hydroxyapatite/chitosan matrix for bone tissue engineering application. Ceram. Int. 41, 3094−3100.

Hsu, S.H., Ni, H.C., 2009. Fabrication of the microgrooved/microporous polylactide substrates as peripheral nerve conduits and in vivo evaluation. Tissue Eng. A. 15, 1381−1390.

Hu, N., Wu, H., Xue, C., Gong, Y., Wu, J., Xiao, Z., et al., 2013. Long-term outcome of the repair of 50 mm long median nerve defects in rhesus monkeys with marrow mesenchymal stem cells-containing, chitosan-based tissue engineered nerve grafts. Biomaterials. 34, 100−111.

Hwang, C.M., Khademhosseini, A., Park, Y., Sun, K., Lee, S.-H., 2008. Microfluidic chip-based fabrication of PLGA microfiber scaffolds for tissue engineering. Langmuir. 24, 6845–6851.

Im, G.I., 2013. Adipose stem cells and skeletal repair. Histol. Histopathol. 28, 557–564.

Jazayeri, H.E., Tahriri, M., Razavi, M., Khoshroo, K., Fahimipour, F., Dashtimoghadam, E., et al., 2017. A current overview of materials and strategies for potential use in maxillofacial tissue regeneration. Mater. Sci. Eng. C: Mater. Biol. Appl. 70, 913–929.

Jeong, C.G., Atala, A., 2015. 3D printing and biofabrication for load bearing tissue engineering. Adv. Exp. Med. Biol. 881, 3–14.

Jones, R.H., 2010. Repair of the trigeminal nerve: a review. Aust. Dent. J. 55, 112–119.

Kanungo, B.P., Silva, E., Van Vliet, K., Gibson, L.J., 2008. Characterization of mineralized collagen-glycosaminoglycan scaffolds for bone regeneration. Acta Biomater. 4, 490–503.

Khojasteh, A., Fahimipour, F., Eslaminejad, M.B., Jafarian, M., Jahangir, S., Bastami, F., et al., 2016a. Development of PLGA-coated β-TCP scaffolds containing VEGF for bone tissue engineering. Mater. Sci. Eng. C. 69, 780–788.

Khojasteh, A., Fahimipour, F., Jafarian, M., Sharifi, D., Jahangir, S., Khayyatan, F., et al., 2016b. Bone engineering in dog mandible: coculturing mesenchymal stem cells with endothelial progenitor cells in a composite scaffold containing vascular endothelial growth factor. J. Biomed. Mater. Res. B: Appl. Biomater.. Available from: http://dx.doi.org/10.1002/jbm.b.33707.

Khoshroo, K., Jafarzadeh Kashi, T.S., Moztarzadeh, F., Tahriri, M., Jazayeri, H.E., Tayebi, L., 2017a. Development of 3D PCL microsphere/TiO_2 nanotube composite scaffolds for bone tissue engineering. Mater. Sci. Eng. C: Mater. Biol. Appl. 70, 586–598.

Khoshroo, K., Kashi, T.S.J., Moztarzadeh, F., Tahriri, M., Jazayeri, H.E., Tayebi, L., 2017b. Development of 3D PCL microsphere/TiO2 nanotube composite scaffolds for bone tissue engineering. Mater. Sci. Eng. C. 70, 586–598.

Kimura, A., Namekata, K., Guo, X., Harada, C., Harada, T., 2016. Neuroprotection, growth factors and BDNF-TrkB signalling in retinal degeneration. Int. J. Mol. Sci. 17, 1584.

Király, M., Porcsalmy, B., Pataki, A., Kádár, K., Jelitai, M., Molnár, B., et al., 2009. Simultaneous PKC and cAMP activation induces differentiation of human dental pulp stem cells into functionally active neurons. Neurochem. Int. 55, 323–332.

Kitada, M., 2012. Mesenchymal cell populations: development of the induction systems for Schwann cells and neuronal cells and finding the unique stem cell population. Anat. Sci. Int. 87, 24–44.

Kokai, L.E., Lin, Y.C., Oyster, N.M., Marra, K.G., 2009. Diffusion of soluble factors through degradable polymer nerve guides: controlling manufacturing parameters. Acta Biomater. 5, 2540–2550.

Li, G.N., Hoffman-Kim, D., 2008. Tissue-engineered platforms of axon guidance. Tissue Eng. B: Rev. 14, 33–51.

Lin, Y.C., Marra, K.G., 2012. Injectable systems and implantable conduits for peripheral nerve repair. Biomed. Mater. 7, 024102.

Lin, Y.L., Jen, J.C., Hsu, S.H., Chiu, I.M., 2008. Sciatic nerve repair by microgrooved nerve conduits made of chitosan-gold nanocomposites. Surg. Neurol. 70 Suppl. 1 (S1), 9–18.

Liu, G.B., Cheng, Y.X., Feng, Y.K., Pang, C.J., Li, Q., Wang, Y., et al., 2011. Adipose-derived stem cells promote peripheral nerve repair. Arch. Med. Sci. 7, 592–596.

Mccaig, C.D., Zhao, M., 1997. Physiological electrical fields modify cell behaviour. Bioessays. 19, 819–826.

Mo, X.M., Xu, C.Y., Kotaki, M., Ramakrishna, S., 2004. Electrospun P(LLA-CL) nanofiber: a biomimetic extracellular matrix for smooth muscle cell and endothelial cell proliferation. Biomaterials. 25, 1883−1890.

Molander, H., Olsson, Y., Engkvist, O., Bowald, S., Eriksson, I., 1982. Regeneration of peripheral nerve through a polyglactin tube. Muscle Nerve. 5, 54−57.

Naghavi Alhosseini, S., Moztarzadeh, F., Kargozar, S., Dodel, M., Tahriri, M., 2015. Development of polyvinyl alcohol fibrous biodegradable scaffolds for nerve tissue engineering applications: In vitro study. Int. J. Polym. Mater. Polym. Biomater. 64, 474−480.

Nguyen, K.T., West, J.L., 2002. Photopolymerizable hydrogels for tissue engineering applications. Biomaterials. 23, 4307−4314.

Novikova, L.N., Pettersson, J., Brohlin, M., Wiberg, M., Novikov, L.N., 2008. Biodegradable poly-beta-hydroxybutyrate scaffold seeded with Schwann cells to promote spinal cord repair. Biomaterials. 29, 1198−1206.

Oliveira, S.L., Pillat, M.M., Cheffer, A., Lameu, C., Schwindt, T.T., Ulrich, H., 2013. Functions of neurotrophins and growth factors in neurogenesis and brain repair. Cytometry A. 83, 76−89.

Orbay, H., Uysal, A.C., Hyakusoku, H., Mizuno, H., 2012. Differentiated and undifferentiated adipose-derived stem cells improve function in rats with peripheral nerve gaps. J. Plast. Reconstr. Aesthet. Surg. 65, 657−664.

Ozgenel, G.Y., 2003. Effects of hyaluronic acid on peripheral nerve scarring and regeneration in rats. Microsurgery. 23, 575−581.

Pan, L., Ren, Y., Cui, F., Xu, Q., 2009. Viability and differentiation of neural precursors on hyaluronic acid hydrogel scaffold. J. Neurosci. Res. 87, 3207−3220.

Parenteau-Bareil, R., Gauvin, R., Berthod, F., 2010. Collagen-based biomaterials for tissue engineering applications. Materials. 3, 1863−1887.

Park, J., Lim, E., Back, S., Na, H., Park, Y., Sun, K., 2010. Nerve regeneration following spinal cord injury using matrix metalloproteinase-sensitive, hyaluronic acid-based biomimetic hydrogel scaffold containing brain-derived neurotrophic factor. J. Biomed. Mater. Res. A. 93, 1091−1099.

Pfister, L.A., Papaloizos, M., Merkle, H.P., Gander, B., 2007. Nerve conduits and growth factor delivery in peripheral nerve repair. J. Peripher. Nerv. Syst. 12, 65−82.

Prabhakaran, M.P., Venugopal, J.R., Ramakrishna, S., 2009. Mesenchymal stem cell differentiation to neuronal cells on electrospun nanofibrous substrates for nerve tissue engineering. Biomaterials. 30, 4996−5003.

Raz, M., Moztarzadeh, F., Shokrgozar, M.A., Azami, M., Tahriri, M., 2014. Development of biomimetic gelatin−chitosan/hydroxyapatite nanocomposite via double diffusion method for biomedical applications. Int. J. Mater. Res. 105, 493−501.

Rider, D.A., Dombrowski, C., Sawyer, A.A., Ng, G.H., Leong, D., Hutmacher, D.W., et al., 2008. Autocrine fibroblast growth factor 2 increases the multipotentiality of human adipose-derived mesenchymal stem cells. Stem Cells. 26, 1598−1608.

Rosén, A., Tardast, A., Shi, T.-J., 2016. How far have we come in the field of nerve regeneration after trigeminal nerve injury? Curr. Oral Health Rep. 3, 309−313.

Sakiyama-Elbert, S.E., Hubbell, J.A., 2000. Development of fibrin derivatives for controlled release of heparin-binding growth factors. J. Control Release. 65, 389−402.

Schlosshauer, B., Muller, E., Schroder, B., Planck, H., Muller, H.W., 2003. Rat Schwann cells in bioresorbable nerve guides to promote and accelerate axonal regeneration. Brain Res. 963, 321−326.

Schmidt, C.E., Shastri, V.R., Vacanti, J.P., Langer, R., 1997. Stimulation of neurite outgrowth using an electrically conducting polymer. Proc. Natl. Acad. Sci. U.S.A. 94, 8948−8953.

Shabafrooz, V., Mozafari, M., Köhler, G.A., Assefa, S., Vashaee, D., Tayebi, L., 2014. The effect of hyaluronic acid on biofunctionality of gelatin–collagen intestine tissue engineering scaffolds. J. Biomed. Mater. Res. A. 102, 3130–3139.

Sicher, H., 1962. Anatomy and oral pathology. Oral. Surg. Oral. Med. Oral. Pathol. 15, 1264–1269.

Sill, T.J., Von Recum, H.A., 2008. Electrospinning: applications in drug delivery and tissue engineering. Biomaterials. 29, 1989–2006.

Stansbury, J.W., Idacavage, M.J., 2016. 3D printing with polymers: Challenges among expanding options and opportunities. Dent. Mater. 32, 54–64.

Stokols, S., Tuszynski, M.H., 2004. The fabrication and characterization of linearly oriented nerve guidance scaffolds for spinal cord injury. Biomaterials. 25, 5839–5846.

Subramanian, A., Krishnan, U.M., Sethuraman, S., 2009. Development of biomaterial scaffold for nerve tissue engineering: Biomaterial mediated neural regeneration. J. Biomed. Sci. 16, 108.

Sun, C., Jin, X., Holzwarth, J.M., Liu, X., Hu, J., Gupte, M.J., et al., 2012. Development of channeled nanofibrous scaffolds for oriented tissue engineering. Macromol. Biosci. 12, 761–769.

Suri, S., Schmidt, C.E., 2010. Cell-laden hydrogel constructs of hyaluronic acid, collagen, and laminin for neural tissue engineering. Tissue Eng. A. 16, 1703–1716.

Tahmasbi Rad, A., Ali, N., Kotturi, H.S.R., Yazdimamaghani, M., Smay, J., Vashaee, D., et al., 2014. Conducting scaffolds for liver tissue engineering. J. Biomed. Mater. Res. A. 102, 4169–4181.

Tierney, C.M., Haugh, M.G., Liedl, J., Mulcahy, F., Hayes, B., O'brien, F.J., 2009. The effects of collagen concentration and crosslink density on the biological, structural and mechanical properties of collagen-GAG scaffolds for bone tissue engineering. J. Mech. Behav. Biomed. Mater. 2, 202–209.

Timnak, A., Gharebaghi, F.Y., Shariati, R.P., Bahrami, S.H., Javadian, S., Emami, S.H.,H., et al., 2011. Fabrication of nano-structured electrospun collagen scaffold intended for nerve tissue engineering. J. Mater. Sci. Mater. Med. 22, 1555–1567.

Tonda-Turo, C., Gentile, P., Saracino, S., Chiono, V., Nandagiri, V.K., Muzio, G., et al., 2011. Comparative analysis of gelatin scaffolds crosslinked by genipin and silane coupling agent. Int. J. Biol. Macromol. 49, 700–706.

Tonda-Turo, C., Gnavi, S., Ruini, F., Gambarotta, G., Gioffredi, E., Chiono, V., et al., 2017. Development and characterization of novel agar and gelatin injectable hydrogel as filler for peripheral nerve guidance channels. J. Tissue Eng. Regen. Med. 11, 197–208.

Uebersax, L., Mattotti, M., Papaloizos, M., Merkle, H.P., Gander, B., Meinel, L., 2007. Silk fibroin matrices for the controlled release of nerve growth factor (NGF). Biomaterials. 28, 4449–4460.

Vasita, R., Katti, D.S., 2006. Nanofibers and their applications in tissue engineering. Int. J. Nanomed. 1, 15–30.

Vindigni, V., Cortivo, R., Iacobellis, L., Abatangelo, G., Zavan, B., 2009. Hyaluronan benzyl ester as a scaffold for tissue engineering. Int. J. Mol. Sci. 10, 2972–2985.

Wang, G., Lu, G., Ao, Q., Gong, Y., Zhang, X., 2010. Preparation of cross-linked carboxymethyl chitosan for repairing sciatic nerve injury in rats. Biotechnol. Lett. 32, 59–66.

Wang, W., Itoh, S., Konno, K., Kikkawa, T., Ichinose, S., Sakai, K., et al., 2009. Effects of Schwann cell alignment along the oriented electrospun chitosan nanofibers on nerve regeneration. J. Biomed. Mater. Res. A. 91, 994–1005.

Wang, W., Itoh, S., Matsuda, A., Ichinose, S., Shinomiya, K., Hata, Y., et al., 2008. Influences of mechanical properties and permeability on chitosan nano/microfiber mesh tubes as a scaffold for nerve regeneration. J. Biomed. Mater. Res. A. 84, 557–566.

Wang, X., Gu, X., Yuan, C., Chen, S., Zhang, P., Zhang, T., et al., 2004. Evaluation of biocompatibility of polypyrrole in vitro and in vivo. J. Biomed. Mater. Res. A. 68, 411−422.

Widmer, M.S., Gupta, P.K., Lu, L., Meszlenyi, R.K., Evans, G.R., Brandt, K., et al., 1998. Manufacture of porous biodegradable polymer conduits by an extrusion process for guided tissue regeneration. Biomaterials. 19, 1945−1955.

Willerth, S.M., Sakiyama-Elbert, S.E., 2007. Approaches to neural tissue engineering using scaffolds for drug delivery. Adv. Drug. Deliv. Rev. 59, 325−338.

Xie, J., Macewan, M.R., Schwartz, A.G., Xia, Y., 2010. Electrospun nanofibers for neural tissue engineering. Nanoscale. 2, 35−44.

Xu, C., Inai, R., Kotaki, M., Ramakrishna, S., 2004. Electrospun nanofiber fabrication as synthetic extracellular matrix and its potential for vascular tissue engineering. Tissue Eng. 10, 1160−1168.

Xu, X., Yee, W.-C., Hwang, P.Y., Yu, H., Wan, A.C., Gao, S., et al., 2003. Peripheral nerve regeneration with sustained release of poly (phosphoester) microencapsulated nerve growth factor within nerve guide conduits. Biomaterials. 24, 2405−2412.

Yang, F., Murugan, R., Ramakrishna, S., Wang, X., Ma, Y.X., Wang, S., 2004. Fabrication of nano-structured porous PLLA scaffold intended for nerve tissue engineering. Biomaterials. 25, 1891−1900.

Yang, Y., Yuan, X., Ding, F., Yao, D., Gu, Y., Liu, J., et al., 2011. Repair of rat sciatic nerve gap by a silk fibroin-based scaffold added with bone marrow mesenchymal stem cells. Tissue Eng. A. 17, 2231−2244.

Yazdimamaghani, M., Razavi, M., Vashaee, D., Pothineni, V.R., Rajadas, J., Tayebi, L., 2015. Significant degradability enhancement in multilayer coating of polycaprolactone-bioactive glass/gelatin-bioactive glass on magnesium scaffold for tissue engineering applications. Appl. Surf. Sci. 338, 137−145.

Yazdimamaghani, M., Razavi, M., Vashaee, D., Tayebi, L., 2014a. Development and degradation behavior of magnesium scaffolds coated with polycaprolactone for bone tissue engineering. Mater. Lett. 132, 106−110.

Yazdimamaghani, M., Razavi, M., Vashaee, D., Tayebi, L., 2014b. Microstructural and mechanical study of PCL coated Mg scaffolds. Surf. Eng. 30, 920−926.

Yazdimamaghani, M., Vashaee, D., Assefa, S., Shabrangharehdasht, M., Rad, A.T., Eastman, M.A., et al., 2014c. Green synthesis of a new gelatin-based antimicrobial scaffold for tissue engineering. Mater. Sci. Eng. C. 39, 235−244.

Yazdimamaghani, M., Vashaee, D., Assefa, S., Walker, K., Madihally, S., Köhler, G., et al., 2014d. Hybrid macroporous gelatin/bioactive-glass/nanosilver scaffolds with controlled degradation behavior and antimicrobial activity for bone tissue engineering. J. Biomed. Nanotechnol. 10, 911−931.

Zamani, Y., Rabiee, M., Shokrgozar, M.A., Bonakdar, S., Tahriri, M., 2013. Response of human mesenchymal stem cells to patterned and randomly oriented poly (vinyl alcohol) nano-fibrous scaffolds surface-modified with Arg-Gly-Asp (RGD) ligand. Appl. Biochem. Biotechnol. 171, 1513−1524.

Zhang, Y., Luo, H., Zhang, Z., Lu, Y., Huang, X., Yang, L., et al., 2010. A nerve graft constructed with xenogeneic acellular nerve matrix and autologous adipose-derived mesenchymal stem cells. Biomaterials. 31, 5312−5324.

Zhao, M., Forrester, J.V., Mccaig, C.D., 1999. A small, physiological electric field orients cell division. Proc. Natl. Acad. Sci. U.S.A. 96, 4942−4946.

Zhu, Y., Wang, A., Patel, S., Kurpinski, K., Diao, E., Bao, X., et al., 2011. Engineering bi-layer nanofibrous conduits for peripheral nerve regeneration. Tissue Eng. C: Methods. 17, 705−715.

Tissue engineering of salivary glands

20

Dewi Borkent[1] *and Keyvan Moharamzadeh*[2]
[1]University of Edinburgh, Edinburgh, United Kingdom,
[2]University of Sheffield, Sheffield, United Kingdom

20.1 Introduction

Salivary glands secretory function can be impaired as a result of several diseases and conditions such as infections (Delli et al., 2014), Sjogren's syndrome (Barone and Colafrancesco, 2016; Cartee et al., 2015), radiotherapy (Acauan et al., 2015), tumors (Green et al., 2016), and certain medications (Villa et al., 2015). Xerostomia (dry mouth) caused by the damage to the salivary gland tissue can have debilitating consequences including dental caries, development of fungal infections, altered taste or burning sensation, halitosis, difficulty swallowing and chewing, and discomfort associated with wearing dentures that can significantly impact the patients quality of life (Tanasiewicz et al., 2016).

In head and neck oncology patients who are treated by radiotherapy involving the oral cavity, salivary glands are at particular risk of tissue injury induced by radiation (Grundmann et al., 2009). In order to reduce radiation-induced damage to the salivary glands, preventative approaches have been the focus of research and significant progress has been made in this field (Vissink et al., 2015). These methods include the modification of radiotherapy techniques to spare the salivary glands from high-dose radiation (Mendenhall et al., 2014) and salivary gland tissue transfer (Wu et al., 2015) if the gland is in the path of radiation and cannot be spared by the new radiotherapy techniques.

Although preventative measures have been successful, there are still a large number of patients who suffer from previously damaged salivary glands. Therefore, repair and regeneration of salivary glands have attracted the attention of research significantly in the recent decade (Lombaert et al., 2017). Different in vivo and in vitro tissue-engineering strategies have been developed employing different types of cells, scaffolds, biological agents, growth factors, and culture conditions for regeneration of salivary glands (Yoo et al., 2014; Amrollahi et al., 2016; Ferreira et al., 2016). However, due to the complexity of the salivary glands structure and the challenges in the clinical application of engineered tissues (Almela et al., 2016), limited progress has been made with clinical transplantation of tissue-engineered salivary glands on patients and the in vivo studies in this field are restricted to animals (Ono et al., 2015). The following sections briefly review the anatomy, structure, and pathology of the salivary glands and discuss the important aspects of damage prevention and preservation of the glands as well as the progress made to date with regard to tissue engineering and regeneration of the salivary glands.

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00020-7
© 2017 Elsevier Ltd. All rights reserved.

20.2 Salivary gland function, anatomy, and histology

Saliva has many functions such as lubrication, facilitating swallowing, and speech. It also acts as a solvent to experience taste of food substances and contains the enzyme amylase which helps to digest food. Furthermore, it prevents infections (IgA, lysozyme, histatins), cleans the mouth, and has a buffering capacity. In addition, saliva plays a role in tissue repair (growth factors) and maintaining tooth integrity (calcium and phosphate) (Nanci, 2008; Dios et al., 2016).

Salivary glands can be grouped into two categories: (1) major salivary glands including parotid, submandibular, and sublingual salivary glands and (2) the minor salivary glands. The major salivary glands are paired with one salivary gland at the left side and one at the right side of the head. The parotid gland is the largest salivary gland and is located near the ear at the posterior part of the mandibular ramus. The saliva produced in the parotid gland is drained into the oral cavity via the parotid duct (Stensen's duct) with its opening located at a papilla opposite the maxillary second molar tooth. The second largest salivary gland is the submandibular salivary gland that is located at the ventromedial aspect of the mandible, surrounding the posterior part of the mylohyoid muscle. Its duct is named the Wharton's duct and opens into the oral cavity at a sublingual caruncle that is situated lateral to the lingual frenulum behind the lower incisor.

Sublingual glands are located in the anterior part of the oral cavity between the mucosa and the mylohyoid muscle. Several small ducts called the ducts of Rivinus connect the sublingual glands with the oral cavity and their openings can be found along the sublingual fold. Bartholin's duct is another duct draining saliva from the sublingual glands into the oral cavity that opens together with the submandibular duct at the sublingual caruncle (Nanci, 2008; Holsinger and Bui, 2007).

The minor salivary glands are spread in the submucosa of almost the entire oral cavity except for the gingiva and the anterior part of the hard palate.

Salivary glands are exocrine glands meaning that the produced secretions are released via ducts into the adjacent external or internal epithelia. Salivary glands have a lobular organization and can be compared to a bunch of grapes, with secretory end pieces representing the grapes and the ducts corresponding to the stems and stalks. The secretory end pieces of salivary glands are called acini and can consist of solely serous cells (serous acinus), solely mucinous cells (mucinous acinus), or a mixture of serous and mucinous cells (mixed acinus). Although serous cells are typically pyramid-shaped with their base closest to the basal lamina, mucinous cells are usually columnar in shape. Myoepithelial cells (basket cells) surround the acini, lie between the basal lamina and the epithelial cells, and saliva is secreted upon their contraction.

The acini of parotid salivary glands only contain serous cells, whereas the submandibular salivary glands and sublingual salivary glands are mixed glands with acini containing both serous and mucous cells as well as serous acini and mucinous acini. The submandibular salivary glands contain mainly serous acini and the sublingual salivary glands contain especially mucinous acini. Most minor salivary glands are either mucinous or seromucinous (Peel and Seethala, 2007).

Additionally, in some salivary glands, extensions of the lumen, which are termed the intercellular canaliculi, can be found between the adjacent secretory cells. From the lumina of the acini, saliva flows into the intercalated ducts, the striated ducts, and finally into the excretory ducts.

The initial isotonic, primary saliva is modified in the striated and excretory ducts by an exchange of electrolytes, with a hypotonic saliva as a result. The acini and ducts make up the parenchyma of the salivary glands and are accompanied by stroma consisting of a connective tissue containing blood vessels and nerves.

20.3 Salivary gland diseases and pathology

Several conditions can impair the function of the salivary glands including inflammation of the salivary glands (sialadenitis) due to bacterial, viral, or parasitic infections; autoimmune disorders such as Sjogren's syndrome; obstruction of the gland (sialolithiasis) and radiation-induced damage; salivary gland tumors; and medication-induced salivary gland dysfunction (MISGD) (Peel and Seethala, 2007; Hellquist and Skalova, 2014).

20.3.1 Infections

Bacterial sialadenitis is characterized by swelling, erythema, and pain in the region of the affected salivary gland and pus may be observed draining from the excretory duct opening. Acute bacterial sialadenitis is often a polymicrobial disease, and the most common bacterium associated with sialadenitis is *Staphylococcus aureus*. Anaerobic bacteria such as Prevotella, Porphyromonas, Fusobacterium, and Peptostreptococcus were also commonly found in bacterial sialadenitis (Brook, 2009; Raad et al., 1990).

Histologically, the main structure of the salivary glands remains the same in case of an acute infection, although lobules may be widened, friable, and colored red to yellow. Acute bacterial infection of the salivary glands commonly gives rise to abscess formation which can cause the affected salivary gland tissue to liquefy and also necrosis is often observed. The acini are destructed and interstitial infiltrates of mainly neutrophils are present (Peel and Seethala, 2007).

Viruses infecting the salivary glands follow a systemic route, typically resulting in a bilateral swelling and tenderness of affected salivary glands, and prodromal symptoms such as fever, headache, and myalgia. The most commonly associated virus with sialadenitis is the mumps virus. Cytomegalovirus and adenovirus are rare causes of viral sialadenitis and occur mainly in HIV-affected persons. Other viruses associated with salivary gland infection include Epstein–Barr virus, human herpes virus 6 and 7, influenza virus, parainfluenza virus, Coxsackie A and B, echovirus, and lymphocytic choriomeningitis virus (Hellquist and Skalova, 2014; Mcquone, 1999). If an acute viral sialadenitis is histologically examined, interstitial infiltrates of lymphocytes and monocytes can be found. Although a salivary gland infected by

cytomegalovirus may not show any gross abnormalities, nor features of inflammation, it can be recognized histologically by the presence of viral inclusions in acini and ducts (Peel and Seethala, 2007).

20.3.2 Sjogren's syndrome

Sjogren's syndrome which is an autoimmune disease is characterized by the inflammation and dysfunction of exocrine glands. The resulting symptoms can be limited to dryness of the affected mucosa, predominantly of the mouth (xerostomia) and eyes (keratoconjunctivitis sicca) in primary Sjogren's syndrome. Symptoms can be more severe in secondary Sjogren's syndrome where the exocrinopathy coincides with another autoimmune connective tissue disorder such as rheumatoid arthritis, systemic lupus erythematosus, or systemic sclerosis (Fox, 2005).

The presence of serum autoantibodies, such as rheumatoid factor, anti-Ro (SS-A), anti-La (SS-B), as well as antinuclear antibodies are important markers in the diagnosis of Sjogren's syndrome (Stott et al., 1998; Tincani et al., 2013). Antibodies against salivary duct antigens can also be found in the blood serum, and 95% of patients with primary Sjogren's syndrome have been reported to have serum antibodies against α-fodrin (Haneji et al., 1997; Macsween et al., 1967).

Histologically, lymphocytic infiltrations of mainly primed CD4-positive T-lymphocytes can be found surrounding the salivary ducts in the early stages of Sjogren's syndrome, whereas B-lymphocytes can increase in number in later stages of the disease with formation of ectopic germinal center-like structures, resulting in acinar atrophy (Singh and Cohen, 2012; Matthews et al., 1991).

20.3.3 Radiation damage

Radiation of the salivary glands can cause degeneration and death of parenchymal cells of the salivary glands, and it can also cause damage to blood vessels, nerves, or the connective tissue (Grundmann et al., 2009). Damage can be caused directly by the radiation or indirectly by infiltration of inflammatory cells in response to radiation (Schaue and Mcbride, 2012). Radiation results in an increased permeability of endothelia of capillaries surrounding the salivary ducts, which leads to interstitial edema and can give rise to obstruction caused by compression of the ducts (Grötz et al., 2001). Serous acini are most susceptible to radiation damage and there can be little or even no regeneration following absorption of high doses of radiation. As mucous acini are less sensitive to radiation than the serous acini, some regeneration may take place in sublingual and submandibular glands (Redman, 2008).

20.3.4 Tumors

Benign and malignant tumors of salivary glands can disrupt the normal function of the gland.

Benign tumors include pleomorphic adenoma (most common salivary gland tumor), basal cell adenoma, myoepitheliomas, Warthin's tumor, oncocytoma, and sclerosing polycystic adenosis.

Malignant tumors include adenoid cystic carcinoma, mucoepidermoid carcinoma, malignant mixed tumors, acinic cell carcinoma, epithelial–myoepithelial carcinoma, basal cell adenocarcinoma, myoepithelial carcinoma, and salivary duct carcinoma (Peel and Seethala, 2007).

20.3.5 Medication-induced salivary gland dysfunction

MISGD includes medication-induced salivary gland hypofunction (decreased saliva production) and objective sialorrhea (excessive saliva production). According to the systematic reviews by the World Workshop on Oral Medicine (Villa et al., 2015, 2016), medication acting on nervous, cardiovascular, genitourinary, musculoskeletal, respiratory, and alimentary systems can cause MISGD, which means that medication influencing almost all body systems can cause possible side effects related to dysfunction of the salivary glands. In particular for the patients with Sjogren's syndrome or patients undergoing radiation of head and neck area, it is important to prescribe medications that do not aggravate the symptoms of the already existing salivary gland dysfunction (Wolff et al., 2017).

20.4 Prevention and preservation of salivary glands

Salivary gland tissue damage caused by radiation can be prevented by several techniques including (1) organ-sparing radiotherapy to reduce the absorbed dose by the salivary glands, (2) surgical relocation of the gland to an area of the body which is not subject to high-dose radiotherapy, and (3) the use of medications that increase the resistance of the salivary glands to radiation (Vissink et al., 2015).

20.4.1 Organ-sparing radiotherapy

Intensity-modulated radiation therapy (IMRT) is an advanced organ-sparing radiotherapy technique that has been used in recent years to reduce the risk of radiation damage to the adjacent structures near malignant tumors. Using computer-aided linear accelerators, it delivers highly accurate radiation doses to the specific areas within the tumor (Lee and Terezakis, 2008). Studies indicate that the use of submandibular gland- and parotid gland-sparing IMRT in oncology patients resulted in a reduction in the long-term radiotherapy side effects such as xerostomia (Mendenhall et al., 2014; Jensen et al., 2010; Nutting et al., 2011).

Although IMRT has been effective in salivary gland damage prevention, 40% of patients still experience xerostomia following treatment by IMRT (Beetz et al., 2014).

Proton beam therapy, using positively charged high-energy proton particles to destroy cancer cells, is another advanced radiotherapy technique with the ability to

spare salivary glands and reduce the risk of xerostomia by up to 70% (Van De Water et al., 2012).

20.4.2 Preventative medication

Several medications have been used to reduce the sensitivity and to increase the resistance of the salivary glands to radiation (Vissink et al., 2015). These drugs are amifostine, pilocarpine, insulin growth factor (IGF), and keratinocyte growth factor (Jensen et al., 2010). Newly studied agents also include roscovitine (Martin et al., 2012) and fibroblast growth factor (FGF) (Kojima et al., 2011b). However, there is some controversy regarding the efficacy and even safety of some of these drugs (Ma et al., 2009).

20.4.3 Salivary gland tissue transfer

Jha et al. (2000) introduced a new technique for submandibular gland tissue transfer into the submental space prior to radiotherapy and shielding the gland to protect the gland's function. Surgical salivary gland tissue transfer has since been widely accepted by the patients and practiced by the clinicians (Seikaly et al., 2001). A recent systematic review and meta-analysis showed that submandibular gland tissue transfer reduced the risk of acute and late postradiation xerostomia by 69% and 81%, respectively, without any serious adverse events (Wu et al., 2015). It appears that the best method to manage radiation-induced xerostomia is the preventative approach.

20.5 Tissue-engineering approaches

Tissue-engineering strategies for the reconstruction of salivary glands can be generally divided into three main categories: (1) cell culture of salivary gland primary cells or cell lines on the surface of the scaffolds mimicking the tubular structure of the glands (Sequeira et al., 2012; Wang et al., 1999; Zhang et al., 2015), (2) three-dimensional (3D) culture of salivary gland stem cells and epithelial cells within hydrogels which has gained popularity in recent years (Shubin et al., 2015, 2017; Nam et al., 2016), and (3) organ culture and guided tissue morphogenesis of embryonic salivary gland tissue buds (Miyajima et al., 2011; Yamada et al., 2016). The following sections describe the cells, bioactive agents, and scaffolds used in tissue engineering and regeneration of salivary glands.

20.5.1 Cells and bioactive agents

Cell-based approaches for potential clinical regeneration of salivary glands include (1) the application of autologous salivary gland primary cells and epithelial stem or progenitor cells and (2) use of other nonepithelial cells to induce regenerative effects on the remaining salivary gland cells and engineered tissue reconstructs (Lombaert et al., 2017).

The use of different types of adult stem cells such as salivary gland-derived stem cells, bone marrow mesenchymal stem cells (MSCs), adipose-derived MSCs, and human amniotic epithelial cells in regeneration salivary glands has been thoroughly reviewed (Yoo et al., 2014).

In addition to the primary cells and progenitor stem-cells, tumor-derived human salivary gland cell lines and virally transformed immortalized rat salivary gland cell lines have been extensively used in in vitro studies for tissue engineering and studies of salivary glands regeneration (Nelson et al., 2013).

Bioactive agents are used to induce environmental-signaling cues and promote stem-cell differentiation and regeneration of salivary glands (Lombaert et al., 2017). These include cytokines (Lombaert et al., 2008), growth factors such as epidermal growth factor (Ohlsson et al., 1997), FGF (Kojima et al., 2011a), insulin growth factor (IGF-1) (Jensen et al., 2010), vascular endothelial growth factor (Xiong et al., 2014), enzymes such as aldehyde dehydrogenase-3 activator (Alda-89) (Xiao et al., 2013), and hormones such as melatonin (Cakmak Karaer et al., 2016).

Gene therapy using viral vectors and nonviral methods to transfer genes into the salivary gland cells has also been considered as a potential treatment modality for salivary gland hypofunction with promising results (Baum et al., 2015). Aquaporins (AQPs) have been the main targets of gene therapy for the salivary glands. AQPs are protein channels that are integrated in the cell membrane and regulate water permeability (Delporte et al., 2016).

Recent human clinical trials have shown increase in saliva production following the administration of adenovirus encoding human aquaporin-1 (AdhAQP1) into radiation-damaged parotid glands (Zheng et al., 2015).

20.5.2 Scaffolds

Many different types of scaffolds have been investigated for their suitability for salivary gland tissue engineering. These scaffolds can be grouped into three categories including naturally derived biomaterials, synthetic scaffolds, and hybrid scaffolds that are discussed in the following sections.

20.5.2.1 Naturally derived scaffolds

Collagen and basement membrane matrix-based scaffolds and hydrogels such as Matrigel have been used frequently for tissue engineering of salivary glands (Maria et al., 2011a,b; Joraku et al., 2007). Matrigel is a solubilized extract of basement membrane proteins from the Engelbreth-Holm-Swarm mouse sarcoma which is rich in extracellular matrix proteins such as laminin, collagen type IV, heparan sulfate proteoglycans, and growth factors and is shown to promote cell attachment and differentiation in vitro (Kleinman and Martin, 2005; Baker et al., 2010).

Laminin, which is the major component of Matrigel, has been shown to be critical for intact salivary gland organization and morphogenesis (Kadoya and Yamashina, 1989; Maruyama et al., 2015). However, clinical application of the full Laminin-1 sequence can lead to unwanted side effects, such as tumorigenesis,

degradation, and immune reactions (Topley et al., 1993). Therefore, Laminin-1 peptide-conjugated fibrin-based hydrogel scaffolds have been investigated recently and demonstrated to promote salivary gland regeneration forming new and functional tissues displaying acinar and ductal cell organization (Nam et al., 2016, 2017).

Human-derived hydrogels based on fibronectin and placenta basement membrane extracts have also been shown to promote morphological and functional differentiation of primary human salivary gland epithelial cells to form polarized salivary acinar-like structures with comparable results to Matrigel (Maria et al., 2016).

Chitin and chitosan-based scaffolds (Patil and Nanduri, 2017) are shown to be capable of providing a suitable environment for salivary gland branch formation enabling cells to produce essential extracellular matrix (Yang and Young, 2008, 2009; Yang and Hsiao, 2015).

Recently studied biomaterials also include silk fibroin scaffolds that have been shown to promote salivary gland epithelial cell growth, differentiation and matrix formation (Zhang et al., 2015), hyaluronic acid-based hydrogels that have been developed for 3D culture of salivary gland spheroids using primary salivary human stem/progenitor cells (Ozdemir et al., 2016b), and arginine-glycine-aspartic acid-modified alginate-based hydrogels which enhanced submandibular gland bud expansion and cleft formation (Taketa et al., 2015).

Denuded rat tracheal preparation with tubular structure has been examined as a biological scaffold for salivary gland tissue engineering in vitro. Coating the tracheae with collagen I or fibronectin has been shown to promote salivary epithelial cell line growth and monolayer formation. Matrigel coating resulted in cell growth in a more organized fashion (Wang et al., 1999).

Whole-organ decellularized scaffolds have been developed by immersion of rat submandibular glands in detergents. Repopulation of the decellularized organ with salivary gland cells has shown evidence of cellular adhesion and differentiation in vitro (Gao et al., 2014).

Residual extracellular matrices prepared by mechanical and enzymatic release of cellular components from human submandibular gland biopsies have been studied as a substrate for seeding and culture of human epithelial cells and fibroblasts. The engineered reconstructs retained their native extracellular matrix proteins and supported cell proliferation (Lilliu et al., 2016).

20.5.2.2 Synthetic biomaterials

The use of synthetic polymers as scaffolds for tissue engineering obviates some of the limitations associated with the use of human or animal-derived biomaterials. The advantages include reduced risk of disease transmission and improved strength and stability. In addition, some other properties of the synthetic scaffolds such as microstructure, stiffness, and porosity can be adjusted to provide desirable environment for cell growth and differentiation.

Synthetic polymers studied for applications in salivary gland tissue engineering include nanofibrous poly lactic-*co*-glycolic acid (PLGA) scaffolds (Sequeira et al., 2012), poly (ethylene glycol) hydrogels (Shubin et al., 2015, 2017), and poly

ethylene glycol hydrogel-micropatterned polycaprolactone nanofibrous microwells which have been shown to promote functional acinar-like organoids from human parotid epithelial cells (Shin et al., 2016).

Polyacrylamide gels have been used to assess the influence of the substrate modulus on submandibular salivary gland regeneration. Physiologically compliant gels demonstrated higher levels of branching morphogenesis in salivary gland explants compared to the stiff gels (Peters et al., 2014).

Attempts have been made to employ advanced manufacturing techniques such as 3D printing using resin-based polymers to replicate laboratory models of rat salivary glands following magnetic resonance imaging scanning of the glands infused with a contrast agent. Due to the structural complexity of salivary glands, the use of this technique is currently limited to only monitoring the overall dimensional and volumetric changes in the salivary gland (Cecchini et al., 2014).

20.5.2.3 Hybrid scaffolds

Hybrid scaffolds containing synthetic polymers coated with natural proteins have been developed to enhance the bioactivity of the polymer surface and improve cell attachment and differentiation. Aframian et al. (2000) compared several biodegradable polymer-based substrates including poly-L-lactic acid (PLLA), polyglycolic acid (PGA), and their copolymers, coated with different extracellular matrix proteins including collagen I, collagen IV, laminin, fibronectin, and gelatin. They showed that a salivary gland epithelial cell line did not attach to the polymer disks in the absence of preadsorbed proteins. The cell behavior was similar on PLLA and PGA substrates. However, fibronectin-coated PLLA disks showed superior results. Laminin, collagen I, and gelatin coating also promoted monolayer cell growth (Aframian et al., 2000).

It has been shown that incorporation of Matrigel coating to the surface of poly (ethylene glycol)-terephthalate/poly (butylene terephthalate) scaffolds improved salivary gland epithelial cell growth and resembled the morphology of normal epithelium (Sun et al., 2006). In a different study, chitosan and laminin coating of nanofiber PLGA scaffolds further enhanced the proliferation of salivary gland acinar and ductal cell lines (Cantara et al., 2012).

20.6 Regeneration challenges

Despite the progress made with regard to in vitro tissue engineering of salivary glands, there are still many challenges ahead before this concept becomes a clinical reality. The limitations of the current tissue-engineered salivary gland reconstructs are listed below:

- Risk of adverse host tissue response to the implanted biomaterials such as inflammation and foreign body reaction as well as potential immune reaction to any biological components with antigenic properties (Anderson et al., 2008).

- The lack of appropriate vascularization, innervation, and the secretory function of the reconstructed salivary gland tissue (Holmberg and Hoffman, 2014).
- Presence of several different types of cells with specialized functions in salivary glands with complex structures which further complicates the reconstruction process due to the paucity of multifunctional biomaterials with ideal mechanical and biological properties to accommodate and support all different types of cellular activities (Ozdemir et al., 2016a).
- Cell-related challenges for clinical application of tissue engineered reconstructs that have been recently reviewed in Almela et al. (2016).

20.7 Conclusion

Although significant progress has been made in tissue engineering and regeneration of salivary glands using in vitro studies and in vivo animal models, clinical transplantation of tissue-engineered salivary gland reconstructs in humans is still a long way ahead. There are many challenges associated with tissue engineering and regeneration of salivary glands including limitations with biomaterials, cells, glands functionality, and the host response. Protection and preservation of salivary glands appear to be viable strategies to prevent radiation damage to the salivary gland tissue and reduce the risk of development of associated xerostomia. Advances in gene therapy offer new therapeutic opportunity, although there is a need for further clinical trials to establish their long-term safety and effectiveness.

References

Acauan, M.D., Figueiredo, M.A., Cherubini, K., Gomes, A.P., Salum, F.G., 2015. Radiotherapy-induced salivary dysfunction: structural changes, pathogenetic mechanisms and therapies. Arch. Oral Biol. 60, 1802–1810.

Aframian, D.J., Cukierman, E., Nikolovski, J., Mooney, D.J., Yamada, K.M., Baum, B.J., 2000. The growth and morphological behavior of salivary epithelial cells on matrix protein-coated biodegradable substrata. Tissue Eng. 6, 209–216.

Almela, T., Brook, I.M., Moharamzadeh, K., 2016. The significance of cell-related challenges in the clinical application of tissue engineering. J. Biomed. Mater. Res. A. 104, 3157–3163.

Amrollahi, P., Shah, B., Seifi, A., Tayebi, L., 2016. Recent advancements in regenerative dentistry: a review. Mater. Sci. Eng. C: Mater. Biol. Appl. 69, 1383–1390.

Anderson, J.M., Rodriguez, A., Chang, D.T., 2008. Foreign body reaction to biomaterials. Semin. Immunol. 20, 86–100.

Baker, O.J., Schulz, D.J., Camden, J.M., Liao, Z., Peterson, T.S., Seye, C.I., et al., 2010. Rat parotid gland cell differentiation in three-dimensional culture. Tissue. Eng. C: Methods. 16, 1135–1144.

Barone, F., Colafrancesco, S., 2016. Sjogren's syndrome: from pathogenesis to novel therapeutic targets. Clin. Exp. Rheumatol. 34, 58–62.

Baum, B.J., Alevizos, I., Chiorini, J.A., Cotrim, A.P., Zheng, C., 2015. Advances in salivary gland gene therapy—oral and systemic implications. Expert Opin. Biol. Ther. 15, 1443–1454.

Beetz, I., Steenbakkers, R.J., Chouvalova, O., Leemans, C.R., Doornaert, P., Van Der Laan, B.F., et al., 2014. The QUANTEC criteria for parotid gland dose and their efficacy to prevent moderate to severe patient-rated xerostomia. Acta Oncol. 53, 597–604.
Brook, I., 2009. The bacteriology of salivary gland infections. Oral Maxillofac. Surg. Clin. North Am. 21, 269–274.
Cakmak Karaer, I., Simsek, G., Yildiz, A., Vardi, N., Polat, A., Tanbek, K., et al., 2016. Melatonin's protective effect on the salivary gland against ionized radiation damage in rats. J. Oral Pathol. Med. 45, 444–449.
Cantara, S.I., Soscia, D.A., Sequeira, S.J., Jean-Gilles, R.P., Castracane, J., Larsen, M., 2012. Selective functionalization of nanofiber scaffolds to regulate salivary gland epithelial cell proliferation and polarity. Biomaterials 33, 8372–8382.
Cartee, D.L., Maker, S., Dalonges, D., Manski, M.C., 2015. Sjogren's syndrome: oral manifestations and treatment, a dental perspective. J. Dent. Hyg. 89, 365–371.
Cecchini, M.P., Parnigotto, M., Merigo, F., Marzola, P., Daducci, A., Tambalo, S., et al., 2014. 3D printing of rat salivary glands: the submandibular-sublingual complex. Anat. Histol. Embryol. 43, 239–244.
Delli, K., Spijkervet, F.K., Vissink, A., 2014. Salivary gland diseases: infections, sialolithiasis and mucoceles. Monogr. Oral Sci. 24, 135–148.
Delporte, C., Bryla, A., Perret, J., 2016. Aquaporins in salivary glands: from basic research to clinical applications. Int. J. Mol. Sci. 17.
Dios, P.D., Scully, C., Almeida, O.P.D., Bagán, J.V. & Taylor, A.M. 2016. Oral Medicine and Pathology at a Glance, Chichester, John Wiley & Sons, Ltd.
Ferreira, J.N., Rungarunlert, S., Urkasemsin, G., Adine, C., Souza, G.R., 2016. Three-dimensional bioprinting nanotechnologies towards clinical application of stem cells and their secretome in salivary gland regeneration. Stem Cells Int. 2016, 7564689.
Fox, R., 2005. Sjögren's syndrome. Lancet. 366, 321–331.
Gao, Z., Wu, T., Xu, J., Liu, G., Xie, Y., Zhang, C., et al., 2014. Generation of bioartificial salivary gland using whole-organ decellularized bioscaffold. Cells Tissues Organs 200, 171–180.
Green, B., Rahimi, S., Brennan, P.A., 2016. Salivary gland malignancies - an update on current management for oral healthcare practitioners. Oral Dis. 22, 735–739.
Grötz, K.A., Wüstenberg, P., Kohnen, R., Al-Nawas, B., Henneicke-Von Zepelin, H.-H., Bockisch, A., et al., 2001. Prophylaxis of radiogenic sialadenitis and mucositis by coumarin/troxerutine in patients with head and neck cancer—a prospective, randomized, placebo-controlled, double-blind study. Br. J. Oral Maxillofac. Surg. 39, 34–39.
Grundmann, O., Mitchell, G.C., Limesand, K.H., 2009. Sensitivity of salivary glands to radiation: from animal models to therapies. J. Dent. Res. 88, 894–903.
Haneji, N., Nakamura, T., Takio, K., Yanagi, K., Higashiyama, H., Saito, I., et al., 1997. Identification of alpha-fodrin as a candidate autoantigen in primary Sjogren's syndrome. Science 276, 604–607.
Hellquist, H., Skalova, A., 2014. Histopathology of the Salivary Glands. Springer, Berlin.
Holmberg, K.V., Hoffman, M.P., 2014. Anatomy, biogenesis and regeneration of salivary glands. Monogr. Oral Sci. 24, 1–13.
Holsinger, F.C., Bui, D.T., 2007. Anatomy, function, and evaluation of the salivary glands. In: Myers, E.C., Ferris, R.L. (Eds.), Salivary Gland Disorders. Springer, Berlin.
Jensen, S.B., Pedersen, A.M., Vissink, A., Andersen, E., Brown, C.G., Davies, A.N., et al., 2010. A systematic review of salivary gland hypofunction and xerostomia induced by cancer therapies: management strategies and economic impact. Support Care Cancer. 18, 1061–1079.

Jha, N., Seikaly, H., Mcgaw, T., Coulter, L., 2000. Submandibular salivary gland transfer prevents radiation-induced xerostomia. Int. J. Radiat. Oncol. Biol. Phys. 46, 7–11.

Joraku, A., Sullivan, C.A., Yoo, J., Atala, A., 2007. In-vitro reconstitution of three-dimensional human salivary gland tissue structures. Differentiation 75, 318–324.

Kadoya, Y., Yamashina, S., 1989. Intracellular accumulation of basement membrane components during morphogenesis of rat submandibular gland. J. Histochem. Cytochem. 37, 1387–1392.

Kleinman, H.K., Martin, G.R., 2005. Matrigel: basement membrane matrix with biological activity. Semin. Cancer Biol. 15, 378–386.

Kojima, T., Kanemaru, S., Hirano, S., Tateya, I., Ohno, S., Nakamura, T., et al., 2011a. Regeneration of radiation damaged salivary glands with adipose-derived stromal cells. Laryngoscope 121, 1864–1869.

Kojima, T., Kanemaru, S., Hirano, S., Tateya, I., Suehiro, A., Kitani, Y., et al., 2011b. The protective efficacy of basic fibroblast growth factor in radiation-induced salivary gland dysfunction in mice. Laryngoscope 121, 1870–1875.

Lee, N.Y., Terezakis, S.A., 2008. Intensity-modulated radiation therapy. J. Surg. Oncol. 97, 691–696.

Lilliu, M.A., Seo, Y.J., Isola, M., Charbonneau, A.M., Zeitouni, A., El-Hakim, M., et al., 2016. Natural extracellular matrix scaffolds recycled from human salivary digests: a morphometric study. Oral Dis. 22, 313–323.

Lombaert, I., Movahednia, M.M., Adine, C., Ferreira, J.N., 2017. Concise review: salivary gland regeneration: therapeutic approaches from stem cells to tissue organoids. Stem Cells. 35, 97–105.

Lombaert, I.M., Brunsting, J.F., Wierenga, P.K., Kampinga, H.H., De Haan, G., Coppes, R.P., 2008. Cytokine treatment improves parenchymal and vascular damage of salivary glands after irradiation. Clin. Cancer Res. 14, 7741–7750.

Ma, C., Xie, J., Chen, Q., Wang, G., Zuo, S., 2009. Amifostine for salivary glands in high-dose radioactive iodine treated differentiated thyroid cancer. Cochrane Database Syst. Rev. 4, CD007956.

Macsween, R.N., Goudie, R.B., Anderson, J.R., Armstrong, E., Murray, M.A., Mason, D.K., et al., 1967. Occurrence of antibody to salivary duct epithelium in Sjogren's disease, rheumatoid arthritis, and other arthritides. A clinical and laboratory study. Ann. Rheum. Dis. 26, 402–411.

Maria, O.M., Liu, Y., El-Hakim, M., Zeitouni, A., Tran, S.D., 2016. The role of human fibronectin- or placenta basement membrane extract-based gels in favouring the formation of polarized salivary acinar-like structures. J. Tissue Eng. Regen. Med. Available from: http://dx.doi.org/10.1002/term.2164.

Maria, O.M., Maria, O., Liu, Y., Komarova, S.V., Tran, S.D., 2011a. Matrigel improves functional properties of human submandibular salivary gland cell line. Int. J. Biochem. Cell Biol. 43, 622–631.

Maria, O.M., Zeitouni, A., Gologan, O., Tran, S.D., 2011b. Matrigel improves functional properties of primary human salivary gland cells. Tissue Eng. A. 17, 1229–1238.

Martin, K.L., Hill, G.A., Klein, R.R., Arnett, D.G., Burd, R., Limesand, K.H., 2012. Prevention of radiation-induced salivary gland dysfunction utilizing a CDK inhibitor in a mouse model. PLoS One. 7, e51363.

Maruyama, C.L., Leigh, N.J., Nelson, J.W., Mccall, A.D., Mellas, R.E., Lei, P., et al., 2015. Stem cell-soluble signals enhance multilumen formation in SMG cell clusters. J. Dent. Res. 94, 1610–1617.

Matthews, J.B., Deacon, E.M., Kitas, G.D., Salmon, M., Potts, A.J., Hamburger, J., et al., 1991. Primed and naive helper T cells in labial glands from patients with Sjogren's syndrome. Virchows Arch. A: Pathol. Anat. Histopathol. 419, 191–197.

Mcquone, S.J., 1999. Acute viral and bacterial infections of the salivary glands. Otolaryngol. Clin. North Am. 32, 793–811.

Mendenhall, W.M., Mendenhall, C.M., Mendenhall, N.P., 2014. Submandibular gland-sparing intensity-modulated radiotherapy. Am. J. Clin. Oncol. 37, 514–516.

Miyajima, H., Matsumoto, T., Sakai, T., Yamaguchi, S., An, S.H., Abe, M., et al., 2011. Hydrogel-based biomimetic environment for in vitro modulation of branching morphogenesis. Biomaterials 32, 6754–6763.

Nam, K., Jones, J.P., Lei, P., Andreadis, S.T., Baker, O.J., 2016. Laminin-111 peptides conjugated to fibrin hydrogels promote formation of lumen containing parotid gland cell clusters. Biomacromolecules 17, 2293–2301.

Nam, K., Wang, C.S., Maruyama, C.L., Lei, P., Andreadis, S.T., Baker, O.J., 2017. L1 Peptide-conjugated fibrin hydrogels promote salivary gland regeneration. J. Dent. Res. 10, 22034517695496.

Nanci, A. 2008. Ten Cate's Oral Histology: Development, Structure, and Function, St. Louis, Mosby Elsevier.

Nelson, J., Manzella, K., Baker, O.J., 2013. Current cell models for bioengineering a salivary gland: a mini-review of emerging technologies. Oral Dis. 19, 236–244.

Nutting, C.M., Morden, J.P., Harrington, K.J., Urbano, T.G., Bhide, S.A., Clark, C., et al., 2011. Parotid-sparing intensity modulated versus conventional radiotherapy in head and neck cancer (PARSPORT): a phase 3 multicentre randomised controlled trial. Lancet Oncol. 12, 127–136.

Ohlsson, B., Jansen, C., Ihse, I., Axelson, J., 1997. Epidermal growth factor induces cell proliferation in mouse pancreas and salivary glands. Pancreas 14, 94–98.

Ono, H., Obana, A., Usami, Y., Sakai, M., Nohara, K., Egusa, H., et al., 2015. Regenerating salivary glands in the microenvironment of induced pluripotent stem cells. Biomed. Res. Int. 2015, 293570.

Ozdemir, T., Fowler, E.W., Hao, Y., Ravikrishnan, A., Harrington, D.A., Witt, R.L., et al., 2016a. Biomaterials-based strategies for salivary gland tissue regeneration. Biomater. Sci. 4, 592–604.

Ozdemir, T., Fowler, E.W., Liu, S., Harrington, D.A., Witt, R.L., Farach-Carson, M.C., et al., 2016b. Tuning hydrogel properties to promote the assembly of salivary gland spheroids in 3D. ACS Biomater. Sci. Eng. 2, 2217–2230.

Patil, S.V., Nanduri, L.S., 2017. Interaction of chitin/chitosan with salivary and other epithelial cells—an overview. Int. J. Biol. Macromol. Available from: http://dx.doi.org/10.1016/j.ijbiomac.2017.03.058.

Peel, R.L., Seethala, R.R., 2007. Pathology of salivary gland disease. In: Meyers, E.N., Ferris, R.L. (Eds.), Salivary Gland Disorders. Berlin Springer.

Peters, S.B., Naim, N., Nelson, D.A., Mosier, A.P., Cady, N.C., Larsen, M., 2014. Biocompatible tissue scaffold compliance promotes salivary gland morphogenesis and differentiation. Tissue Eng. A 20, 1632–1642.

Raad, I.I., Sabbagh, M.F., Caranasos, G.J., 1990. Acute bacterial sialadenitis: a study of 29 cases and review. Rev. Infect. Dis. 12, 591–601.

Redman, R.S., 2008. On approaches to the functional restoration of salivary glands damaged by radiation therapy for head and neck cancer, with a review of related aspects of salivary gland morphology and development. Biotech. Histochem. 83, 103–130.

Schaue, D., Mcbride, W.H., 2012. T lymphocytes and normal tissue responses to radiation. Front. Oncol. 2, 119.

Seikaly, H., Jha, N., Mcgaw, T., Coulter, L., Liu, R., Oldring, D., 2001. Submandibular gland transfer: a new method of preventing radiation-induced xerostomia. Laryngoscope 111, 347–352.

Sequeira, S.J., Soscia, D.A., Oztan, B., Mosier, A.P., Jean-Gilles, R., Gadre, A., et al., 2012. The regulation of focal adhesion complex formation and salivary gland epithelial cell organization by nanofibrous PLGA scaffolds. Biomaterials 33, 3175–3186.

Shin, H.S., Kook, Y.M., Hong, H.J., Kim, Y.M., Koh, W.G., Lim, J.Y., 2016. Functional spheroid organization of human salivary gland cells cultured on hydrogel-micropatterned nanofibrous microwells. Acta Biomater. 45, 121–132.

Shubin, A.D., Felong, T.J., Graunke, D., Ovitt, C.E., Benoit, D.S., 2015. Development of poly(ethylene glycol) hydrogels for salivary gland tissue engineering applications. Tissue Eng. A. 21, 1733–1751.

Shubin, A.D., Felong, T.J., Schutrum, B.E., Joe, D.S., Ovitt, C.E., Benoit, D.S., 2017. Encapsulation of primary salivary gland cells in enzymatically degradable poly(ethylene glycol) hydrogels promotes acinar cell characteristics. Acta Biomater. 50, 437–449.

Singh, N., Cohen, P.L., 2012. The T cell in Sjogren's syndrome: force majeure, not spectateur. J. Autoimmun. 39, 229–233.

Stott, D.I., Hiepe, F., Hummel, M., Steinhauser, G., Berek, C., 1998. Antigen-driven clonal proliferation of B cells within the target tissue of an autoimmune disease. The salivary glands of patients with Sjogren's syndrome. J. Clin. Invest. 102, 938–946.

Sun, T., Zhu, J., Yang, X., Wang, S., 2006. Growth of miniature pig parotid cells on biomaterials in vitro. Arch. Oral Biol. 51, 351–358.

Taketa, H., Sathi, G.A., Farahat, M., Rahman, K.A., Sakai, T., Hirano, Y., et al., 2015. Peptide-modified substrate for modulating gland tissue growth and morphology in vitro. Sci. Rep. 5, 11468.

Tanasiewicz, M., Hildebrandt, T., Obersztyn, I., 2016. Xerostomia of various etiologies: a review of the literature. Adv. Clin. Exp. Med. 25, 199–206.

Tincani, A., Andreoli, L., Cavazzana, I., Doria, A., Favero, M., Fenini, M.G., et al., 2013. Novel aspects of Sjogren's syndrome in 2012. BMC Med. 11, 93.

Topley, P., Jenkins, D.C., Jessup, E.A., Stables, J.N., 1993. Effect of reconstituted basement membrane components on the growth of a panel of human tumour cell lines in nude mice. Br. J. Cancer 67, 953–958.

Van De Water, T.A., Lomax, A.J., Bijl, H.P., Schilstra, C., Hug, E.B., Langendijk, J.A., 2012. Using a reduced spot size for intensity-modulated proton therapy potentially improves salivary gland-sparing in oropharyngeal cancer. Int. J. Radiat. Oncol. Biol. Phys. 82, e313–e319.

Villa, A., Wolff, A., Aframian, D., Vissink, A., Ekstrom, J., Proctor, G., et al., 2015. World Workshop on Oral Medicine VI: a systematic review of medication-induced salivary gland dysfunction: prevalence, diagnosis, and treatment. Clin. Oral Investig. 19, 1563–1580.

Villa, A., Wolff, A., Narayana, N., Dawes, C., Aframian, D.J., Lynge Pedersen, A.M., et al., 2016. World Workshop on Oral Medicine VI: a systematic review of medication-induced salivary gland dysfunction. Oral Dis. 22, 365–382.

Vissink, A., Van Luijk, P., Langendijk, J.A., Coppes, R.P., 2015. Current ideas to reduce or salvage radiation damage to salivary glands. Oral Dis. 21, e1–e10.

Wang, S., Cukierman, E., Swaim, W.D., Yamada, K.M., Baum, B.J., 1999. Extracellular matrix protein-induced changes in human salivary epithelial cell organization and proliferation on a model biological substratum. Biomaterials 20, 1043−1049.

Wolff, A., Joshi, R.K., Ekstrom, J., Aframian, D., Pedersen, A.M., Proctor, G., et al., 2017. A guide to medications inducing salivary gland dysfunction, xerostomia, and subjective sialorrhea: a systematic review sponsored by the World Workshop on Oral Medicine VI.. Drugs R D. 17, 1−28.

Wu, F., Weng, S., Li, C., Sun, J., Li, L., Gao, Q., 2015. Submandibular gland transfer for the prevention of postradiation xerostomia in patients with head and neck cancer: a systematic review and meta-analysis. ORL J. Otorhinolaryngol. Relat. Spec. 77, 70−86.

Xiao, N., Cao, H., Chen, C.H., Kong, C.S., Ali, R., Chan, C., et al., 2013. A novel aldehyde dehydrogenase-3 activator (Alda-89) protects submandibular gland function from irradiation without accelerating tumor growth. Clin. Cancer Res. 19, 4455−4464.

Xiong, X., Shi, X., Chen, F., 2014. Human adipose tissuederived stem cells alleviate radiationinduced xerostomia. Int. J. Mol. Med. 34, 749−755.

Yamada, A., Futagi, M., Fukumoto, E., Saito, K., Yoshizaki, K., Ishikawa, M., et al., 2016. Connexin 43 is necessary for salivary gland branching morphogenesis and FGF10-induced ERK1/2 phosphorylation. J. Biol. Chem. 291, 904−912.

Yang, T.L., Hsiao, Y.C., 2015. Chitosan facilitates structure formation of the salivary gland by regulating the basement membrane components. Biomaterials 66, 29−40.

Yang, T.L., Young, T.H., 2008. The enhancement of submandibular gland branch formation on chitosan membranes. Biomaterials 29, 2501−2508.

Yang, T.L., Young, T.H., 2009. Chitosan cooperates with mesenchyme-derived factors in regulating salivary gland epithelial morphogenesis. J. Cell. Mol. Med. 13, 2853−2863.

Yoo, C., Vines, J.B., Alexander, G., Murdock, K., Hwang, P., Jun, H.W., 2014. Adult stem cells and tissue engineering strategies for salivary gland regeneration: a review. Biomater. Res. 18, 9.

Zhang, B.X., Zhang, Z.L., Lin, A.L., Wang, H., Pilia, M., Ong, J.L., et al., 2015. Silk fibroin scaffolds promote formation of the ex vivo niche for salivary gland epithelial cell growth, matrix formation, and retention of differentiated function. Tissue Eng. A. 21, 1611−1620.

Zheng, C., Baum, B.J., Liu, X., Goldsmith, C.M., Perez, P., Jang, S.I., et al., 2015. Persistence of hAQP1 expression in human salivary gland cells following AdhAQP1 transduction is associated with a lack of methylation of hCMV promoter. Gene Ther. 22, 758−766.

This page intentionally left blank

Facial muscle tissue engineering

21

Michael Del Monico, Mohammadreza Tahriri, Zach Nicholson,
Kimia Khoshroo and Lobat Tayebi
Marquette University School of Dentistry, Milwaukee, WI, United States

21.1 Introduction

Tissue engineering (TE) itself is the long-awaited answer to the problems faced by many patients who experience dysfunction or defects of their facial muscles, resulting in significant functional, physical, and social anxiety (Terzis and Noah, 1997; Kumar and Hassan, 2002; Koning et al., 2009). The use of stem and progenitor cells in combination with different types of scaffolds and biomaterials designed to be compatible with the natural oral environment offer new therapeutic opportunities for patients suffering from the loss of facial and masticatory muscles due to trauma, cancer, and other pathological conditions (Holzapfel et al., 2016; Nie et al., 2014; Razavi et al., 2014a). This relies on the great ability of the facial muscle to be customized for the needs of each specific patient. TE does, however, have its challenges, such as the high specificity of the myogenic progenitor cells, scaffold-related limitations, and the bioactive factors required to ensure the proper differentiation and growth of the myogenic progenitor cells on the scaffold. The tissue should also be capable of becoming fully innervated and vascularized, while maintaining its biocompatibility (Vandenburgh, 2002; Bach et al., 2003). Ultimately, the facial muscle itself must be fully functional as a whole, requiring the correct parallel alignment of the muscle fibers, consisting of the correct myosin/actin filaments and acetylcholine receptors so that direct forces can be created (Terzis and Noah, 1997).

21.2 Anatomy and structure of facial muscles

Facial muscles include a group of 20 flat skeletal muscles, which reside under flat facial skin. They originate from the skull and fibrous structures and use an elastic tendon in order to radiate to the skin. These muscles are unique in the way that they are positioned surrounding facial openings, such as the ears, eyes, nose, and mouth, while also having to stretch across the entirety of the skull and neck. They are also categorized into muscles of the mouth, nose, eyelid, cranium and neck, and the external ear. In addition to the facial muscles, other related skeletal muscles include the muscles of mastication and the tongue which are responsible for the vital functions including mastication, swallowing, and speech.

Each muscle includes of a bundle of muscle fibers each consisting of long multinucleated cells which are composed of fused single mononucleated cells called

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00021-9
© 2017 Elsevier Ltd. All rights reserved.

myoblasts. Skeletal muscles contain myofibrils that are cylindrical bundles of thin myofilaments called actin and thick myofilaments called myosin which are all organized into contraction units named sarcomeres (Norton, 2016).

21.3 Tissue engineering approaches

TE and regenerative medicine attempt to recreate or repair the damaged or lost tissues through using cells, biomaterial scaffolds, growth factors, and bioactive agents combined and cultured in a suitable environment that promotes cell growth, signaling, and differentiation (Tahriri and Moztarzadeh, 2014; Yazdimamaghani et al., 2014c,b; Khoshroo et al., 2017; Rasoulianboroujeni et al., 2017; Nojehdehian et al., 2010). The biomaterial scaffold itself and its coating are the tools used to guide and control tissue regeneration by providing a three-dimensional matrix that can be made of natural or synthetic materials resembling the properties of the intended tissue and capable of supporting cells to grow and produce extracellular matrix (ECM) (Heidari et al., 2015; Yazdimamaghani et al., 2013, 2014a, 2015; Razavi et al., 2013, 2015, 2014b; Mozafari et al., 2010). Bioactive agents such as growth factors and proteins should be able to be retained by the scaffolds and their effects be sustained, allowing the cells to adhere to the scaffold, proliferate, and secrete their own ECM in the same manner that the original tissue would have prior to its loss (Khojasteh et al., 2016). Delivery, regulation of stem cell fate, and cell guidance are all direct results of the fact that scaffolds can be created from a wide range of established biomaterials in combination with the most fitting structural, mechanical, physicochemical, and biological properties in order to make tissue engineered facial constructs (Jazayeri et al., 2017).

21.4 Cells

The specific cell type used for muscle TE is the stem/progenitor cells, which must be cultured, expanded, and induced to differentiate into the intended muscle cells (Usas and Huard, 2007). Myogenic potency is crucial for the proper regeneration of muscle tissue through TE using mesenchymal stem cells (MSCs) derived from several different sources. In conjunction with this, bone marrow-derived MSCs are specifically able to differentiate into myoblasts with particularly high efficiency (Dezawa et al., 2005). The intention is for the myoblasts to fuse with one another to form myotubes, which then differentiate into specific muscle fibers capable of performing their specific, intended function. MSCs themselves are also capable of self-renewal, which in combination with their high differentiation rate makes them ideal for muscle tissue regeneration (Dezawa et al., 2005; Caplan, 2005). Sequestered between the sarcolemma and mature muscle fibers are satellite cells, which normally do not proliferate unless they are responding to specific local changes such as muscle damage. These myogenic satellite cells move through the

basal lamina sheets to the areas of injury, where they differentiate into myoblasts in order to fuse with the preexisting, damaged fibers or with each other in order to differentiate into muscle fibers. Failure to fuse on the part of the satellite cells results in them differentiating back into quiescent satellite cells (Bach et al., 2004). This proneness of satellite cells for myogenic differentiation suggests their potential use for the TE of facial muscles as appropriate muscle-derived progenitor cells (Koning et al., 2009; Shadrach and Wagers, 2011).

21.5 Scaffolds

The primary function of the scaffolds is to serve as a biocompatible matrix for cell growth and differentiation resembling the natural muscle fibers architecture to support function and development (Kamelger et al., 2004). The types of scaffolds vary depending on the biological characteristics, as well as their physiochemical features and compositions. Although nonbiodegradable scaffolds, such as phosphate-based glass fiber (Shah et al., 2005) have been used, biodegradable scaffolds can be preferred due to the fact that they can remodel the natural muscular ECM upon degradation, allowing for more precise function of the muscle tissue. Different types of synthetic and natural scaffolds have been developed for skeletal muscle TE that have been thoroughly reviewed recently by Qazi et al. (2015) and summarized in Table 21.1.

Synthetic biodegradable three-dimensional scaffolds such as polylactic-co-glycolic acid fiber mesh sheets (Saxena et al., 1999) provide appropriate rigidity and connection, promoting the correct alignment of nanoscale and microscale topographic features of the polymer scaffold itself, allowing myotube assembly along the nanofibers and microgrooves to mimic the way in which the myotubes are assembled in the muscle fibers. Proper myotube striation can also be achieved, as well as the restriction of cell spreading, promoting the assembly of longer myotubes as opposed to microscale features (Huang et al., 2006). Natural biodegradable three-dimensional scaffolds such as collagen, consist of aligned topographic features that facilitate alignment of myoblasts and cytoskeletal proteins (Yan et al., 2007; Kroehne et al., 2008). Although this is beneficial, these natural biodegradable three-dimensional scaffolds are extremely fragile and are not easily handled, particularly when acellular muscle is used (Borschel et al., 2004).

21.6 Laboratory and clinical challenges

Although culturing cells on a biomaterial substrate until it has evolved into a functional tissue that can immediately be transplanted into a patient seems beneficial for successful TE, in vitro process consists of a few major issues, such as ensuring that sufficient vascularization to maintain cellular viability in large constructs,

Table 21.1 A summary of properties that are desirable in a biomaterial designed for skeletal muscle tissue engineering and their resulting benefits (Qazi et al., 2015)

Desirable biomaterial property	Materials utilized	Benefits	Key parameters for optimization	Reference
Porosity	• Alginate • Collagen	• High surface area resulting from porosity allows seeded cells to proliferate. • Macroporous structure promotes outward migration of transplanted cells and inward migration of host cells. • Migrated cells then interact with cells from native tissue and participate in the regeneration process.	• Pore size and interconnectivity • Cell adhesive cues (e.g., RGD peptide motifs when using alginate)	Borselli et al. (2011), Hill et al. (2006a), Hill et al. (2006b), Ma et al. (2011)
2D topographical cues (patterned substrates, electrospun aligned fiber patches)	• Poly-L-lactic acid (PLLA) • Poly lactic-co-glycolic acid (PLGA) • Polycaprolactone (PCL) • Collagen • Poly(lactide-co-caprolactone) (PLCL) • Polyaniline (PANI)	• Potential to combine different materials to form composites of desirable mechanical and physicochemical properties • Electrically conductive polymers can be incorporated for additional stimuli to seeded cells • Myoblast differentiation can be enhanced by synergic effects of electroactivity and fiber alignment	For patterned substrates: • Groove width • Groove depth • Ridge width For electrospun fibers: • Polymer composition • Fiber alignment	Huang et al. (2006), Dugan et al. (2013), Beier et al. (2009), Choi et al. (2008), Aviss et al. (2010), Jun et al. (2009), Chen et al. (2013), Ku et al. (2012), Page et al. (2011), Neumann et al. (2003), Huang et al. (2010), Hosseini et al. (2012), Monge et al. (2012), Zhao et al. (2009), Yang et al. (2014)

	• Fibrin
	• Gelatin
		• Cell sheets composed of differentiated myotubes can be detached from patterned substrates for direct transplantation
		• Topographical cues promote:
		• Cytoskeletal alignment
		  ◦ Myotube assembly and fusion
		  ◦ Myotube striation		

3D topographical cues (grooved scaffolds, aligned pores)	• Collagen • Chitosan	• Contact guidance for alignment and fusion of myoblasts • Mechanical properties can be varied without affecting porous structure • Structural parameters (pore size) can be varied without influencing pore orientation • Large diameter myotubes (similar to native muscle fiber) can be grown on such scaffolds in vitro	• Continuous channels to facilitate myoblast fusion and formation of long myotubes • Ensuring no change in structure (due to degradation and/or swelling) takes place during cell culture	Jana et al. (2013), Kroehne et al. (2008)
Injectable	Hydrogels including: • Alginate • Collagen • Hyaluronic acid • Polyethylene glycol (PEG)	• Minimally invasive application of gel • Encapsulated cells can migrate and interact with cells from injured tissue as gel degrades over time	• Hydrogel concentration optimized to maintain high cell viability • Final volume of gel should ensure sufficient nutrient diffusion to encapsulated cells	Natsu et al. (2004), Wang et al. (2014), Rossi et al. (2011), Liu et al. (2013), Beier et al. (2006), Liu et al. (2012), Kim et al. (2010)

(*Continued*)

Table 21.1 **(Continued)**

Desirable biomaterial property	Materials utilized	Benefits	Key parameters for optimization	Reference
	• Fibrin	• Tunable mechanical properties permit optimization of cellular microenvironment • Encapsulated cells can be protected from direct effects of immune cells in the injury environment • Encapsulated cell-based drug delivery (e.g., paracrine signaling from MSCs)		
Native structural and biochemical cues	Decellularized tissues/ECM including: • Tibialis Anterior • Extensor digitorum longus • Abdominal muscles • Porcine bladder • Porcine intestinal submucosa	• Pro-myogenic environment • Intact native ECM structure, basement membrane structural proteins, cytokines and growth factors • High clinical potential due to utilization of natural tissues (autografts/xenografts) • Maintenance of structural integrity	• Effective removal of all cells and debris • Minimization of undesirable effects of chemical agents and detergents used during the decellularization process	Perniconi et al. (2011), Wolf et al. (2012), Coppi et al. (2006), Borschel et al. (2004), Conconi et al. (2005), Machingal et al. (2011), Corona et al. (2013), Merritt et al. (2010), Dequach et al. (2012), Mase et al. (2010), Sicari et al. (2014), Crapo et al. (2011))

		• Generation of full thickness in vitro tissue engineered skeletal muscle tissues • Potential treatment option for volumetric muscle loss • Can be formed into an injectable hydrogel without loss of bioactivity		
Presentation of growth factors	• Alginate [stromal cell-derived factor-1 • (SDF-1)] • Alginate (VEGF, IGF-1) • Alginate (HGF, FGF)	• Growth factors can be released locally over a period of time and therefore have a long lasting, localized effect at injury site • Growth factors can either stimulate transplanted cells, or directly influence injured tissue	• Growth factor release kinetics	Hill et al. (2006a), Kuraitis et al. (2011), Borselli et al. (2010))

fabricating large muscle constructs while maintaining high myofiber packing, density, alignment (Kannan et al., 2005), and creating engineered tissues that are able to generate physiologically relevant contraction forces (Griffith and Naughton, 2002). Thus in vivo process might be necessary, in which the TE relies on transplanting cells either unaided or in combination with a biomaterial scaffold into the body. The intention is to create a local niche at the location of the injury so that the cells can influence muscle regeneration through either their own regenerative mechanisms to promote new tissue formation, or by stimulating the host tissue (Mccullen et al., 2011). The in vivo implantation also limits the degree of manipulation of cells during ex vivo culture so that they can retain their functional properties. This approach, however, also has its downfalls in that it leaves the transplanted cells vulnerable to immune response, especially since these immune cells are abundantly present within the environment of the site of injury which can reduce the tissue viability drastically (Reinke et al., 2013; Liu et al., 2011). Therefore identifying the most appropriate cell type for successful muscle TE, is a crucial step towards the clinical application of the engineered muscle reconstructs (Fishman et al., 2013; Shadrach and Wagers, 2011).

21.7 Conclusion/future trends

Progress has been made to date with regards to TE of facial muscles for potential in vitro and in vivo applications. A large number of different biomaterials and processing techniques have been developed to allow for the selection of the most optimal strategy throughout all levels of facial muscle TE. Scientists are currently working with clinicians to bridge the gap between the laboratory and the clinical approaches in this field. This chapter has summarized the relevant biomaterials, cells, the processing techniques, and challenges of facial muscle TE.

References

Aviss, K., Gough, J., Downes, S., 2010. Aligned electrospun polymer fibres for skeletal muscle regeneration. Eur. Cell. Mater. 19, 193–204.

Bach, A., Beier, J., Stern-Staeter, J., Horch, R., 2004. Skeletal muscle tissue engineering. J. Cell. Mol. Med. 8, 413–422.

Bach, A., Stern-Straeter, J., Beier, J., Bannasch, H., Stark, G., 2003. Engineering of muscle tissue. Clin. Plastic Surg. 30, 589–599.

Beier, J.P., Klumpp, D., Rudisile, M., Dersch, R., Wendorff, J.H., Bleiziffer, O., et al., 2009. Collagen matrices from sponge to nano: new perspectives for tissue engineering of skeletal muscle. BMC Biotechnol. 9, 34.

Beier, J.P., Stern-Straeter, J., Foerster, V.T., Kneser, U., Stark, G.B., Bach, A.D., 2006. Tissue engineering of injectable muscle: three-dimensional myoblast-fibrin injection in the syngeneic rat animal model. Plastic Reconstruct. Surg. 118, 1113–1121.

Borschel, G.H., Dennis, R.G., Kuzon, J.R., William, M., 2004. Contractile skeletal muscle tissue-engineered on an acellular scaffold. Plastic Reconstruct. Surg. 113, 595−602.

Borselli, C., Cezar, C.A., Shvartsman, D., Vandenburgh, H.H., Mooney, D.J., 2011. The role of multifunctional delivery scaffold in the ability of cultured myoblasts to promote muscle regeneration. Biomaterials. 32, 8905−8914.

Borselli, C., Storrie, H., Benesch-Lee, F., Shvartsman, D., Cezar, C., Lichtman, J.W., et al., 2010. Functional muscle regeneration with combined delivery of angiogenesis and myogenesis factors. Proc. Natl. Acad. Sci. 107, 3287−3292.

Caplan, A.I., 2005. Review: mesenchymal stem cells: cell-based reconstructive therapy in orthopedics. Tissue Eng. 11, 1198−1211.

Chen, M.-C., Sun, Y.-C., Chen, Y.-H., 2013. Electrically conductive nanofibers with highly oriented structures and their potential application in skeletal muscle tissue engineering. Acta Biomater. 9, 5562−5572.

Choi, J.S., Lee, S.J., Christ, G.J., Atala, A., Yoo, J.J., 2008. The influence of electrospun aligned poly (epsilon-caprolactone)/collagen nanofiber meshes on the formation of self-aligned skeletal muscle myotubes. Biomaterials. 29, 2899−2906.

Conconi, M.T., De Coppi, P., Bellini, S., Zara, G., Sabatti, M., Marzaro, M., et al., 2005. Homologous muscle acellular matrix seeded with autologous myoblasts as a tissue-engineering approach to abdominal wall-defect repair. Biomaterials. 26, 2567−2574.

Coppi, P.D., Bellini, S., Conconi, M.T., Sabatti, M., Simonato, E., Gamba, P.G., et al., 2006. Myoblast-acellular skeletal muscle matrix constructs guarantee a long-term repair of experimental full-thickness abdominal wall defects. Tissue Eng. 12, 1929−1936.

Corona, B.T., Ward, C.L., Baker, H.B., Walters, T.J., Christ, G.J., 2013. Implantation of in vitro tissue engineered muscle repair constructs and bladder acellular matrices partially restore in vivo skeletal muscle function in a rat model of volumetric muscle loss injury. Tissue Eng. A. 20, 705−715.

Crapo, P.M., Gilbert, T.W., Badylak, S.F., 2011. An overview of tissue and whole organ decellularization processes. Biomaterials. 32, 3233−3243.

Dequach, J.A., Lin, J.E., Cam, C., HU, D., Salvatore, M.A., Sheikh, F., et al., 2012. Injectable skeletal muscle matrix hydrogel promotes neovascularization and muscle cell infiltration in a hindlimb ischemia model. Eur. Cells Mater. 23, 400.

Dezawa, M., Ishikawa, H., Itokazu, Y., Yoshihara, T., Hoshino, M., Takeda, S.-I., et al., 2005. Bone marrow stromal cells generate muscle cells and repair muscle degeneration. Science. 309, 314−317.

Dugan, J.M., Collins, R.F., Gough, J.E., Eichhorn, S.J., 2013. Oriented surfaces of adsorbed cellulose nanowhiskers promote skeletal muscle myogenesis. Acta Biomater. 9, 4707−4715.

Fishman, J.M., Tyraskis, A., Maghsoudlou, P., Urbani, L., Totonelli, G., Birchall, M.A., et al., 2013. Skeletal muscle tissue engineering: which cell to use? Tissue Eng. B: Rev. 19, 503−515.

Griffith, L.G., Naughton, G., 2002. Tissue engineering--current challenges and expanding opportunities. Science. 295, 1009−1014.

Heidari, F., Bahrololoom, M.E., Vashaee, D., Tayebi, L., 2015. In situ preparation of iron oxide nanoparticles in natural hydroxyapatite/chitosan matrix for bone tissue engineering application. Ceram. Int. 41, 3094−3100.

Hill, E., Boontheekul, T., Mooney, D.J., 2006a. Designing scaffolds to enhance transplanted myoblast survival and migration. Tissue Eng. 12, 1295−1304.

Hill, E., Boontheekul, T., Mooney, D.J., 2006b. Regulating activation of transplanted cells controls tissue regeneration. Proc. Natl. Acad. Sci. U.S.A. 103, 2494−2499.

Holzapfel, B.M., Wagner, F., Martine, L.C., Reppenhagen, S., Rudert, M., Schuetz, M., et al., 2016. Tissue engineering and regenerative medicine in musculoskeletal oncology. Cancer Meta. Rev. 35, 475−487.

Hosseini, V., Ahadian, S., Ostrovidov, S., Camci-Unal, G., Chen, S., Kaji, H., et al., 2012. Engineered contractile skeletal muscle tissue on a microgrooved methacrylated gelatin substrate. Tissue Eng. A. 18, 2453−2465.

Huang, N.F., Lee, R.J., Li, S., 2010. Engineering of aligned skeletal muscle by micropatterning. Am. J. Transl. Res. 2, 43−55.

Huang, N.F., Patel, S., Thakar, R.G., Wu, J., Hsiao, B.S., Chu, B., et al., 2006. Myotube assembly on nanofibrous and micropatterned polymers. Nano Lett. 6, 537−542.

Jana, S., Cooper, A., Zhang, M., 2013. Chitosan scaffolds with unidirectional microtubular pores for large skeletal myotube generation. Adv. Healthc. Mater. 2, 557−561.

Jazayeri, H.E., Tahriri, M., Razavi, M., Khoshroo, K., Fahimipour, F., Dashtimoghadam, E., et al., 2017. A current overview of materials and strategies for potential use in maxillofacial tissue regeneration. Mater. Sci. Eng. C. 70, 913−929.

Jun, I., Jeong, S., Shin, H., 2009. The stimulation of myoblast differentiation by electrically conductive sub-micron fibers. Biomaterials. 30, 2038−2047.

Kamelger, F., Marksteiner, R., Margreiter, E., Klima, G., Wechselberger, G., Hering, S., et al., 2004. A comparative study of three different biomaterials in the engineering of skeletal muscle using a rat animal model. Biomaterials. 25, 1649−1655.

Kannan, R.Y., Salacinski, H.J., Sales, K., Butler, P., Seifalian, A.M., 2005. The roles of tissue engineering and vascularisation in the development of micro-vascular networks: a review. Biomaterials. 26, 1857−1875.

Khojasteh, A., Fahimipour, F., Eslaminejad, M.B., Jafarian, M., Jahangir, S., Bastami, F., et al., 2016. Development of PLGA-coated β-TCP scaffolds containing VEGF for bone tissue engineering. Mater. Sci. Eng. C. 69, 780−788.

Khoshroo, K., Kashi, T.S.J., Moztarzadeh, F., Tahriri, M., Jazayeri, H.E., Tayebi, L., 2017. Development of 3D PCL microsphere/TiO_2 nanotube composite scaffolds for bone tissue engineering. Mater. Sci. Eng. C. 70, 586−598.

Kim, M.H., Hong, H.N., Hong, J.P., Park, C.J., Kwon, S.W., Kim, S.H., et al., 2010. The effect of VEGF on the myogenic differentiation of adipose tissue derived stem cells within thermosensitive hydrogel matrices. Biomaterials. 31, 1213−1218.

Koning, M., Harmsen, M.C., Van Luyn, M.J., Werker, P.M., 2009. Current opportunities and challenges in skeletal muscle tissue engineering. J. Tissue Eng. Regener. Med. 3, 407.

Kroehne, V., Heschel, I., Schügner, F., Lasrich, D., Bartsch, J., Jockusch, H., 2008. Use of a novel collagen matrix with oriented pore structure for muscle cell differentiation in cell culture and in grafts. J. Cell. Mol. Med. 12, 1640−1648.

Ku, S.H., Lee, S.H., Park, C.B., 2012. Synergic effects of nanofiber alignment and electroactivity on myoblast differentiation. Biomaterials. 33, 6098−6104.

Kumar, P., Hassan, K.M., 2002. Cross-face nerve graft with free-muscle transfer for reanimation of the paralyzed face: a comparative study of the single-stage and two-stage procedures. Plastic Reconstruct. Surg. 109, 451−462, discussion 463-4.

Kuraitis, D., Zhang, P., Zhang, Y., Padavan, D., Mcewan, K., Sofrenovic, T., et al., 2011. A stromal cell-derived factor-1 releasing matrix enhances the progenitor cell response and blood vessel growth in ischaemic skeletal muscle. Eur. Cell Mater. 22, e23.

Liu, J., Xu, H.H., Zhou, H., Weir, M.D., Chen, Q., Trotman, C.A., 2013. Human umbilical cord stem cell encapsulation in novel macroporous and injectable fibrin for muscle tissue engineering. Acta Biomater. 9, 4688−4697.

Liu, J., Zhou, H., Weir, M.D., Xu, H.H., Chen, Q., Trotman, C.A., 2012. Fast-degradable microbeads encapsulating human umbilical cord stem cells in alginate for muscle tissue engineering. Tissue Eng. A. 18, 2303−2314.

Liu, Y., Wang, L., Kikuiri, T., Akiyama, K., Chen, C., Xu, X., et al., 2011. Mesenchymal stem cell-based tissue regeneration is governed by recipient T lymphocytes via IFN-[gamma] and TNF-[alpha]. Nat. Med. 17, 1594−1601.

Ma, J., Holden, K., Zhu, J., Pan, H., Li, Y., 2011. The application of three-dimensional collagen-scaffolds seeded with myoblasts to repair skeletal muscle defects. BioMed Res. Int. 2011.

Machingal, M.A., Corona, B.T., Walters, T.J., Kesireddy, V., Koval, C.N., Dannahower, A., et al., 2011. A tissue-engineered muscle repair construct for functional restoration of an irrecoverable muscle injury in a murine model. Tissue Eng. A. 17, 2291−2303.

Mase, V.J., Hsu, J.R., Wolf, S.E., Wenke, J.C., Baer, D.G., Owens, J., et al., 2010. Clinical application of an acellular biologic scaffold for surgical repair of a large, traumatic quadriceps femoris muscle defect. Orthopedics. 33 (7), 511.

Mccullen, S.D., Chow, A.G., Stevens, M.M., 2011. In vivo tissue engineering of musculo-skeletal tissues. Curr. Opin. Biotechnol. 22, 715−720.

Merritt, E.K., Cannon, M.V., Hammers, D.W., Le, L.N., Gokhale, R., Sarathy, A., et al., 2010. Repair of traumatic skeletal muscle injury with bone-marrow-derived mesenchymal stem cells seeded on extracellular matrix. Tissue Eng. A. 16, 2871−2881.

Monge, C., Ren, K., Berton, K., Guillot, R., Peyrade, D., Picart, C., 2012. Engineering muscle tissues on microstructured polyelectrolyte multilayer films. Tissue Eng. A. 18, 1664−1676.

Mozafari, M., Moztarzadeh, F., Rabiee, M., Azami, M., Nezafati, N., Moztarzadeh, Z., et al., 2010. Development of 3D bioactive nanocomposite scaffolds made from gelatin and nano bioactive glass for biomedical applications. Adv. Compos. Lett. 19, 91−96.

Nojehdehian, H., Moztarzadeh, F., Baharvand, H., Zare Mehrjerdi, N., Nazarian, H., Tahriri, M., 2010. Effect of poly-L-lysine coating on retinoic acid-loaded PLGA microspheres in the differentiation of carcinoma stem cells into neural cells. Int. J. Artif. Organs. 33, 721−730.

Natsu, K., Ochi, M., Mochizuki, Y., Hachisuka, H., Yanada, S., Yasunaga, Y., 2004. Allogeneic bone marrow-derived mesenchymal stromal cells promote the regeneration of injured skeletal muscle without differentiation into myofibers. Tissue Eng. 10, 1093−1112.

Neumann, T., Hauschka, S.D., Sanders, J.E., 2003. Tissue engineering of skeletal muscle using polymer fiber arrays. Tissue Eng. 9, 995−1003.

Nie, X., Xing, Y., Deng, M., Gang, L., Liu, R., Zhang, Y., et al., 2014. Ecto-mesenchymal stem cells from facial process: potential for muscle regeneration. Cell Biochem. Biophys. 70, 615−622.

Norton, N.S., 2016. Netter's head and neck anatomy for dentistry. Elsevier Health Sciences, Amsterdam.

Page, R.L., Malcuit, C., Vilner, L., Vojtic, I., Shaw, S., Hedblom, E., et al., 2011. Restoration of skeletal muscle defects with adult human cells delivered on fibrin micro-threads. Tissue Eng. A. 17, 2629−2640.

Perniconi, B., Costa, A., Aulino, P., Teodori, L., Adamo, S., Coletti, D., 2011. The pro-myogenic environment provided by whole organ scale acellular scaffolds from skeletal muscle. Biomaterials. 32, 7870−7882.

Qazi, T.H., Mooney, D.J., Pumberger, M., Geissler, S., Duda, G.N., 2015. Biomaterials based strategies for skeletal muscle tissue engineering: existing technologies and future trends. Biomaterials. 53, 502−521.

Rasoulianboroujeni, M., Yazdimamaghani, M., Khoshkenar, P., Pothineni, V.R., Kim, K.M., Murray, T.A., et al., 2017. From solvent-free microspheres to bioactive gradient scaffolds, Nanomed. Nanotechnol. Biol. Med., 13. pp. 1157−1169.

Razavi, M., Fathi, M., Savabi, O., Razavi, S.M., Beni, B.H., Vashaee, D., et al., 2013. Surface modification of magnesium alloy implants by nanostructured bredigite coating. Mater. Lett. 113, 174−178.

Razavi, M., Fathi, M., Savabi, O., Razavi, S.M., Heidari, F., Manshaei, M., et al., 2014a. In vivo study of nanostructured diopside ($CaMgSi_2O_6$) coating on magnesium alloy as biodegradable orthopedic implants. Appl. Surf. Sci. 313, 60−66.

Razavi, M., Fathi, M., Savabi, O., Vashaee, D., Tayebi, L., 2015. In vivo assessments of bioabsorbable AZ91 magnesium implants coated with nanostructured fluoridated hydroxyapatite by MAO/EPD technique for biomedical applications. Mater. Sci. Eng. C. 48, 21−27.

Razavi, M., Fathi, M.H., Savabi, O., Vashaee, D., Tayebi, L., 2014b. Biodegradation, bioactivity and in vivo biocompatibility analysis of plasma electrolytic oxidized (PEO) biodegradable Mg implants. Phys. Sci. Int. J. 4, 708.

Reinke, S., Geissler, S., Taylor, W.R., Schmidt-Bleek, K., Juelke, K., Schwachmeyer, V., et al., 2013. Terminally differentiated CD8 + T cells negatively affect bone regeneration in humans. Sci. Trans. Med. 5, 177ra36-177ra36.

Rossi, C.A., Flaibani, M., Blaauw, B., Pozzobon, M., Figallo, E., Reggiani, C., et al., 2011. In vivo tissue engineering of functional skeletal muscle by freshly isolated satellite cells embedded in a photopolymerizable hydrogel. FASEB J. 25, 2296−2304.

Saxena, A.K., Marler, J., Benvenuto, M., Willital, G.H., Vacanti, J.P., 1999. Skeletal muscle tissue engineering using isolated myoblasts on synthetic biodegradable polymers: preliminary studies. Tissue Eng. 5, 525−531.

Shadrach, J.L., Wagers, A.J., 2011. Stem cells for skeletal muscle repair. Philos. Trans. R. Soc. Lond. B Biol. Sci. 366, 2297−2306.

Shah, R., Sinanan, A., Knowles, J., Hunt, N., Lewis, M., 2005. Craniofacial muscle engineering using a 3-dimensional phosphate glass fibre construct. Biomaterials. 26, 1497−1505.

Sicari, B.M., Rubin, J.P., Dearth, C.L., Wolf, M.T., Ambrosio, F., Boninger, M., et al., 2014. An acellular biologic scaffold promotes skeletal muscle formation in mice and humans with volumetric muscle loss. Sci. Trans. Med. 6, 234ra58-234ra58.

Tahriri, M., Moztarzadeh, F., 2014. Preparation, characterization, and in vitro biological evaluation of PLGA/nano-fluorohydroxyapatite (FHA) microsphere-sintered scaffolds for biomedical applications. Appl. Biochem. Biotechnol. 172, 2465−2479.

Terzis, J.K., Noah, M.E., 1997. Analysis of 100 cases of free-muscle transplantation for facial paralysis. Plastic Reconstr. Surg. 99, 1905−1921.

Usas, A., Huard, J., 2007. Muscle-derived stem cells for tissue engineering and regenerative therapy. Biomaterials. 28, 5401−5406.

Vandenburgh, H.H., 2002. Functional assessment and tissue design of skeletal muscle. Annal. N. Y. Acad. Sci. 961, 201−202.

Wang, L., Cao, L., Shansky, J., Wang, Z., Mooney, D., Vandenburgh, H., 2014. Minimally invasive approach to the repair of injured skeletal muscle with a shape-memory scaffold. Mol. Ther. 22, 1441−1449.

Wolf, M.T., Daly, K.A., Reing, J.E., Badylak, S.F., 2012. Biologic scaffold composed of skeletal muscle extracellular matrix. Biomaterials. 33, 2916−2925.

Yan, W., George, S., Fotadar, U., Tyhovych, N., Kamer, A., Yost, M.J., et al., 2007. Tissue engineering of skeletal muscle. Tissue Eng. 13, 2781−2790.

Yang, H.S., Ieronimakis, N., Tsui, J.H., Kim, H.N., Suh, K.-Y., Reyes, M., et al., 2014. Nanopatterned muscle cell patches for enhanced myogenesis and dystrophin expression in a mouse model of muscular dystrophy. Biomaterials. 35, 1478−1486.

Yazdimamaghani, M., Pourvala, T., Motamedi, E., Fathi, B., Vashaee, D., Tayebi, L., 2013. Synthesis and characterization of encapsulated nanosilica particles with an acrylic copolymer by in situ emulsion polymerization using thermoresponsive nonionic surfactant. Materials. 6, 3727−3741.

Yazdimamaghani, M., Razavi, M., Vashaee, D., Tayebi, L., 2014a. Microstructural and mechanical study of PCL coated Mg scaffolds. Surf. Eng. 30, 920−926.

Yazdimamaghani, M., Razavi, M., Vashaee, D., Pothineni, V.R., Rajadas, J., Tayebi, L., 2015. Significant degradability enhancement in multilayer coating of polycaprolactone-bioactive glass/gelatin-bioactive glass on magnesium scaffold for tissue engineering applications. Appl. Surf. Sci. 338, 137−145.

Yazdimamaghani, M., Vashaee, D., Assefa, S., Shabrangharehdasht, M., Rad, A.T., Eastman, M.A., et al., 2014b. Green synthesis of a new gelatin-based antimicrobial scaffold for tissue engineering. Mater. Sci. Eng. C. 39, 235−244.

Yazdimamaghani, M., Vashaee, D., Assefa, S., Walker, K., Madihally, S., Köhler, G., et al., 2014c. Hybrid macroporous gelatin/bioactive-glass/nanosilver scaffolds with controlled degradation behavior and antimicrobial activity for bone tissue engineering. J. Biomed. Nanotechnol. 10, 911−931.

Zhao, Y., Zeng, H., Nam, J., Agarwal, S., 2009. Fabrication of skeletal muscle constructs by topographic activation of cell alignment. Biotechnol. Bioeng. 102, 624−631.

Vascularization

22

Mostafa Yazdimamaghani[1], *Jose Gonzalez*[2] *and Lobat Tayebi*[2]
[1]University of Utah, Salt Lake City, UT, United States,
[2]Marquette University School of Dentistry, Milwaukee, WI, United States

22.1 Introduction

Recently, there is a widespread effort in the research community to address clinical orthopedic issues. This would be more evident considering that bone is the second most prevailing transplanted tissue. Comparing small-to-large bone defects, there is less concern in small bone defects due to spontaneous regenerative and remodeling capabilities of bone tissue toward repair. However, large bone defects created by different causes such as tumors, trauma, car accidents, and osteoporotic fracture require major clinical intervention. Allografts and autografts are the foremost approaches in clinical application. Interestingly, both of these strategies have downsides such as availability, morbidity, risk of immuno-rejection, infection, and diseases transmission (Yazdimamaghani et al., 2014b, 2017; Kurien et al., 2013) ultimately pushing for another alternative. In this regard, tissue engineering and synthetic graft substitutes and implants have emerged to address the great demand for orthopedic implants and bone regeneration. A successful synthetic bone graft substitute nominee is required to have number of optimized properties; among others, the ability to induce adequate amount of vascularization is one of the foremost (Pina et al., 2015; Yazdimamaghani et al., 2014a, 2015a,b, 2016; Denry and Kuhn, 2016; Bose et al., 2017; Tarafder and Bose, 2014; Rasoulianboroujeni et al., 2016). In fact, the main pitfall of developing regenerative strategies is compromised cell viability due to both lack of delivery of oxygen and nutritious materials and clearance of metabolized byproducts (Wang et al., 2015; Mercado-pagán et al., 2015; Horch et al., 2014).

Neovascularization can come about by two different processes—vasculogenesis and angiogenesis. Vasculogenesis is the course of new vascular development in the absence of preexisting blood vessels. Angiogenesis is formation of new blood vessels from preexisting vessels. Although angiogenesis predominantly occurs in tumor growth, vasculogenesis primarily takes place at slow rates (<1 mm/d) in menstrual cycle or wound healing (Auger et al., 2013; Nomi et al., 2002). Most often, angiogenesis spontaneously happens upon scaffold implantation in response to inflammation and the wound-healing process (Probst and Spiegel, 1997). However, these induced capillary formations are impermanent and will revert within a few weeks (Santos and Reis, 2010). On the other hand, vasculogenesis's slow rate infiltration creates neovascularization complication in clinically relevant tissue size. To address these shortages, emergence of new approaches to accelerate the onset of tissue-engineered neovascularization is indispensable (Nomi et al., 2002; Laschke et al., 2006).

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00022-0
© 2017 Elsevier Ltd. All rights reserved.

Commonly, it is recommended to style scaffolds with interconnected pores over 300 μm permitting vascularization, cell attachment, immigration, mechanical fixation, and tissue ingrowth (Leukers et al., 2005; Karageorgiou and Kaplan, 2005). Viability of cells delivered by implanted scaffolds or the ingrowth of host tissue by immigration of cells toward middle of scaffold is limited by a lack of nutrients in the absence of a vascular supply. Lacking the necessary vascular supply, the nutrients and waste transport happen only over short distances by diffusion which could not support large scaffolds implanted for bone defect treatments and would lead to central necrosis. Bone is also a metabolically active place, being repaired and remodeled constantly by osteoblasts, ostoclasts, and star-shaped osteocytes. Osteoblasts create newly mineralized bone matrix, ostoclasts disassemble the matrix, and star-shaped osteocytes maintain the matrix (Marks and Odgren, 2002). Normally, osteocytes can be found 100 μm from a capillary. Implanted scaffolds should mimic the complexity of the host tissue and provide proper three-dimensional (3D) setting for cell attachment, cell proliferation, and tissue formation. This could happen in the presence of support toward strategies that would augment vascular network formation to prevent cell nutrient deprivation (Leukers et al., 2005).

22.2 Craniofacial and dental tissue-engineering approaches

Four main approaches, recombinant protein therapy, cell-based therapy, cell-seeded scaffold, and gene therapy, are interrelated strategies for regeneration of craniofacial and dental tissue engineering. In recombinant protein therapy, growth factors are loaded on the appropriately designed scaffold to stimulate tissue cell growth (Lieberman et al., 2002). Another approach, cell-based therapy, entails injecting particular cells such as stem cells into defected sites. Interestingly, the efficacy of this method is limited by host immune system response, low engraftment, and poor localization of injected cells (Abou Neel et al., 2014). Alternatively, cell-based therapy could be combined with other strategies. For example, cell-based therapy with scaffold tissue engineering might be combined in which appropriate cell type, normally human oral mucosa-/gingiva-derived mesenchymal stem cell (MSC) extracted from a biopsy obtained from the patient, are seeded on a synthetic scaffold (Zhang et al., 2012; Abou Neel et al., 2014). Lastly, a relatively new emerging approach, gene therapy uses genetically modified cells that secrete certain protein products and injects them into damaged sites to induce tissue regeneration (Hannallah et al., 2002). It is noteworthy to mention that potentially all four approaches of craniofacial and dental tissue engineering may end in defeat without considering the need for vascularization to prevent hypoxia and necrosis in the center of an implanted scaffold. Vascularization is important for the success and regeneration of tissue posttrauma or surgery. The rationale behind promoting vascularization in craniofacial and dental tissue engineering is to encourage success of newly regenerated bone. The promotion of vascularization may facilitate the healing process.

Significantly, oxygen affects certain vascularization factors function. Human MSCs (hMSCs) proliferate more in hypoxic conditions. However, specific optimum oxygen levels facilitate the metabolic activity of hMSC, angiogenic factors (AGFs), vascular endothelial growth factor (VEGF), and angiopoietin (ANG-1). VEGF is a strong AGF essential for vascular endothelial cells (ECs). The ANG-1 gene codes for a glycoprotein, one which contributes to vascular development and angiogenesis. The role of vascularization in biotissue engineering is to provide healing factors and growth factors to the newly implemented biograft. Ideally upon implantation, proteins and inflammatory factors disperse and distribute from vasculature adjacent to the bioengineered dental tissue into the new scaffold. Evidence supporting the push toward vascularization states that in biotissue-engineered tooth roots, dental pulp and dentine regeneration is essential for the viability of the roots. The dental pulp contains the vasculature of a tooth. Therefore, vascularization is important for the viability of dental biotissue engineering. The encouragement of angiogenesis postsurgery may be indispensable for proper tissue healing and integration (Buizer et al., 2017; Duffy et al., 2004; Liu et al., 2017; Na et al., 2013).

22.3 Clinical transplantation of engineered blood vessels

Clinically, prosthetic grafts and vascular grafts have used tissue-engineered blood vessels with diverse results. Prosthetic grafts are commonly made of synthetic polymers. Their properties include poor elasticity, low compliance, and thrombogenicity of synthetic surfaces. These properties limit the use of prosthetic grafts to conditions of high flow and low resistance. Unfortunately, placement of the synthetic grafts often leads to failure, when the graft is less than 6 mm in diameter, in areas of low blood flow due to early formation of thrombosis. In addition, the materials lack growth potential (Mori and Matsuda, 2005). Alternatively, vascular grafts have been altered to form blood vessels with collagen and cultured vascular cells. This formation came about by Weinburg and Bell where smooth muscle cells, fibroblasts, and bovine ECs form collagen gel layers supported by a Dacron mesh. The result is the construction of biocompatible vessel grafts with growth potential (Weinberg and Bell, 1986; Ravi and Chaikof, 2010; Kazemzadeh-narbat et al., 2017).

22.4 Current strategies to increment vascularization

While there are several strategies to increment vascular network formation, attention to particular settings such as scaffolds architecture, appropriate porosity, pore size, and biodegradability would enhance vascularization in engineered bone scaffolds. In addition, there are two concepts not entirely researched. “Is vasculature structure incorporation in the scaffold required before transplantation?” or “Would induction of neovascularization by regenerative cells after transplantation be sufficient?” In the former, connecting prefabricated vasculature to host tissue

vasculature, and in the latter induction of neovascularization simultaneously with tissue growth require further attention (Phelps and García, 2010). Vasculature structure incorporation is also called inosculation, which is anastomoses of preexisting scaffold's vasculature network to host's microvascular network (Laschke et al., 2006; Auger et al., 2013). Inosculation happens relatively fast (2−4 days), whereas neovascularization happens later (weeks) (Tremblay et al., 2005; Young et al., 1996). Thus, inosculation promptly provides vital oxygen and nutrients to cells and further induces capillary remodeling to accomplish tissue homeostasis by infiltration of essential cell types for inflammatory and in next-step antiinflammatory responses (Laschke et al., 2009; Rothenfluh et al., 2004). Prevascularization within scaffolds can be conducted under in vitro and in vivo prevascularization. In this regard, one can use cell sheet engineering or arteriovenous loops (Chen et al., 2008; Laschke et al., 2008; Sekine et al., 2008; Hobo et al., 2008; Khouri et al., 1991; Lokmic et al., 2007).

The major approaches, their principles, advantages, and limitations in addition to most up-to-date research efforts in the pursuit of achieving developed vascular network in bone tissue engineering are being reviewed in this section.

22.4.1 Growth factor and cytokines

Angiogenic growth factors are one of powerful therapeutic agents to induce neovascularization and bone regeneration, thereby forming a close relationship between angiogenesis and osteogenesis (Street et al., 2002; Dai and Rabie, 2007). Endothelial (progenitor) cells will be activated by angiogenic growth factors, triggering them to migrate toward the release site of growth factor, and provoking cell maturation and vessel formation. Among others, VEGF, hepatocyte growth factor, and basic fibroblast growth factor are important growth factors. Moreover, cytokines such as angiopoietin, transforming growth factor beta, and platelet-derived growth factor indirectly stimulate neovascularization. VEGF, for instance, participates in angiogenesis and bone healing simultaneously by activating osteoblasts, chondrocytes, and osteoclasts (Tarkka et al., 2003; Novosel et al., 2011). In addition to growth factor and cytokines research, new sophisticated studies have investigated gene therapy and upstream activators as master switch such as HIF-1a to stimulate upper pathways of induction of entire growth factors (Chen et al., 2007; Sarkar et al., 2009; Shyu et al., 2002).

There are some challenges in utilizing growth factor and cytokines for induction of neovascularization. Angiogenic growth factors lack stability in in vivo conditions. One main approach to overcome instability of growth factors is using localized and sustained delivery to prolong release time in implantation site, decreasing circulation in off-target areas, and upsurging availability (Chen et al., 2007; Santos and Reis, 2010; Zisch et al., 2003). The other approach to circumvent the instability of growth factors is using high doses. However, this approach will increase the cost of therapy. In addition, other disadvantages of growth factors are their tendencies to augment pathological effects such as cancer, proliferative retinopathies, atherosclerosis, and tumor growth (Barralet et al., 2009). Specifically, high dosages of VEGF

may produce malformed and leaky vasculature which is a characteristic of tumor site vasculature. A new method involving angiogenic growth factors embraces the idea of using combinations of growth factors sets. In summary, localized and sustained delivery of multiple growth factors presents promising results in formation of vasculature network in the scaffolds.

22.4.2 Mature and primary cell delivery

ECs incorporation into a scaffold in bone tissue engineering can further improve neovascularization (Lee et al., 2015; Haug et al., 2015; Kinzer et al., 2014; Tsukada et al., 2013; Mao et al., 2014; Zigdon-giladi et al., 2015; Ma et al., 2014). First off, a successful scaffold requires appropriate structural and molecular level characteristics; having proper porosity, roughness, functional groups pertaining to the former, and biocompatibility; and ability for cell attachment, growth, viability, differentiation, and phenotypic expression pertaining to the latter. Enhanced ECs attachment, migration, and endothelialization to increase angiogenesis on scaffolds can be obtained by effective immobilization of proteins on a scaffold surface (Conconi et al., 2010; Santos et al., 2007; Hamada et al., 2003). For instance, laminin, fibronectin (RGD or REDV), or adhesive ligands (CGRGES) can be used for surface modification of scaffolds with peptides (Von der mark et al., 2010; Miller et al., 2010). However, the difficulty of surface modification with peptides is related to their stability over time. Alternative approaches to enhance ECs attachment are chemical treatment, plasma treatment, bioactive molecules incorporation, and ultraviolet (UV) radiation (Santos et al., 2009; Chua et al., 2005). Human umbilical vein endothelial cells, human dermal microvascular ECs, bovine capillary ECs, and bovine aortic ECs are varieties in which ECs have been used. Mature ECs can be secluded from a different site such as the bone marrow, umbilical cord, skin, peripheral blood, and fat tissue. Disadvantages of using mature ECs include low proliferation capacity, availability, and remarkable phenotypic and genotypic heterogeneity which results in different responses from mature ECs based on their site of extraction (Mercado-pagán et al., 2015; Stegen et al., 2015; Roberts et al., 2016; Chi et al., 2003; Sumpio et al., 2002; Garlanda and Dejana, 1997). An alternative source is progenitor cells with the ability to differentiate into mature ECs and capability for angiogenesis, verified by the detection of three cell markers of CD133, CD34, and VEGFR2 (Moschetta et al., 2014; Herrmann et al., 2014; Bobryshev et al., 2015). The driving force behind progenitor cells application is the high population doublings compared to mature ECs, and mimicking embryonic vasculogenesis by MSCs to produce vasculature network (Loffredo and Lee, 2008).

22.4.3 Vascular inductive scaffold

Optimal tissue regeneration can be generally obtained by utilizing interconnected porous scaffolds fabricated from a smart design of biomaterials representing native extracellular matrices (ECM) of tissue. Different natural and synthetic biomaterials in fabricating scaffold have been used. Among those biomaterials, a polymer's mechanical properties, capability to support vascularity, low immunogenicity, and

ease of manufacturing, were extensively investigated. Although natural biomaterials have advantages of biocompatibility, disadvantages such as inadequate mechanical properties and immunogenicity limit their applications. On the other hand, synthetic biomaterials exhibit tailored made structure, good mechanical strength, and controlled degradation rate. However, these types of scaffolds need modification to increase cell attachment. Table 22.1 summarizes biomaterials extensively used for scaffold fabrication in craniofacial and dental applications (Sharma et al., 2014).

The ability to induce proper vascularization of cell-free smart materials takes priority. In this approach, intelligently designed vascular networks within a scaffold recruit vascular ingrowth upon implantation from surrounding host tissue. There are several methods such as electrostatic discharge, lamination of two-dimensional (2D) structures, and 3D printing to incorporate vascular architectures in scaffolds (Gergely et al., 2015). Moreover, a smart 3D scaffold design is achieved by microfabrication techniques to obtain desired flow regimes, oxygen gradients, angiogenesis, and cell alignment (Bueno and Glowacki, 2009; Yu et al., 2009; Chen et al., 2016; Robinson et al., 2016). One of emerging microfabrication techniques for fashioning vascular networks is sacrificial templating. In this regards, the sacrificial element would be shaped as desired architecture of vasculature network and subsequently removed through different approaches such as dissolution, etching, melting, or thermal vaporization. Several materials such as molded gelatin, sugar glass, aqueous Pluronic fugitive ink, and bioprinted agarose template have been used as sacrificial element to fabricate vascularized network within the scaffolds (Golden and Tien, 2007; Miller et al., 2012; Bertassoni et al., 2014; Kolesky et al., 2014; Bellan et al., 2012). In an attempt to building and mimicking a capillary network, the carnival's sweet cotton candy may offer some help. Cotton candy forms a complex network of strands similar to that of a capillary bed. In a study using cotton candy, a polymer resin is poured over the thin sugar strands to harden. The sacrificial cotton candy is dissolved with water and alcohol solutions to leave a network of channels similar in size to that of a capillary network, between 1 and 100 μm wide with a few hundred micrometers between channels. The goal is to create a cast using a biodegradable resin mixed with cells of a particular tissue. The cells will coat the cast's channels, an endothelium, and the resin will biodegrade leaving the intact channel of blood vessel cells. To test blood flow, fluorescent labeling was used to pump blood through the network. The advantage to this procedure is that material cost is low, production is fast and highly scalable. Clinically, the insertion of a capillary network may support the proper healing and oxygen and nutrient supply to an area lacking vasculature (Bellan et al., 2009).

The imprinted networks with the best hydrodynamic properties include having multibranches with less than two nodes in each branch (Wang and Hsu, 2006). Furthermore, these microvascular networks may be seeded by ECs in vitro to induce fully functional vascularized scaffolds. In addition, scaffolds could have growth factor monolithically embedded in biodegradable matrix to have sustainable release in a cell-demanded manner. Moreover, bioactive ligand attachment on the scaffolds such as RGD peptide for enhanced cell adherence would further promote vascular network formation.

Table 22.1 Summary of biomaterials used in fabricating craniofacial and dental scaffolds, their advantages and disadvantages

Biomaterial	Advantages	Disadvantages	References
Collagen	• Available from several allogenic sources • Similar to structural protein of dental tissue ECM • Biocompatible, degradable, and bioactive • Adequate cellular adhesion • High tensile strength	• Low physical strength	Kim et al. (2010), Wu et al. (2007), Wahl et al. (2007), Nakashima (1994), Chan et al. (2005)
Fibrin	• Available from autologous sources • Adequate cellular adhesion • Induce low immune response • Cost-effective • Biocompatible and injectable • Provides appropriate condition for angiogenesis	• Rapid shrinkage in size • Fast degradation • Low mechanical properties	Jockenhoevel et al. (2001), Lee and Kurisawa (2013), Linnes et al. (2007), Clark et al. (1995), Ehrbar et al. (2004)
Alginate	• Adequate cellular adhesion • Biocompatible and nontoxic	• Low-mechanical properties • Uncontrolled degradation rates	Sakai and Kawakami (2007), Dobie et al. (2002)
Hyaluronic acid	• Similar to structural protein of connective tissue ECM • Adequate cellular adhesion • Induce low immune response • Biocompatible and injectable • Nontoxic degradation products • Provides appropriate condition for angiogenesis • Lower inflammatory response compared to collagen	• Low mechanical properties • Fast degradation	Ouasti et al. (2011), Ouasti et al. (2011), Park et al. (2003), Inuyama et al. (2010)
Poly (ethylene glycol) (PEG)	• Tunable mechanical strength by adjusting molecular weight • Biodegradable • Induce low immune response • Cost effective • Biocompatible and nontoxic	• Resists protein adsorption • Low cell adhesion	Burdick and Anseth (2002), Zhu (2010), Papadopoulos et al. (2010), Alcantar et al. (2000)

(*Continued*)

Table 22.1 **(Continued)**

Biomaterial	Advantages	Disadvantages	References
Chitosan	• Biocompatible and biodegradable • Antimicrobial • Osteoinductive • Bioactive through specific interactions with growth factors, adhesion proteins, and receptors • Chemoattractive effects • Minimal foreign body reaction response in vivo • Provides appropriate condition for angiogenesis	• Low mechanical properties	Suh and Matthew (2000), Boynueğri et al. (2009), Tanase et al. (2013), Tahmasbi rad et al. (2014)
Synthetic polyester polymers; polylactic acid (PLA), polylactide-co-glycolide (PLGA), polyglycolic acid (PGA)	• Biocompatible • Biodegradable • Nontoxic • Tunable physiochemical properties by adjusting molecular weight, crystallinity, and polymer ratio • Adequate cellular adhesion • Capability to differentiate stem cells into endothelial like cells and odontoblast cells • Minimal immune response • Controlled release of bioactive agents	• Induce mild inflammatory response	Jeong et al. (2004), Tonomura et al. (2010), Bohl et al. (1998)
Ceramics; bioactive-glasses, bioactive-glass-ceramics, calcium/ phosphate materials	• Biocompatible • Biodegradable • Osteoconductive • Osteoproductive • Low immunogenicity • Promote cell proliferation • Activate osteoblasts • Able to induce hydroxycarbonate apatite layer deposition	• Slow degradation rate • High density • Brittle • Difficult to construct complex architectures • Poor mechanical strength	Yazdimamaghani et al. (2014b), Mozafari et al. (2013), Yazdimamaghani et al. (2015b), Nam et al. (2011), Fielding et al. (2012)

22.4.4 Decellularized matrix

A developing body of research confirmed the pivotal role of decellularized osteoinductive cell-free ECM in the regenerative courses of bone tissue-engineering development both in preclinical and clinical applications (Crapo et al., 2011; Badylak, 2002; Badylak et al., 2009). A 3D microstructure of an organ or tissue contains entirely specific ECM properties; however, an exact copy has not been replicated. Most importantly, decellularized ECM would contain a precise vascular network structure that is crucial for bone reconstruction and regeneration. Goals of eradicating the cellular component while maintaining the 3D microstructure have been met in some tissues and organs such as intervertebral disks, skeletal muscle, blood vessels, heart, liver, etc. (Badylak et al., 2009; Wolf et al., 2012; Chan et al., 2013; Price et al., 2010; Baiguera et al., 2014; Uygun et al., 2010; Funamoto et al., 2010). Decellularized ECM can be obtained from native bone tissue (autograft), from a donor (allograft) or even from animals (xenograft). The advantages of using decellularized mature tissue are having a developed and preserved structure and architecture that could induce an appropriate spatial vascular network structure. Along this line, decellularized ECM is a promising product establishing more standardized scaffolds without immunogenic responses and a reduced risk of disease transmission (Zimmermann and Moghaddam, 2011; Greenwald et al., 2001; Laurencin and Khan, 2012).

22.5 Key challenges and future trend

Since the first use of the term "tissue engineering" in 1985 by Yuan-Cheng Fung, only a small number of engineered tissues such as cartilage, skin, and bladder have successfully transitioned to clinical applications. Still, other more complex organs require much work before commercialization. For the past 30 years, research in bone tissue engineering has focused on osteoconductivity, osteoblasts, development of biomaterials, and establishment of fabrication methods. However, the importance of functional bone vascular network formation has required more attention. Recently, an apparent shift in research of bone tissue engineering has been observed toward an establishment of bone vasculature formation. In light of a large body of emerged evidence, there is now doubt that functional vasculature plays a crucial role in successful clinical outcomes of engineered tissue constructs. Bone is a metabolically dynamic multicomponent tissue, repaired and remodeled constantly by osteoblasts, osteoclasts, and star-shaped osteocytes. Oxygen and nutrient transfer and metabolic waste withdrawal through a vascular network in bone are essential for implant survival and integration. There is a close relation between angiogenesis and osteogenesis, and between osteoblasts and ECs, all which regulate bone formation and vasculature development. As overviewed in this work, there are a number of approaches for tackling the neovascularization concern:

- Growth factor or cytokine signaling for regulating neovascularization
- Mature and primary EC delivery for self-assembling vascular network

- Vascular inductive scaffold application
- Utilizing native organ or tissue decellularized matrix.

While considering vascularization a key design factor and applying mentioned strategies, capillary formation has been achieved. However, instability and regression of formed vascular structures has occurred. This new challenge further requires new momentum in the research community to establish approaches toward stabilization of formed capillaries to develop long-standing vascular network. We believe that the future directions of tissue-engineering research are as follows:

- obtain ability to quickly stabilize vascular structures to develop long-lasting vascular networks to prevent ischemic events in organ or implanted scaffold;
- novel approaches to promote and modulate angiogenesis in an implant in response to different physiological conditions that occur along transplantation and fixation to host tissue;
- long-term assessment of vascular networks functionality in vivo;
- development of technologies that surgeons would practically apply in clinical practice to successfully anastomose implanted scaffold with host circulation;
- enhance control over systematic modulation of spatial characteristics of vascularization to obtain thick enough scaffolds;
- considering bone as a metabolically dynamic multicomponent tissue and facilitating the cross talk of these different components to make vascularization attainable.

References

Abou Neel, E.A., Chrzanowski, W., Salih, V.M., Kim, H.-W., Knowles, J.C., 2014. Tissue engineering in dentistry. J. Dent. 42, 915–928.

Alcantar, N.A., Aydil, E.S., Israelachvili, J.N., 2000. Polyethylene glycol-coated biocompatible surfaces. J. Biomed. Mater. Res. 51, 343–351.

Auger, F.A., Gibot, L., Lacroix, D., 2013. The pivotal role of vascularization in tissue engineering. Ann. Rev. Biomed. Eng. 15, 177–200.

Badylak, S.F., 2002. The extracellular matrix as a scaffold for tissue reconstruction. Seminars in Cell & Developmental Biology. 15, 377–383.

Badylak, S.F., Freytes, D.O., Gilbert, T.W., 2009. Extracellular matrix as a biological scaffold material: structure and function. Acta Biomater. 5, 1–13.

Baiguera, S., Del gaudio, C., Kuevda, E., Gonfiotti, A., Bianco, A., Macchiarini, P., 2014. Dynamic decellularization and cross-linking of rat tracheal matrix. Biomaterials 35, 6344–6350.

Barralet, J., Gbureck, U., Habibovic, P., Vorndran, E., Gerard, C., Doillon, C.J., 2009. Angiogenesis in calcium phosphate scaffolds by inorganic copper ion release. Tissue Eng. A. 15, 1601–1609.

Bellan, L.M., Kniazeva, T., Kim, E.S., Epshteyn, A.A., Cropek, D.M., Langer, R., et al., 2012. Fabrication of a hybrid microfluidic system incorporating both lithographically patterned microchannels and a 3D fiber-formed microfluidic network. Adv. Healthcare Mater. 1, 164–167.

Bellan, L.M., Singh, S.P., Henderson, P.W., Porri, T.J., Craighead, H.G., Spector, J.A., 2009. Fabrication of an artificial 3-dimensional vascular network using sacrificial sugar structures. Soft Matter. 5, 1354–1357.

Bertassoni, L.E., Cecconi, M., Manoharan, V., Nikkhah, M., Hjortnaes, J., Cristino, A.L., et al., 2014. Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs. Lab Chip. 14, 2202−2211.

Bobryshev, Y.V., Orekhov, A.N., Chistiakov, D.A., 2015. Vascular stem/progenitor cells: current status of the problem. Cell Tissue Res. 362, 1−7.

Bohl, K.S., Shon, J., Rutherford, B., Mooney, D.J., 1998. Role of synthetic extracellular matrix in development of engineered dental pulp. J. Biomater. Sci. Polym. Ed. 9, 749−764.

Bose, S., Tarafder, S., Bandyopadhyay, A., 2017. Effect of chemistry on osteogenesis and angiogenesis towards bone tissue engineering using 3D printed scaffolds. Ann. Biomed. Eng. 45, 261−272.

Boynueğri, D., Özcan, G., Şenel, S., Uç, D., Uraz, A., Öğüş, E., et al., 2009. Clinical and radiographic evaluations of chitosan gel in periodontal intraosseous defects: a pilot study. J. Biomed. Mater. Res. B: Appl. Biomater. 90, 461−466.

Bueno, E.M., Glowacki, J., 2009. Cell-free and cell-based approaches for bone regeneration. Nat. Rev. Rheumatol. 5, 685−697.

Buizer, A.T., Bulstra, S.K., Veldhuizen, A.G., Kuijer, R., 2017. The balance between proliferation and transcription of angiogenic factors of mesenchymal stem cells in hypoxia. Connect. Tissue Res, 1−9.

Burdick, J.A., Anseth, K.S., 2002. Photoencapsulation of osteoblasts in injectable RGD-modified PEG hydrogels for bone tissue engineering. Biomaterials 23, 4315−4323.

Chan, C., Lan, W., Chang, M., Chen, Y., Lan, W., Chang, H., et al., 2005. Effects of TGF-βs on the growth, collagen synthesis and collagen lattice contraction of human dental pulp fibroblasts in vitro. Arch. Oral Biol. 50, 469−479.

Chan, L.K., Leung, V.Y., Tam, V., Lu, W.W., Sze, K., Cheung, K.M., 2013. Decellularized bovine intervertebral disc as a natural scaffold for xenogenic cell studies. Acta Biomater. 9, 5262−5272.

Chen, G., Yang, L., Lv, Y., 2016. Cell-free scaffolds with different stiffness but same microstructure promote bone regeneration in rabbit large bone defect model. J. Biomed. Mater. Res. A 104, 833−841.

Chen, R.R., Silva, E.A., Yuen, W.W., Mooney, D.J., 2007. Spatio-temporal VEGF and PDGF delivery patterns blood vessel formation and maturation. Pharm. Res. 24, 258−264.

Chen, X., Aledia, A.S., Ghajar, C.M., Griffith, C.K., Putnam, A.J., Hughes, C.C., et al., 2008. Prevascularization of a fibrin-based tissue construct accelerates the formation of functional anastomosis with host vasculature. Tissue Eng. A 15, 1363−1371.

Chi, J.-T., Chang, H.Y., Haraldsen, G., Jahnsen, F.L., Troyanskaya, O.G., Chang, D.S., et al., 2003. Endothelial cell diversity revealed by global expression profiling. Proc. Natl. Acad. Sci. USA 100, 10623−10628.

Chua, K.-N., Lim, W.-S., Zhang, P., Lu, H., Wen, J., Ramakrishna, S., et al., 2005. Stable immobilization of rat hepatocyte spheroids on galactosylated nanofiber scaffold. Biomaterials. 26, 2537−2547.

Clark, R., Nielsen, L.D., Welch, M.P., Mcpherson, J.M., 1995. Collagen matrices attenuate the collagen-synthetic response of cultured fibroblasts to TGF-beta. J. Cell Sci. 108, 1251−1261.

Conconi, M.T., Ghezzo, F., Dettin, M., Urbani, L., Grandi, C., Guidolin, D., et al., 2010. Effects on in vitro and in vivo angiogenesis induced by small peptides carrying adhesion sequences. J. Pept. Sci. 16, 349−357.

Crapo, P.M., Gilbert, T.W., Badylak, S.F., 2011. An overview of tissue and whole organ decellularization processes. Biomaterials 32, 3233−3243.

Dai, J., Rabie, A., 2007. VEGF: an essential mediator of both angiogenesis and endochondral ossification. J. Dent. Res. 86, 937–950.
Denry, I., Kuhn, L.T., 2016. Design and characterization of calcium phosphate ceramic scaffolds for bone tissue engineering. Dent. Mater. 32, 43–53.
Dobie, K., Smith, G., Sloan, A., Smith, A., 2002. Effects of alginate hydrogels and TGF-β1 on human dental pulp repair in vitro. Connect. Tissue Res. 43, 387–390.
Duffy, A.M., Bouchier-Hayes, D.J., Harmey, J.H., 2004. Vascular endothelial growth factor (VEGF) and its role in non-endothelial cells: autocrine signalling by VEGF. In: Judith H, Harmey (Ed.), VEGF Cancer. Kluwer academic/plenum publishers, New York, pp. 133–144. ISBN 0-306-47988-5.
Ehrbar, M., Djonov, V.G., Schnell, C., Tschanz, S.A., Martiny-Baron, G., Schenk, U., et al., 2004. Cell-demanded liberation of VEGF121 from fibrin implants induces local and controlled blood vessel growth. Circ. Res. 94, 1124–1132.
Fielding, G.A., Bandyopadhyay, A., Bose, S., 2012. Effects of silica and zinc oxide doping on mechanical and biological properties of 3D printed tricalcium phosphate tissue engineering scaffolds. Dent. Mater. 28, 113–122.
Funamoto, S., Nam, K., Kimura, T., Murakoshi, A., Hashimoto, Y., Niwaya, K., et al., 2010. The use of high-hydrostatic pressure treatment to decellularize blood vessels. Biomaterials 31, 3590–3595.
Garlanda, C., Dejana, E., 1997. Heterogeneity of endothelial cells. Arterioscler. Thromb. Vasc. Biol. 17, 1193–1202.
Gergely, R.C., Pety, S.J., Krull, B.P., Patrick, J.F., Doan, T.Q., Coppola, A.M., et al., 2015. Multidimensional vascularized polymers using degradable sacrificial templates. Adv. Funct. Mater. 25, 1043–1052.
Golden, A.P., Tien, J., 2007. Fabrication of microfluidic hydrogels using molded gelatin as a sacrificial element. Lab Chip. 7, 720–725.
Greenwald, A.S., Boden, S.D., Goldberg, V.M., Khan, Y., Laurencin, C.T., Rosier, R.N., 2001. Bone-graft substitutes: facts, fictions, and applications. J. Bone Joint Surg. 83, S98–S103.
Hamada, Y., Nokihara, K., Okazaki, M., Fujitani, W., Matsumoto, T., Matsuo, M., et al., 2003. Angiogenic activity of osteopontin-derived peptide SVVYGLR. Biochem. Biophys. Res. Commun. 310, 153–157.
Hannallah, D., Peterson, B., Lieberman, J.R., Fu, F.H., Huard, J., 2002. Gene therapy in orthopaedic surgery. J. Bone. Joint Surg. Am. 84, 1046–1061.
Haug, V., Torio-padron, N., Stark, G.B., Finkenzeller, G., Strassburg, S., 2015. Comparison between endothelial progenitor cells and human umbilical vein endothelial cells on neovascularization in an adipogenesis mouse model. Microvasc. Res. 97, 159–166.
Herrmann, M., Binder, A., Menzel, U., Zeiter, S., Alini, M., Verrier, S., 2014. CD34/CD133 enriched bone marrow progenitor cells promote neovascularization of tissue engineered constructs in vivo. Stem Cell Res. 13, 465–477.
Hobo, K., Shimizu, T., Sekine, H., Shin'oka, T., Okano, T., Kurosawa, H., 2008. Therapeutic angiogenesis using tissue engineered human smooth muscle cell sheets. Arterioscler. Thromb. Vasc. Biol. 28, 637–643.
Horch, R.E., Beier, J.P., Kneser, U., Arkudas, A., 2014. Successful human long-term application of in situ bone tissue engineering. J. Cell. Mol. Med. 18, 1478–1485.
Inuyama, Y., Kitamura, C., Nishihara, T., Morotomi, T., Nagayoshi, M., Tabata, Y., et al., 2010. Effects of hyaluronic acid sponge as a scaffold on odontoblastic cell line and amputated dental pulp. J. Biomed. Mater. Res. B: Appl. Biomater. 92, 120–128.

Jeong, J., Kim, S., Park, T., 2004. Biodegradable triblock copolymer of PLGA-PEG-PLGA enhances gene transfection efficiency. Pharm. Res. 21, 50–54.

Jockenhoevel, S., Zund, G., Hoerstrup, S.P., Chalabi, K., Sachweh, J.S., Demircan, L., et al., 2001. Fibrin gel—advantages of a new scaffold in cardiovascular tissue engineering. Eur. J. Cardiothorac. Surg. 19, 424–430.

Karageorgiou, V., Kaplan, D., 2005. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 26, 5474–5491.

Kazemzadeh-narbat, M., Rouwkema, J., Annabi, N., Cheng, H., Ghaderi, M., Cha, B.H., et al., 2017. Engineering photocrosslinkable bicomponent hydrogel constructs for creating 3D vascularized bone. Adv. Healthcare Mater.

Khouri, R.K., Upton, J., Shaw, W.W., 1991. Prefabrication of composite free flaps through staged microvascular transfer: an experimental and clinical study. Plast. Reconstr. Surg. 87, 108–115.

Kim, J.Y., Xin, X., Moioli, E.K., Chung, J., Lee, C.H., Chen, M., et al., 2010. Regeneration of dental-pulp-like tissue by chemotaxis-induced cell homing. Tissue Eng. A 16, 3023–3031.

Kinzer, M., Hingerl, K., König, J., Reinisch, A., Strunk, D., Huppertz, B., et al., 2014. Mesenchymal stromal cells from the human placenta promote neovascularization in a mouse model in vivo. Placenta 35, 517–519.

Kolesky, D.B., Truby, R.L., Gladman, A., Busbee, T.A., Homan, K.A., Lewis, J.A., 2014. 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. Adv. Mater. 26, 3124–3130.

Kurien, T., Pearson, R., Scammell, B., 2013. Bone graft substitutes currently available in orthopaedic practice. Bone Joint J. 95, 583–597.

Laschke, M.W., Harder, Y., Amon, M., Martin, I., Farhadi, J., Ring, A., et al., 2006. Angiogenesis in tissue engineering: breathing life into constructed tissue substitutes. Tissue Eng. 12, 2093–2104.

Laschke, M.W., Rücker, M., Jensen, G., Carvalho, C., Mülhaupt, R., Gellrich, N.-C., et al., 2008. Improvement of vascularization of PLGA scaffolds by inosculation of in situ-preformed functional blood vessels with the host microvasculature. Ann. Surg. 248, 939–948.

Laschke, M.W., Vollmar, B., Menger, M.D., 2009. Inosculation: connecting the life-sustaining pipelines. Tissue Eng. B: Rev. 15, 455–465.

Laurencin, C.T., Khan, Y., 2012. Regenerative Engineering. Am. Assoc. Adv. Sci. 4, 1–2.

Lee, F., Kurisawa, M., 2013. Formation and stability of interpenetrating polymer network hydrogels consisting of fibrin and hyaluronic acid for tissue engineering. Acta Biomater. 9, 5143–5152.

Lee, S., Valmikinathan, C.M., Byun, J., Kim, S., Lee, G., Mokarram, N., et al., 2015. Enhanced therapeutic neovascularization by CD31-expressing cells and embryonic stem cell-derived endothelial cells engineered with chitosan hydrogel containing VEGF-releasing microtubes. Biomaterials 63, 158–167.

Leukers, B., Gülkan, H., Irsen, S.H., Milz, S., Tille, C., Schieker, M., et al., 2005. Hydroxyapatite scaffolds for bone tissue engineering made by 3D printing. J. Mater. Sci.: Mater. Med. 16, 1121–1124.

Lieberman, J.R., Daluiski, A., Einhorn, T.A., 2002. The role of growth factors in the repair of bone. J. Bone Joint Surg. Am. 84, 1032–1044.

Linnes, M.P., Ratner, B.D., Giachelli, C.M., 2007. A fibrinogen-based precision microporous scaffold for tissue engineering. Biomaterials 28, 5298–5306.

Liu, W.C., Chen, S., Zheng, L., Qin, L., 2017. Angiogenesis assays for the evaluation of angiogenic properties of orthopaedic biomaterials—a general review. Adv. Healthcare Mater. 6.

Loffredo, F., Lee, R.T., 2008. Therapeutic Vasculogenesis. Circ. Res. 103, 128−130.

Lokmic, Z., Stillaert, F., Morrison, W.A., Thompson, E.W., Mitchell, G.M., 2007. An arterio-venous loop in a protected space generates a permanent, highly vascular, tissue-engineered construct. FASEB J. 21, 511−522.

Ma, J., Both, S.K., Yang, F., Cui, F.-Z., Pan, J., Meijer, G.J., et al., 2014. Concise review: cell-based strategies in bone tissue engineering and regenerative medicine. Stem Cells Transl. Med. 3, 98−107.

Mao, L., Huang, M., Chen, S.C., Li, Y.N., Xia, Y.P., He, Q.W., et al., 2014. Endogenous endothelial progenitor cells participate in neovascularization via CXCR4/SDF-1 axis and improve outcome after stroke. CNS Neurosci. Ther. 20, 460−468.

Marks, S.C., Odgren, P.R., 2002. Structure and development of the skeleton. Princ. Bone Biol. 1, 3−15.

Mercado-pagán, Á.E., Stahl, A.M., Shanjani, Y., Yang, Y., 2015. Vascularization in bone tissue engineering constructs. Ann. Biomed. Eng. 43, 718−729.

Miller, J.S., Shen, C.J., Legant, W.R., Baranski, J.D., Blakely, B.L., Chen, C.S., 2010. Bioactive hydrogels made from step-growth derived PEG−peptide macromers. Biomaterials 31, 3736−3743.

Miller, J.S., Stevens, K.R., Yang, M.T., Baker, B.M., Nguyen, D.-H.T., Cohen, D.M., et al., 2012. Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. Nat. Mater. 11, 768−774.

Mori, H., Matsuda, H., 2005. Cardiovascular Regeneration Therapies Using Tissue Engineering Approaches. Springer, Tokyo.

Moschetta, M., Mishima, Y., Sahin, I., Manier, S., Glavey, S., Vacca, A., et al., 2014. Role of endothelial progenitor cells in cancer progression. Biochim. Biophys. Acta: Rev. Cancer 1846, 26−39.

Mozafari, M., Salahinejad, E., Shabafrooz, V., Yazdimamaghani, M., Vashaee, D., Tayebi, L., 2013. Multilayer bioactive glass/zirconium titanate thin films in bone tissue engineering and regenerative dentistry. Int. J. Nanomed. 8, 1665−1672.

Na, S., Zhang, H., Huang, F., Wang, W., Ding, Y., Li, D., et al., 2013. Regeneration of dental pulp/dentine complex with a three-dimensional and scaffold-free stem-cell sheet-derived pellet. J. Tissue Eng. Regener. Med. 10, 261−270.

Nakashima, M., 1994. Induction of dentine in amputated pulp of dogs by recombinant human bone morphogenetic proteins-2 and-4 with collagen matrix. Arch. Oral Biol. 39, 1085−1089.

Nam, S., Won, J.-E., Kim, C.-H., Kim, H.-W., 2011. Odontogenic differentiation of human dental pulp stem cells stimulated by the calcium phosphate porous granules. J. Tissue Eng. 2011, 812547.

Nomi, M., Atala, A., De coppi, P., Soker, S., 2002. Principals of neovascularization for tissue engineering. Mol. Aspects Med. 23, 463−483.

Novosel, E.C., Kleinhans, C., Kluger, P.J., 2011. Vascularization is the key challenge in tissue engineering. Adv. Drug Deliv. Rev. 63, 300−311.

Ouasti, S., Donno, R., Cellesi, F., Sherratt, M.J., Terenghi, G., Tirelli, N., 2011. Network connectivity, mechanical properties and cell adhesion for hyaluronic acid/PEG hydrogels. Biomaterials. 32, 6456−6470.

Papadopoulos, A., Bichara, D.A., Zhao, X., Ibusuki, S., Randolph, M.A., Anseth, K.S., et al., 2010. Injectable and photopolymerizable tissue-engineered auricular cartilage using poly (ethylene glycol) dimethacrylate copolymer hydrogels. Tissue Eng. A 17, 161–169.

Park, Y.D., Tirelli, N., Hubbell, J.A., 2003. Photopolymerized hyaluronic acid-based hydrogels and interpenetrating networks. Biomaterials 24, 893–900.

Phelps, E.A., García, A.J., 2010. Engineering more than a cell: vascularization strategies in tissue engineering. Curr. Opin. Biotechnol. 21, 704–709.

Pina, S., Oliveira, J.M., Reis, R.L., 2015. Natural-based nanocomposites for bone tissue engineering and regenerative medicine: a review. Adv. Mater. 27, 1143–1169.

Price, A.P., England, K.A., Matson, A.M., Blazar, B.R., Panoskaltsis-mortari, A., 2010. Development of a decellularized lung bioreactor system for bioengineering the lung: the matrix reloaded. Tissue Eng. A 16, 2581–2591.

Probst, A., Spiegel, H.-U., 1997. Cellular mechanisms of bone repair. J. Invest. Surg. 10, 77–86.

Rasoulianboroujeni, M., Yazdimamaghani, M., Khoshkenar, P., Pothineni, V.R., Kim, K.M., Murray, T.A., et al., 2017. From solvent-free microspheres to bioactive gradient scaffolds. Nanomed.: Nanotechnol. Biol. Med. 13, 1157–1169.

Ravi, S., Chaikof, E.L., 2010. Biomaterials for vascular tissue engineering. Regener. Med. 5, 107–120.

Roberts, M.A., Tran, D., Coulombe, K.L., Razumova, M., Regnier, M., Murry, C.E., et al., 2016. Stromal cells in dense collagen promote cardiomyocyte and microvascular patterning in engineered human heart tissue. Tissue Eng. A 22, 633–644.

Robinson, S.T., Douglas, A.M., Chadid, T., Kuo, K., Rajabalan, A., Li, H., et al., 2016. A novel platelet lysate hydrogel for endothelial cell and mesenchymal stem cell-directed neovascularization. Acta Biomater. 36, 86–98.

Rothenfluh, D., Demhartner, T., Fraitzl, C., Cecchini, M., Ganz, R., Leunig, M., 2004. Potential role of pre-existing blood vessels for vascularization and mineralization of osteochondral grafts: an intravital microscopic study in mice. Acta Orthop. Scand. 75, 359–365.

Sakai, S., Kawakami, K., 2007. Synthesis and characterization of both ionically and enzymatically cross-linkable alginate. Acta Biomater. 3, 495–501.

Santos, M., Pashkuleva, I., Alves, C., Gomes, M.E., Fuchs, S., Unger, R.E., et al., 2009. Surface-modified 3D starch-based scaffold for improved endothelialization for bone tissue engineering. J. Mater. Chem. 19, 4091–4101.

Santos, M.I., Reis, R.L., 2010. Vascularization in bone tissue engineering: physiology, current strategies, major hurdles and future challenges. Macromol. Biosci. 10, 12–27.

Santos, M.I., Fuchs, S., Gomes, M.E., Unger, R.E., Reis, R.L., Kirkpatrick, C.J., 2007. Response of micro-and macrovascular endothelial cells to starch-based fiber meshes for bone tissue engineering. Biomaterials 28, 240–248.

Sarkar, K., Fox-talbot, K., Steenbergen, C., Bosch-marcé, M., Semenza, G.L., 2009. Adenoviral transfer of HIF-1α enhances vascular responses to critical limb ischemia in diabetic mice. Proc. Natl. Acad. Sci. USA 106, 18769–18774.

Sekine, H., Shimizu, T., Hobo, K., Sekiya, S., Yang, J., Yamato, M., et al., 2008. Endothelial cell coculture within tissue-engineered cardiomyocyte sheets enhances neovascularization and improves cardiac function of ischemic hearts. Circulation 118, S145–S152.

Sharma, S., Srivastava, D., Grover, S., Sharma, V., 2014. Biomaterials in tooth tissue engineering: a review. J. Clin. Diagn. Res. 8, 309.

Shyu, K.-G., Wang, M.-T., Wang, B.-W., Chang, C.-C., Leu, J.-G., Kuan, P., et al., 2002. Intramyocardial injection of naked DNA encoding HIF-1α/VP16 hybrid to enhance angiogenesis in an acute myocardial infarction model in the rat. Cardiovasc. Res. 54, 576–583.

Stegen, S., Van gastel, N., Carmeliet, G., 2015. Bringing new life to damaged bone: the importance of angiogenesis in bone repair and regeneration. Bone 70, 19–27.

Street, J., Bao, M., Bunting, S., Peale, F.V., Ferrara, N., Steinmetz, H., et al., 2002. Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. Proc. Natl. Acad. Sci. USA 99, 9656–9661.

Suh, J.-K.F., Matthew, H.W., 2000. Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review. Biomaterials 21, 2589–2598.

Sumpio, B.E., Riley, J.T., Dardik, A., 2002. Cells in focus: endothelial cell. Int. J. Biochem. Cell Biol. 34, 1508–1512.

Tahmasbi rad, A., Ali, N., Kotturi, H.S.R., Yazdimamaghani, M., Smay, J., Vashaee, D., et al., 2014. Conducting scaffolds for liver tissue engineering. J. Biomed. Mater. Res. A 102, 4169–4181.

Tanase, C., Sartoris, A., Popa, M., Verestiuc, L., Unger, R., Kirkpatrick, C., 2013. In vitro evaluation of biomimetic chitosan–calcium phosphate scaffolds with potential application in bone tissue engineering. Biomed. Mater. 8, 025002.

Tarafder, S., Bose, S., 2014. Polycaprolactone-coated 3D printed tricalcium phosphate scaffolds for bone tissue engineering: in vitro alendronate release behavior and local delivery effect on in vivo osteogenesis. ACS Appl. Mater. Interf. 6, 9955–9965.

Tarkka, T., Sipola, A., Jämsä, T., Soini, Y., Ylä-Herttuala, S., Tuukkanen, J., et al., 2003. Adenoviral VEGF-A gene transfer induces angiogenesis and promotes bone formation in healing osseous tissues. J. Gene Med. 5, 560–566.

Tonomura, A., Mizuno, D., Hisada, A., Kuno, N., Ando, Y., Sumita, Y., et al., 2010. Differential effect of scaffold shape on dentin regeneration. Ann. Biomed. Eng. 38, 1664–1671.

Tremblay, P.L., Hudon, V., Berthod, F., Germain, L., Auger, F.A., 2005. Inosculation of tissue-engineered capillaries with the host's vasculature in a reconstructed skin transplanted on mice. Am. J. Transplant. 5, 1002–1010.

Tsukada, S., Kwon, S.-M., Matsuda, T., Jung, S.-Y., Lee, J.-H., Lee, S.-H., et al., 2013. Identification of mouse colony-forming endothelial progenitor cells for postnatal neovascularization: a novel insight highlighted by new mouse colony-forming assay. Stem Cell Res. Ther. 4, 20.

Uygun, B.E., Soto-gutierrez, A., Yagi, H., Izamis, M.-L., Guzzardi, M.A., Shulman, C., et al., 2010. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. Nat. Med. 16, 814–820.

Von der mark, K., Park, J., Bauer, S., Schmuki, P., 2010. Nanoscale engineering of biomimetic surfaces: cues from the extracellular matrix. Cell Tissue Res. 339, 131.

Wahl, D.A., Sachlos, E., Liu, C., Czernuszka, J.T., 2007. Controlling the processing of collagen-hydroxyapatite scaffolds for bone tissue engineering. J. Mater. Sci.: Mater. Med. 18, 201–209.

Wang, G.-J., Hsu, Y.-F., 2006. Structure optimization of microvascular scaffolds. Biomed. Microdevices 8, 51–58.

Wang, M.O., Vorwald, C.E., Dreher, M.L., Mott, E.J., Cheng, M.H., Cinar, A., et al., 2015. Evaluating 3D-Printed biomaterials as scaffolds for vascularized bone tissue engineering. Adv. Mater. 27, 138–144.

Weinberg, C.B., Bell, E., 1986. A blood vessel model constructed from collagen and cultured vascular cells. Science 231, 397–401.

Wolf, M.T., Daly, K.A., Reing, J.E., Badylak, S.F., 2012. Biologic scaffold composed of skeletal muscle extracellular matrix. Biomaterials 33, 2916–2925.

Wu, X., Black, L., Santacana-Laffitte, G., Patrick, C.W., 2007. Preparation and assessment of glutaraldehyde-crosslinked collagen−chitosan hydrogels for adipose tissue engineering. J. Biomed. Mater. Res. Part A 81, 59−65.

Yazdimamaghani, M., Razavi, M., Mozafari, M., Vashaee, D., Kotturi, H., Tayebi, L., 2015a. Biomineralization and biocompatibility studies of bone conductive scaffolds containing poly (3, 4-ethylenedioxythiophene): poly (4-styrene sulfonate)(PEDOT: PSS). J. Mater. Sci.: Mater. Med. 26, 274.

Yazdimamaghani, M., Razavi, M., Vashaee, D., Moharamzadeh, K., Boccaccini, A.R., Tayebi, L., 2017. Porous magnesium-based scaffolds for tissue engineering. Mater. Sci. Eng.: C 71, 1253−1266.

Yazdimamaghani, M., Razavi, M., Vashaee, D., Pothineni, V.R., Assefa, S., Köhler, G.A., et al., 2016. In vitro analysis of Mg scaffolds coated with polymer/hydrogel/ceramic composite layers. Surf. Coat. Technol. 301, 126−132.

Yazdimamaghani, M., Razavi, M., Vashaee, D., Pothineni, V.R., Rajadas, J., Tayebi, L., 2015b. Significant degradability enhancement in multilayer coating of polycaprolactone-bioactive glass/gelatin-bioactive glass on magnesium scaffold for tissue engineering applications. Appl. Surf. Sci. 338, 137−145.

Yazdimamaghani, M., Vashaee, D., Assefa, S., Shabrangharehdasht, M., Rad, A.T., Eastman, M.A., et al., 2014a. Green synthesis of a new gelatin-based antimicrobial scaffold for tissue engineering. Mater. Sci. Eng.: C 39, 235−244.

Yazdimamaghani, M., Vashaee, D., Assefa, S., Walker, K., Madihally, S., Köhler, G., et al., 2014b. Hybrid macroporous gelatin/bioactive-glass/nanosilver scaffolds with controlled degradation behavior and antimicrobial activity for bone tissue engineering. J. Biomed. Nanotechnol. 10, 911−931.

Young, D., Greulich, K., Weier, H., 1996. Species-specific in situ hybridization with fluorochrome-labeled DNA probes to study vascularization of human skin grafts on athymic mice. J. Burn Care Res. 17, 305−310.

Yu, H., Vandevord, P.J., Mao, L., Matthew, H.W., Wooley, P.H., Yang, S.-Y., 2009. Improved tissue-engineered bone regeneration by endothelial cell mediated vascularization. Biomaterials 30, 508−517.

Zhang, Q., Nguyen, A., Yu, W., Le, A., 2012. Human oral mucosa and gingiva a unique reservoir for mesenchymal stem cells. J. Dent. Res. 0022034512461016.

Zhu, J., 2010. Bioactive modification of poly (ethylene glycol) hydrogels for tissue engineering. Biomaterials. 31, 4639−4656.

Zigdon-giladi, H., Bick, T., Lewinson, D., Machtei, E.E., 2015. Co-transplantation of endothelial progenitor cells and mesenchymal stem cells promote neovascularization and bone regeneration. Clin. Implant Denti. Relat. Res. 17, 353−359.

Zimmermann, G., Moghaddam, A., 2011. Allograft bone matrix versus synthetic bone graft substitutes. Injury. 42, S16−S21.

Zisch, A.H., Lutolf, M.P., Hubbell, J.A., 2003. Biopolymeric delivery matrices for angiogenic growth factors. Cardiovasc. Pathol. 12, 295−310.

This page intentionally left blank

Part IV

Oral and Dental Hard Tissue Engineering

This page intentionally left blank

Bone tissue engineering in maxillofacial region

23

Thafar Almela, Ian Brook and Keyvan Moharamzadeh
University of Sheffield, Sheffield, United Kingdom

23.1 Introduction

Bone loss in the maxillofacial region is a commonly encountered problem that can range from small periodontal defect to a complex, difficult to manage structural defects (Elsalanty and Genecov, 2009a). Data from National Health and Nutrition Examination Survey (NHNES) showed that the prevalence of sever periodontitis that warrant surgery reached approximately 15% of US population (Eke et al., 2012). Likewise, in 2010, the British Association of Oral and Maxillofacial Surgeons (BAOMS) reported that for a population of 500,000 there were more than 4000 facial injuries annually, 250 of which were facial fractures (CWFI, 2010). In addition, the increased demand for bone regenerative therapies is expected to rise with the increasing population and life expectancy (Beller, 2010).

The current conventional approach for the treatment of bone deficiency is by using various types of bone grafts, guided tissue regeneration, or osteodistraction. Bone grafting can be traced back to several centuries when the earliest xeno grafting procedure was reported in 1668 by Job Janszoon van Meekeren who attempted to perform cranioplasty using dog bone (Hjorting-Hansen, 2002). In 1821, Walther was credited with the first documented autograft procedure although wound suppuration prevented full healing (Sanan and Haines, 1997). Sixty years later the first published case report of successful interhuman bone transfer was performed by William MacEwen (Macewen, 1881) whereas the first introduction of synthetic calcium phosphate as alloplastic bone substitute material was at the outset of 20th century (Albee, 1920).

As it contains all the essential elements for osteogenesis, autograft is considered the “gold standard.” Nevertheless, it is associated with difficulty to obtain the desired bone quantity and postoperative complications such as infection, dehiscence, and nonunion particularly with nonvascularized bone grafts which may be as high as 69% (Miloro and Kolokythas, 2012). In addition, unpredictable bone resorption may reach up to 50% of the initial volume (Buser et al., 2009). Use of allograft or xenograft provides available, low cost alternative, but there have been some concern with the risk of disease transmission although the overall chance is 1:1 million (Miloro and Kolokythas, 2012). Osteoconductive synthetic materials are another potential option yet their use is limited due to suboptimal mechanical properties and resorption rate (Scheller et al., 2009).

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00023-2
© 2017 Elsevier Ltd. All rights reserved.

Although tissue engineering of bone has potential to offer an alternative strategy to conventional bone grafting techniques for replacement of missing hard tissues in the head and neck region, the clinical use is still limited. It is restricted by regulatory requirements, high cost, insufficient clinical trials, and unpredictable long-term results (Elsalanty and Genecov, 2009b). Experimental applications of tissue engineering of bone offer unique opportunities to investigate the interactions among cells, matrix, biomolecules, and environmental factors that cannot be otherwise studied. Such observations provide insights into cell behavior and add to the fund of knowledge improving the understanding of tissue from biological, physiological, and pathological prospects (Spector, 2002). In addition, translation of research from animal to human construct may minimize the need for lengthy, costly, and controversial animal studies which can be misleading due to interspecies molecular and physiological differences (van der Worp et al., 2010).

This chapter aims to discuss the strategies used to date for tissue engineering of the alveolar bone as well as the laboratory and clinical challenges associated with bone tissue engineering.

23.2 Natural alveolar bone structure

Human alveolar bone comprises of outer dense compact bone and inner cancellous bone interrupting the medullary cavity with its trabeculae. Microscopically, mature compact or cancellous bone consists of three distinct layers: circumferential, interstitial, and concentric. The latter forming the basic unit of bone called osteon or Haversian system which consists of a bony cylinder surrounding the Haversian canal. Adjacent canals are unified by Volkmann canals which in turn house a nourishing capillaries. Overlying the external aspect of every alveolar bone is a mucoperiosteum; an arrangement composed of keratinized gingiva covering the periosteum. Periosteum in turn has an outer fibrous layer and inner cellular layer harboring bony cells precursors and microvascular supply (Nanci, 2013).

23.2.1 Bone components

Similar to other connective tissues, bone consists of cells and matrix. However, two essential features distinguish it from other specialized connective tissues. First is matrix mineralization which produces an extremely hard tissue providing support, protection as well as storage and homeostasis of calcium and phosphate (Ross, 2016). Second is its plasticity that allows modeling and remodeling that results in the change of the external shape and/or size and the internal structure, respectively (Berkovitz, 2009).

23.2.2 Bone matrix

The extracellular matrix (ECM) consists of about 15% water, 60% inorganic phase in the form of hydroxyapatite crystals $Ca_{10}(PO_4)_6(OH)_2$ providing rigidity through

distribution within and on the surface of the 25% organic material. The latter consists of 90% collagen (predominantly type I) that provides resiliency and load resistance and about 200 noncollagenous proteins forming the remaining 10% such as the proteoglycans, glycoproteins, and serum proteins (Berkovitz, 2009).

23.2.3 Bone cells

Osteoarchitechture synthesis and resorption are regulated by different cell types belonging to two families each with specific functions: mesenchymal and haemopoietic. The mesenchymal lineage includes; osteoprogenitor, osteoblasts, osteocytes, and bone lining cells which are responsible for osteogenesis. The haemopoietic lineage, on the other hand, includes osteoclast, the bone resorbing cells, which derived from the granulocyte/monocyte progenitor (Ross, 2016).

Three identified transcription factors are necessary for osteoblastogenesis: runt-related transcription factor-2 (Runx2), Ostrix (OSX), and ATF4. Once the mesenchymal stem cells (MSCs) commit to the osteoblast lineage, an orderly progression from osteoprogenitor cells to osteoblasts, osteocytes, and bone lining cells is initiated. Ultimately, the matrix contains only 4%−6% osteoblasts and 1%−2% osteoclasts while the vast matrix volume is occupied by osteocytes 90%−95% which can persist for years (McCauley and Somerman, 2012). The low percentage of osteoblasts may be attributed to its relatively short life span which is approximately 1−3 months (Franz-odendaal et al., 2006). Subsequently, osteoblast will stop matrix secretion and its potential fate will be one of three possibilities: embedding in matrix as osteocytes (approximately 30%), converting to inactive bone lining cells, or undergoing apoptosis (Manolagas, 2000).

Bone formation is controlled by the interdependent actions of mesenchymal cells lineage. By its unique self-renewal ability, osteoprogenitor cells maintain their number and provide a source to generate osteoblasts throughout the life. Once osteoblasts are differentiated the matrix secretion and subsequent mineralization of osteoid (prebone) is established. The secretions include collagen I and other noncollagenous osteoblast specific proteins such as osteocalcin, osteoblast transcription factor, alkaline phosphatase, and different cytokines and growth factors controlling osteoblasts and osteoclasts activity. Osteoblasts surface receptors also help in bone metabolism by interaction with many hormones such as parathyroid hormone, calcitonin, estrogen, and 1,25-dihydroxyvitamin D. When osteoblasts secret matrix, some become entrapped in it and then are called osteocytes which have no role in osteoid secretion, yet they act as a sensor detecting any mechanical or biochemical stimuli and response by signal transduction that affecting other cells to maintain bone integrity. Failure to do so results in hypermineralization and bone death which is resorbed during the turnover process (Nanci, 2013). In quiescent stage, some osteoblasts flatten and extend along the bone surface to form bone lining cell layers which may play a role in metabolism of calcium and phosphate, protecting the surface from osteoclasts resorptive activity, participating in bone remodeling, and reactivating osteoprogenitor cells to form osteoblasts (Ross, 2016).

Bone resorption, on the other hand, is performed by osteoclasts that are possibly recruited by chemotaxis only when they are needed hence there is no reservoir for preosteoclasts. The life span lasts 10−14 days, after which the cells undergo apoptosis (Berkovitz, 2009). In contrast to the osteoblast which appose bone in a rate of 1 μm/day, osteoclasts can resorb bone to a depth of 10 μm/day through bone demineralization by acids and then collagen dissolving by enzymes (Martin, 1998).

23.3 Strategies for bone tissue engineering

Broadly, the strategies for bone engineering can be divided into in vivo and in vitro tissue engineering depending on where the tissue construction takes place (Elsalanty and Genecov, 2009b). In vivo method includes in situ bone engineering involving implantation of osteoconductive resorbable scaffold in the bone defect to provide mechanical support of host cells (Khan et al., 2008) and ectopic implantation of scaffold in muscle which then transplanted as a free bone-muscle tissue flap (Kokemueller et al., 2010). In vitro approach, on the other hand, involves osteogenic cells harvesting, expansion, and seeding into an appropriate scaffold that should be already approved for clinical use if the engineered construct is intended for clinical application. The seeded scaffold is then cultured in vitro ideally in the presence of biophysical and biochemical signals that enhance osteogenesis (Mangano et al., 2009; Pradel and Lauer, 2012). While in vivo technique provides a physiological environment that is difficult to reproduce in laboratory, in vitro tissue engineering offers two distinct advantages: strict control of culture parameters thereby the results variability is minimized, and the ability to use human cells which are quite different from animal source (Gibbons et al., 2013).

Whether in vivo or in vitro strategy is being applied, the success largely depends on the technique used for scaffold fabrication and to what extent this technique can produce a surrogate for ECM. A wide range of conventional techniques commonly used in bone engineering such as solvent casting, freeze drying, gas foaming, phase inversion, and salt leaching. However, these methods can generate matrix with random structure, unpredictable pore sizes, and reduced interconnections. Moreover, it is difficult to control the variation in porosity, mechanical strength, structural stability, and reproducibility (Haycock, 2011).

Recently, the additive manufacturing (AM) approaches allow tailoring a more complex structure directly from a computer-aided design (CAD) file (Melchels et al., 2012). 3D printing, for instance, enables layer-by-layer scaffold fabrication with one of the following techniques: inkjet printing, laser-assisted printing such as selective laser sintering (SLS), and extrusion printing such as fused deposition modeling (FDM) (Obregon et al., 2015). This range of the available techniques facilitate precise fabrication of 3D scaffolds with defined shape, size, porosity, and pore size distribution which can have a significant impact on cell proliferation, differentiation, and vascularization (Wang et al., 2015; Shrivats et al., 2014; Ferlin et al., 2016; Cavo and Scaglione, 2016). They also allow scalable fabrication of

complex designs using various biocompatible materials thereby provide an optimal cell microenvironment. AM approaches can be applicable in fabricating constructs customizable to patient-specific needs (Fedorovich et al., 2011). The physical properties of the scaffolds such as compressive strength, toughness, and modulus can be optimized by adjusting the layer thickness and printing orientation (Farzadi et al., 2014).

Aside from the scaffold printing using AM, it is also possible to explore the potential of tissue biofabrication by deposition of materials with live cells (bioprinting). In this technique the cell-laden prepolymer suspension (bioink) is deposited layer by layer onto a substrate (biopaper) to build a 3D construct analogous to the native tissue (Bajaj et al., 2014). Catros et al. (2011) investigated the patterning and assembling of nanohydroxyapatite and human osteoprogenitors using laser-assisted bioprinting. This strategy allowed tissue construction without changing the physicochemical properties of biomaterial nor the viability, proliferation and phenotype of cells over 15 days. Nevertheless, long-term in vivo and in vitro studies with mature human osteoblasts may require to prove the efficacy of such techniques in terms of maintaining the cell phenotype.

In spite of the above advantages, there are some technological limitations that should be addressed for future improvements in printing methods. First, each 3D-printing process has a specific form of materials. For example, fine powder for SLS and thermoplastics for FDM. Printing of the material can be challenging if the material is deemed suitable for a particular application but cannot be easily prepared to meet the required 3D-printing process. Second, as successful printing relies on bonding strength between the layers, preparation of material in the desired form does not necessarily guarantee its printability. The material needs to be self-supporting for layer-by-layer fabrication. Third, for bioprinting, new methods of material solidification should be developed that preserve the integrity of the printed construct without compromising the cell survival (An et al., 2015). For example, in extrusion printing, hydrogels are solidified through either thermal processes or post-print cross-linking which may have potential harmful effects on the cells. Lastly, the flexibility of the printing parameters such as temperature or dispensing pressure (shear stress) have become limited when using cell-laden materials because sudden changes in the environment around the cells may significantly reduce the cell viability (Nair et al., 2009).

23.4 Tissue-engineered bone

Tissue-engineered bone, like most engineered tissues, is based on three essential components: cells, scaffold, and signals. The advances in tissue engineering technologies have involved this triad by providing in vitro culture conditions that favor cell proliferation with relative cell phenotype maintenance. Improvement in scaffold fabrication that allows precise control in porosity, volume, orientation, and chemical composition is the second enabling factor. Another remarkable advance is the

recombinant technology that permits production of different cytokines such as recombinant human bone morphogenetic proteins (rhBMPs) in large quantities.

23.4.1 Cells

Stem and mature differentiated cells are the main cell sources in tissue engineering including bone. Compared to the stem cells, osteoblasts are capable to secret and mineralize ECM at a higher rate, however, the proliferative potential is limited and further attenuated by increasing age which pose the problem of cells expansion to high quantities (Fisher, 2013). MSCs, on the other hand, are self-renewed and existing in numerous tissues, including bone marrow, periosteum, adipose tissue, and periodontal ligament, yet their availability is extremely limited. The amount in bone marrow, for instance, is 1:100,000 nucleated cells (El Tamer and Reis, 2009). In addition, thorough investigations have pointed out to the complex interactions affecting MSCs osteodifferentiation which include: culture conditions in term of cell size, cells contact, and induction type (Peng et al., 2012), seeding density (Zhou et al., 2011), coculture with different cell types (Henrich et al., 2013; Seebach et al., 2010), tissue source (Niemeyer et al., 2010; Yang et al., 2013), and growth factors (Biver et al., 2012).

Aside from the ethical concern, pluripotent human embryonic stem cells (hESCs) have been investigated for bone engineering, but the evidence for the clinical feasibility is still inconsistent. Several studies showed the possibility of teratoma formation and failure to engineer bone in vivo using hESCs regardless of different differentiation conditions (Kim et al., 2008; Kuznetsov et al., 2011). On the other hand, other studies demonstrated that engineering of compact and homogenous bone like tissue can be induced by implantation of differentiated hESCs using osteoconductive scaffolds. However, the long-term evaluation of safety and stability of engineered bone remains questionable (Kim et al., 2008). Likewise, the utility of induced pluripotent stem cells (iPSCs) in BE remains under investigations with a positive evidence referring to successful bone induction, yet the major concern for clinical application is the possibility of tumorigenesis associated with using genetically modified cells (de Peppo et al., 2013; Jin et al., 2013; Liu et al., 2013).

23.4.2 Scaffolds

Contrary to what was thought for many years that any synthetic biomaterial should be inert to avoid any foreign-body reaction or scar formation, trend has shifted toward the concept of bioactive materials that have bio instructive role in stimulating the body's own repair process through guiding stem cells proliferation and differentiation to regenerate lost tissues (Ben-Nissan et al., 2014). Ideally, several characteristics should be available in the chosen material to serve as a scaffold for bone engineering or drug delivery (Table 23.1). Due to these requirements, no absolute advantageous biomaterial could be used for scaffold fabrication because every type has inherent drawbacks, but instead the selection depends on the

Table 23.1 The ideal characteristics of scaffold for bone engineering

	Criterion	Definition	Reference
1	Biocompatible	Capable of supporting normal cellular activity without local or systemic side effect	Williams (2008)
2	Biodegradable	Varies according to the application ex. 3−6 months in the craniomaxillofacial area or 9 months in spinal fusion	Lichte et al. (2011)
3	Porous	Interconnected porosity with an optimal size of 200−350 μm.	Murphy et al. (2010)
		Multiscale of micro and macroporosity	Woodard et al. (2007)
4	Osteoconductive	Allows cells adherence, proliferation and matrix secretion	Daculsi et al. (2013)
5	Osteoinductive	Induces progenitor cells recruitment and differentiation via biomolecules signaling	Daculsi et al. (2013)
6	Mechanical properties	Comparable to the natural compressive strength of cancellous (2−20 MPa) and compact (100−200 MPa) bones	Olszta et al. (2007)
7	Induce vasculogenesis	Forms new blood plexuses following the implantation and supports nutrient transport	Gu et al. (2013)

intended application. Table 23.2 shows the classification of the currently used bone scaffolds.

The current available types of materials used in bone engineering can be grouped into inorganic ceramics such as calcium phosphate, organic polymers either natural scaffolds or synthetic materials, hydrogel, metal, or composite of organic and non-organic materials. However, the new generation of scaffolds involves loading it with different growth factors or drugs rather than using a pure template (Bose et al., 2012).

As calcium phosphate constitutes the main inorganic phase of bone matrix and because of its properties in term of porosity, biocompatibility, and bioactivity, it has been thoroughly investigated as osteoconductive scaffold for culturing bone cells (Feng et al., 2014; Chen et al., 2014; Sulaiman et al., 2013). In addition, although controversial, it has been reported that the osteoinductive property of calcium phosphate can be promoted with the appropriate composition and geometry (Sun et al., 2011).

23.4.3 Bioactive factors

While the role of numerous bioactive molecules such as fibroblasts growth factors, insulin-like growth factors (IGF-I), and vascular endothelial growth factors in bone formation has been investigated (Allori et al., 2008), only two recombinant human

Table 23.2 Types of biomaterials used as a scaffold in bone engineering

Class	Example	Advantages	Disadvantage	Reference
Ceramic	Hydroxyapatite (HA)	Biocompatible Osteoconductive Similar to the chemical structure of inorganic phase of bone	Slow biodegradation Difficult to shape due to hardness, fragility, and brittleness	Petrovic et al. (2012)
	Tricalcium phosphate	Same to above	Rigid and fragile Faster resorption rate	Miño Fariña et al. (2012)
	Bioglass	Biocompatible. Osteoconductive Bioactive Promote angiogenesis Enhance cell adhesion and proteins adsorption Easy to control the chemical composition Controlled degradation rate	Brittleness Low resistance to crack due to low strength and fracture toughness	Fu et al. (2011)
Polymer				
Natural proteins	Collagen, fibrin, alginate, silk fibroin, hyaluronic acid	Biocompatible Biodegradable without inflammation Bioactive	Poor mechanical strength Rapid resorption	Polo-Corrales et al. (2014)
Natural Polysaccharides	Chitosan	Biodegradable biocompatible Has as antibacterial and bioadhesive properties Promote wound healing	Same to above	Same to above

Synthetic	Polyglycolic acid (PGA)	Versatile Reproducible Thermoplastic so it can be shaped easily	Inflammatory or immune reaction due to acid release in enzymatic biodegradation Mechanical stability is of limited duration Less biocompatible than natural Not bioactive Rapid resorption Low solubility in organic solvent	(Carletti et al., 2011)
	Poly-L-lactide acid (PLLA)	Degrades slower and dissolves easier than PGA Reproducible	The potential to cause immune and foreign-body reactions because it does nor degrade completely The mechanical stability is of limited duration	
	Poly-ε-caprolactone (PCL)	Slow degradation rate Reproducible Good workability	Inflammatory or immune reaction mechanical stability is of limited duration	
	Hydrogel	Modified easily Biocompatible Biodegradable	Contracted Lack stiffness	Polo-Corrales et al. (2014)
Metal	Titanium mesh	High mechanical strength and fracture toughness Biocompatible	Corrosion may release toxic particles affecting the biocompatibility and induce an inflammatory reaction Poor stimulation of new bone formation due to the elastic moduli which does not correspond with natural bone	Chen et al. (2007)

(*Continued*)

Table 23.2 **(Continued)**

Class	Example	Advantages	Disadvantage	Reference
Hybrid	PGA/β-TCP	Enhanced ability for osteogenesis mineralization and biodegradation than HA	Lack osteoinductivity	Cao and Kuboyama (2009)
	Bioglass 45S5 and poly(D,L-lactide) polymer	Improved mechanical properties and resorption rate	Reaction with polymer changes the bioglass surface properties and compromised its bioactivity	Abdollahi et al. (2013)
	Poly (b-hydroxybutyrate-co-b-hydroxyvalerate) (PHBV) microsphere and poly (L-lactic-coglycolic acid) (PLGA)	Supports drugs and growth factors delivery	Changes in the surface topography and decrease porosity due to dehydration shrinkage	Huang et al. (2010)
	Hyaluronic acid-gelatine	Good mechanical property. Biocompatible High porosity Hydrophilic	Suboptimal cell adhesion due to negative cell-scaffold interaction	Linh et al. (2013)
	Nanoscale hydroxyapatite/polymer	Promote better cell adhesion and distribution No significant inflammatory response Biocompatible Improved mechanical properties	Unknown mechanism of cellular proliferation and differentiation	Sun et al. (2011)

proteins have been widely used and proved by Food and Drug Administration (FDA) for clinical purposes: bone morphogenic protein-2,-7 (rhBMP-2,-7) and Platelet-derived growth factor BB (rhPDGF-BB).

BMPs, which belong to transforming growth factor-B superfamily, are members of 30-member family with various cellular effects. For instance, BMP-2,4,6,7, and 9 showed evidence to induce mineralization, osteocalcin production, and orthotopic ossification. However, BMP-3 negatively regulated bone formation and exerted an inhibitory effect of orthotopic ossification induced by BMP-2,6,7 (Kang et al., 2004). PDGFs, by contrast, are the strongest chemotactic factors for on osteoblasts and stem cell precursors and have a potent mitogenic and activating effect on osteoclasts, fibroblasts, and endothelial cells (Allori et al., 2008).

Although these bioactive cues have an advantageous effect on bone formation, their application in bone engineering may be complicated by two factors. First: evidence indicates that the significant effect of these biomolecules is dose and duration dependent which may be difficult to achieve particularly in clinical application (Lieberman et al., 2002). For example, high risk of cancer has been associated with the clinical use of concentrated BMP products (AMPLIFYTM, rhBMP-2,40mg) which may suggest the relation of high dose with the carcinogenic effect (Devine et al., 2012). Second: in order to recapitulate the complex process of embryonic bone formation or bone regeneration, a simultaneous and/or stepwise delivery of a combination or cocktail of factors is required because these molecules act synergistically rather than solely. For example, IGF enhances bone cells migration whereas BMPs induce osteoprogenitor cells differentiation and proliferation (Allori et al., 2008).

To support prolong release and activity of multiple growth factors, several delivery strategies are envisioned. Multilayer fibrous scaffold incorporating more than one growth factor achieve a better result by simultaneous and sustained delivery of multiple biological signals (Shah et al., 2014; Yilgor et al., 2009). Microspheres carriers and nanostructured colloidal gelatine gels loaded with numerous bioactive cues are another controlled release strategies (Buket Basmanav et al., 2008; Van Der Stok et al., 2013).

23.4.4 Environmental factors

In tissue engineering the concept of simulating the native environment is derived from understanding of normal cell behavior. Normally, to survive and grow, cells must be able to import nutrients from their surrounding and regulate the concentration of various inorganic ions. This could be achieved by simple diffusion through which only few small nonpolar molecules can transfer across the cell membrane while the vast majority are unable to pass. Instead, their movement depends on active transport which need a driving force with an expenditure of energy to move the solute "uphill" against its concentration gradient (Alberts, 2013).

Static cell culture techniques rely on the diffusion transport which is sufficient to nourish only thin superficial layer, approximately 100–200 μm, contacting the medium. As the cells increase in number, so does the metabolic demand

and the build-up of waste products. Consequently, the cells in the tissue interior are deprived of oxygen and nutrients source. As such, maintenance of cell viability entails an effective vascular supply replicating the normal convective-diffusive transport. To circumvent this problem, several bioreactor technologies have been developed such as spinner flask, rotating wall vessels, and perfusion system (Chen and Hu, 2006; Dermenoudis and Missirlis, 2010; Martin et al., 2004; Zhong, 2010). A comparison of three systems carried out by Goldstein et al. (2001) showed that perfusion bioreactor yielded the most uniform cell distribution throughout the scaffold with a significant expression of alkaline phosphatase while cell density demonstrated preferential distribution at the scaffold exterior in spinner and rotating vessels. In bone engineering the bioreactor provides another advantage of exposing the cells to mechanical conditioning caused by fluid shear stress and this enhances osteogenic expression and produces more minerals and proteins (Gaspar et al., 2012; Martina and Giuseppe Maria de, 2014).

23.5 Challenges in bone tissue engineering

Despite the significant progress that has been achieved in bone engineering, many hurdles are still ahead. Identification of reliable and scalable cell source, optimization of scaffold properties, controlling of the in vitro microenvironment, and vascularization of the engineered bone are the main challenges for which the solutions continue to evolve (Logeart-avramoglou et al., 2005). This may explain the reason for lacking a proved and marketable cell-based engineered bone that can address the unmet clinical need regardless of the extensive research being conducted in this field. However, even the availability of such product does not imply its successful application. Possibly, a straightforward example in this context is BioSeed-oral bone which was first reported as a commercial engineered bone containing viable mandibular periosteal cells and has been used for augmentation of edentulous maxilla (Schmelzeisen et al., 2003). Nevertheless the outcome was not encouraging due to high resorption rate of the bone product that reached up to 90% compared to 29% of the autologous graft (Zizelmann et al., 2007). Such unfavorable result may pose a question regarding the long-term stability of any off-the-shelf cellular bone product and whether or not this postoperative complication can be encountered by using the current materials and techniques.

In spite of the aforementioned challenges the progress in bone engineering may be considered promising and it has encouraged investigators to reconstruct composite structure comprised of multiple tissues. For instance, an attempt has been carried out for osteochondral tissue engineering to exemplify the temporomandibular joint condyle (Alhadlaq et al., 2004; Rahaman and Mao, 2005) and the author's group has recently developed a full-thickness tissue-engineered bone and oral mucosa composite model (Almela et al., 2016) which has the potential to be further optimized for various in vitro and in vivo applications.

23.6 Future trends

While the vast majority of the research conducted in bone tissue engineering, relied on conventional methods, the new trend has directed toward the utilization of microfabrication technologies. In the commonly used top-down approach the cells and biomaterial are combined and cultured until the cells grow and form the engineered structure. Bottom up or modular tissue approach, by contrast, addresses the biomimetic concept by designing microscale building blocks through different ways such as cell aggregation, cell sheet, cell-laden hydrogel, or tissue printing which are then assembled to create macroscale tissue with specific microarchitectures (Nichol and Khademhosseini, 2009).

Bottom-up approach has been applied by Lima et al. (2014) to construct 3D biodegradable scaffolds by stacking 2D microfabricated polycaprolactone sheets of 200 μm thickness with precise microcavities and microcapillaries of 300 μm in diameter. The templates were seeded with human bone marrow derived mesenchymal stem cells (hBMSCs), human umbilical cord vein endothelial cells, and osteoblasts. The cells demonstrated attachment, proliferation, and maintenance of progenitor marker expression. This approach can be adopted to engineer a well-organized and vascularized bone in a precise, reproducible, and inexpensive way with the advantage of incorporating various materials, cells, and biological cues.

References

Abdollahi, S., Ma, A.C.C., Cerruti, M., 2013. Surface transformations of Bioglass 45S5 during scaffold synthesis for bone tissue engineering. Langmuir. 29, 1466.

Albee, F.H., 1920. Studies in bone growth: triple calcium phosphate as a stimulus to osteogenesis. Annal. Surg. 71, 32.

Alberts, B., 2013. Essential Cell Biology. Garland Science, New York, NY.

Alhadlaq, A., Elisseeff, J., Hong, L., Williams, C., Caplan, A., Sharma, B., et al., 2004. Adult stem cell driven genesis of human-shaped articular condyle. Annal. Biomed. Eng. 32, 911−923.

Allori, A., Sailon, A., Warren, S., 2008. Biological basis of bone formation, remodeling, and repair—Part I: biochemical signaling molecules. Tissue Eng. B: Rev. 14 (3), 259−273.

Almela, T., Brook, I.M., Moharamzadeh, K., 2016. Development of three-dimensional tissue engineered bone-oral mucosal composite models. J. Mater. Sci. Mater. Med. 27, 65.

An, B.W., Kim, K., Lee, H., Kim, S.Y., Shim, Y., Lee, D.Y., et al., 2015. High-resolution printing of 3D structures using an electrohydrodynamic inkjet with multiple functional inks. Adv. Mater. 27, 4322−4328.

Bajaj, P., Schweller, R.M., Khademhosseini, A., West, J.L., Bashir, R., 2014. 3D biofabrication strategies for tissue engineering and regenerative medicine. Annu. Rev. Biomed. Eng. 16, 247−276.

Beller, G., 2010. The cost of our aging population. J. Nucl. Cardiol. 17, 345−346.

Ben-Nissan, B., Nissan, B.B., Ben-Nissan, B., Nissan, B.B., 2014. Advances in Calcium Phosphate Biomaterials. Springer-Verlag, Berlin.

Berkovitz, B.K.B., 2009. Oral Anatomy, Histology and Embryology [Electronic resOurce]. Mosby, Edinburgh.

Biver, E., Soubrier, A.-S., Thouverey, C., Cortet, B., Broux, O., Caverzasio, J., et al., 2012. Fibroblast growth factor 2 inhibits up- regulation of bone morphogenic proteins and their receptors during osteoblastic differentiation of human mesenchymal stem cells. Biochem. Biophys. Res. Commun. 427, 737–742.

Bose, S., Roy, M., Bandyopadhyay, A., 2012. Recent advances in bone tissue engineering scaffolds. Trends Biotechnol. 30 (10), 546–554.

Buket Basmanav, F., Kose, G.T., Hasirci, V., 2008. Sequential growth factor delivery from complexed microspheres for bone tissue engineering. Biomaterials. 29, 4195–4204.

Buser, D., Halbritter, S., Hart, C., Bornstein, M.M., Grutter, L., Chappuis, V., et al., 2009. Early implant placement with simultaneous guided bone regeneration following single-tooth extraction in the esthetic zone: 12-month results of a prospective study with 20 consecutive patients. J. Periodontol. 80, 152–162.

Cao, H., Kuboyama, N., 2009. A biodegradable porous composite scaffold of PGA/β-TCP for bone tissue engineering. Bone. 46, 386–395.

Carletti, E., Motta, A., Migliaresi, C., 2011. Scaffold for tissue engineering and 3D cell culture. In: Haycock, J.W. (Ed.), 3d Cell Culture: Methods and Protocols. *Humana Press, New York, NY*, pp. 17–39.

Catros, S., Fricain, J.C., Guillotin, B., Pippenger, B., Bareille, R., Remy, M., et al., 2011. Laser-assisted bioprinting for creating on-demand patterns of human osteoprogenitor cells and nano-hydroxyapatite. Biofabrication. 3, 025001.

Cavo, M., Scaglione, S., 2016. Scaffold microstructure effects on functional and mechanical performance: Integration of theoretical and experimental approaches for bone tissue engineering applications. Mater. Sci. Eng. C: Mater. Biol. Appl. 68, 872–879.

Chen, F., Feng, X., Wu, W., Ouyang, H., Gao, Z., Cheng, X., et al., 2007. Segmental bone tissue engineering by seeding osteoblast precursor cells into titanium mesh-coral composite scaffolds. Int. J. Oral Maxillofac. Surg. 36, 822–827.

Chen, H.-C., Hu, Y.-C., 2006. Bioreactors for tissue engineering. Biotechnol. Lett. 28, 1415–1423.

Chen, L., Hu, J., Ran, J., Shen, X., Tong, H., 2014. Preparation and evaluation of collagen-silk fibroin/hydroxyapatite nanocomposites for bone tissue engineering. Int. J. Biol. Macromol. 65, 1–7.

CWFI 2010. Center of workforce intellegence (CWFI). Oral and Maxillofacial Surgery: CfWI medical fact sheet and summary sheet. Available at: http://www.cfwi.org.uk/publications/oral-maxillofacial-surgery-cfwi-medical-fact-sheet-and-summary-sheet-august-2010.

Daculsi, G., Fellah, B.H., Miramond, T., Durand, M., 2013. Osteoconduction, osteogenicity, osteoinduction, what are the fundamental properties for a smart bone substitutes. IRBM. 34 (4–5), 346–348.

de Peppo, G.M., Marcos-Campos, I., Kahler, D.J., Alsalman, D., Shang, L., Vunjak-Novakovic, G., et al., 2013. Engineering bone tissue substitutes from human induced pluripotent stem cells. Proc. Natl. Acad. Sci. U.S.A. 110, 8680.

Dermenoudis, S., Missirlis, Y.F., 2010. Bioreactors in tissue engineering. Adv. Eng. Mater. 12, B592–B608.

Devine, J.G., Dettori, J.R., France, J.C., Brodt, E., Mcguire, R.A., 2012. The use of rhBMP in spine surgery: is there a cancer risk? Evidence-Based Spine-Care J. 3, 35.

Eke, P.I., Dye, B.A., Wei, L., Thornton-Evans, G.O., Genco, R.J., 2012. Prevalence of periodontitis in adults in the United States: 2009 and 2010. J. Dental Res. 91, 914–920.

El Tamer, M.K., Reis, R.L., 2009. Progenitor and stem cells for bone and cartilage regeneration. J. Tissue Eng. Regen. Med. 3, 327−337.
Elsalanty, M.E., Genecov, D.G., 2009a. Bone grafts in craniofacial surgery. Craniomaxillofac. Trauma Reconstruct. 2, 125.
Elsalanty, M.E., Genecov, D.G., 2009b. Bone grafts in craniofacial surgery. Craniomaxillofac. Trauma Reconstr. 2, 125−134.
Farzadi, A., Solati-Hashjin, M., Asadi-Eydivand, M., Osman, N., 2014. Effect of layer thickness and printing orientation on mechanical properties and dimensional accuracy of 3D printed porous samples for bone tissue engineering: e108252. PLoS One. 9 (9), e108252.
Fedorovich, N.E., Alblas, J., Hennink, W.E., Oner, F.C., Dhert, W.J., 2011. Organ printing: the future of bone regeneration? Trends Biotechnol. 29, 601−606.
Feng, P., Wei, P.P., Li, P., Gao, C.D., Shuai, C.J., Peng, S., 2014. Calcium silicate ceramic scaffolds toughened with hydroxyapatite whiskers for bone tissue engineering. Mater. Characterization. 97, 47−56.
Ferlin, K.M., Prendergast, M.E., Miller, M.L., Kaplan, D.S., Fisher, J.P., 2016. Influence of 3D printed porous architecture on mesenchymal stem cell enrichment and differentiation. Acta Biomater. 32, 161−169.
Fisher, J.P., 2013. Tissue Engineering: Principles and Practices. CRC Press, Boca Raton.
Franz-odendaal, T.A., Hall, B.K., Witten, P.E., 2006. Buried alive: how osteoblasts become osteocytes. Dev. Dyn. 235 (1), 176−190.
Fu, Q., Saiz, E., Rahaman, M.N., Tomsia, A.P., 2011. Bioactive glass scaffolds for bone tissue engineering: state of the art and future perspectives. Mater. Sci. Eng. C. 31, 1245−1256.
Gaspar, D.A., Gomide, V., Monteiro, F.J., 2012. The role of perfusion bioreactors in bone tissue engineering. Biomatter. 2, 167.
Gibbons, M.C., Foley, M.A., Cardinal, K., 2013. Thinking inside the box: keeping tissue-engineered constructs in vitro for use as preclinical models. Tissue Eng. B-Rev. 19 (1), 14−30.
Goldstein, A.S., Juarez, T.M., Helmke, C.D., Gustin, M.C., Mikos, A.G., 2001. Effect of convection on osteoblastic cell growth and function in biodegradable polymer foam scaffolds. Biomaterials. 22, 1279−1288.
Gu, Z., Zhang, X., Liu, F., Yu, X., Li, L., Xie, H., 2013. Application of strontium-doped calcium polyphosphate scaffold on angiogenesis for bone tissue engineering. J. Mater. Sci.: Mater. Med. 24 (5), 1251−1260.
Haycock, J.W., 2011. 3D Cell Culture: Methods and Protocols. Humana Press, New York, NY.
Henrich, D., Wilhelm, K., Warzecha, J., Frank, J., Barker, J., Marzi, I., et al., 2013. Human endothelial-like differentiated precursor cells maintain their endothelial characteristics when cocultured with mesenchymal stem cell and seeded onto human cancellous bone. Mediators Inflamm. 2013, Article ID: 364591.
Hjorting-Hansen, E., 2002. Bone grafting to the jaws with special reference to reconstructive preprosthetic surgery. A historical review. Mund Kiefer Gesichtschir. 6, 6−14.
Huang, W., Shi, X., Ren, L., Du, C., Wang, Y., 2010. PHBV microspheres—PLGA matrix composite scaffold for bone tissue engineering. Biomaterials. 31, 4278−4285.
Jin, G.Z., Kim, T.H., Kim, J.H., Won, J.E., Yoo, S.Y., Choi, S.J., et al., 2013. Bone tissue engineering of induced pluripotent stem cells cultured with macrochanneled polymer scaffold. J. Biomed. Mater. Res. A. 101, 1283−1291.
Kang, Q., Sun, M.H., Cheng, H., Peng, Y., Montag, A.G., Deyrup, A.T., et al., 2004. Characterization of the distinct orthotopic bone- forming activity of 14 BMPs using recombinant adenovirus-mediated gene delivery. Gene Ther. 11, 1312.

Khan, Y., Yaszemski, M., Mikos, A., Laurencin, C., 2008. Tissue engineering of bone: material and matrix considerations. J. Bone Joint Surg. Am. 90((Suppl 1), 36–42.

Kim, S., Kim, S.-S., Lee, S.-H., Eun Ahn, S., Gwak, S.-J., Song, J.-H., et al., 2008. In vivo bone formation from human embryonic stem cell-derived osteogenic cells in poly(d, l-lactic- co-glycolic acid)/hydroxyapatite composite scaffolds. Biomaterials. 29, 1043–1053.

Kokemueller, H., Spalthoff, S., Nolff, M., Tavassol, F., Essig, H., Stuehmer, C., et al., 2010. Prefabrication of vascularized bioartificial bone grafts in vivo for segmental mandibular reconstruction: experimental pilot study in sheep and first clinical application. Int. J. Oral Maxillofac. Surg. 39, 379–387.

Kuznetsov, S.A., Cherman, N., Robey, P.G., 2011. In vivo bone formation by progeny of human embryonic stem cells. Stem Cells Dev. 20, 269–287.

Lichte, P., Pape, H.C., Pufe, T., Kobbe, P., Fischer, H., 2011. Scaffolds for bone healing: concepts, materials and evidence. Injury. 42, 569–573.

Lieberman, J.R., Daluiski, A., Einhorn, T.A., 2002. The role of growth factors in the repair of bone. Biology and clinical applications. J. Bone Joint Surg. Am. 84-A, 1032–1044.

Lima, M.J., Pirraco, R.P., Sousa, R.A., Neves, N.M., Marques, A.P., Bhattacharya, M., et al., 2014. Bottom-up approach to construct microfabricated multi-layer scaffolds for bone tissue engineering. Biomed. Microdev. 16, 69–78.

Linh, N., Min, Y., Lee, B.-T., 2013. Fabrication and in vitro evaluations with osteoblast-like MG-63 cells of porous hyaluronic acid-gelatin blend scaffold for bone tissue engineering applications. J. Mater. Sci. 48, 4233–4242.

Liu, J., Chen, W., Zhao, Z., Xu, H.H.K., 2013. Reprogramming of mesenchymal stem cells derived from iPSCs seeded on biofunctionalized calcium phosphate scaffold for bone engineering. Biomaterials. 34, 7862–7872.

Logeart-avramoglou, D., Anagnostou, F., Bizios, R., Petite, H., 2005. Engineering bone: challenges and obstacles. J. Cell. Mol. Med. 9, 72–84.

Macewen, W., 1881. Observations concerning transplantation of bone. Illustrated by a case of inter-human osseous transplantation, whereby over two-thirds of the shaft of a humerus was restored. Proc. R. Soc. Lond. 32, 232–247.

Mangano, C., Piattelli, A., Mangano, A., Mangano, F., Mangano, A., Iezzi, G., et al., 2009. Combining scaffolds and osteogenic cells in regenerative bone surgery: a preliminary histological report in human maxillary sinus augmentation. Clin. Implant Dent. Related Res. 11, e92–e102.

Manolagas, S.C., 2000. Birth and death of bone cells: Basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr. Rev. 21 (2), 115–137.

Martin, I., Wendt, D., Heberer, M., 2004. The role of bioreactors in tissue engineering. Trends Biotechnol. 22, 80–86.

Martin, R.B., 1998. Skeletal Tissue Mechanics. Springer, New York/London.

Martina, S., Giuseppe Maria De, P., 2014. Bioreactor systems for human bone tissue engineering. Processes. 2, 494–525.

McCauley, L.K., Somerman, M.J., 2012. Mineralized Tissues in Oral and Craniofacial Science: Biological Principles and Clinical Correlates. *Wiley-Blackwell*, *Ames, Iowa, Chichester*.

Melchels, F.P.W., Domingos, M.A.N., Klein, T.J., Malda, J., Bartolo, P.J., Hutmacher, D.W., 2012. Additive manufacturing of tissues and organs. Progress in Polymer Science. 37, 1079–1104.

Miloro, M., Kolokythas, A., 2012. Management of Complications in Oral and Maxillofacial Surgery. Wiley-Blackwell, Chichester, West Sussex, UK.

Miño Fariña, N., Muñoz Guzón, F., López Peña, M., Ginebra Molins, M.P., Valle Fresno, S.D., Ayala Vallespí, M.D., et al., 2012. Quantitative analysis of the resorption and osteoconduction of a macroporous calcium phosphate bone cement for the repair of a critical size defect in the femoral condyle. Vet. J. 179 (2), 264−272.

Murphy, C.M., Haugh, M.G., Amp, A.P.O.S., Brien, F.J., 2010. The effect of mean pore size on cell attachment, proliferation and migration in collagen− glycosaminoglycan scaffolds for bone tissue engineering. Biomaterials. 31, 461−466.

Nair, K., Gandhi, M., Khalil, S., Yan, K.C., Marcolongo, M., Barbee, K., et al., 2009. Characterization of cell viability during bioprinting processes. Biotechnol. J. 4, 1168−1177.

Nanci, A., 2013. Ten Cate's Oral Histology: Development, Structure, and Function. Elsevier, St. Louis, Mo.

Nichol, J.W., Khademhosseini, A., 2009. Modular tissue engineering: engineering biological tissues from the bottom up. Soft Matter. 5, 1312−1319.

Niemeyer, P., Fechner, K., Milz, S., Richter, W., Suedkamp, N.P., Mehlhorn, A.T., et al., 2010. Comparison of mesenchymal stem cells from bone marrow and adipose tissue for bone regeneration in a critical size defect of the sheep tibia and the influence of platelet-rich plasma. Biomaterials. 31, 3572−3579.

Obregon, F., Vaquette, C., Ivanovski, S., Hutmacher, D.W., Bertassoni, L.E., 2015. Three-dimensional bioprinting for regenerative dentistry and craniofacial tissue engineering. J. Dent. Res. 94, 143S−152S.

Olszta, M.J., Cheng, X., Jee, S.S., Kumar, R., Kim, Y.-Y., Kaufman, M.J., et al., 2007. Bone structure and formation: a new perspective, Mater. Sci. Eng. R, 58. pp. 77−116.

Peng, R., Yao, X., Cao, B., Tang, J., Ding, J., 2012. The effect of culture conditions on the adipogenic and osteogenic inductions of mesenchymal stem cells on micropatterned surfaces. Biomaterials. 33, 6008−6019.

Petrovic, V., Zivkovic, P., Petrovic, D., Stefanovic, V., 2012. Craniofacial bone tissue engineering. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 114, E1−E9.

Polo-Corrales, L., Latorre-Esteves, M., Ramirez-Vick, J.E., 2014. Scaffold design for bone regeneration. J. Nanosci. Nanotechnol. 14, 15−56.

Pradel, W., Lauer, G., 2012. Tissue-engineered bone grafts for osteoplasty in patients with cleft alveolus. Annal. Anat. 194, 545−548.

Rahaman, M.N., Mao, J.J., 2005. Stem cell-based composite tissue constructs for regenerative medicine. Biotechnol. Bioeng. 91 (3), 261−284.

Ross, M.H., 2016. Histology: A Text and Atlas: with Correlated Cell and Molecular Biology. Wolters Kluwer, Philadelphia.

Sanan, A., Haines, S.J., 1997. Repairing holes in the head: a history of cranioplasty. Neurosurgery. 40 (3), 588−603.

Scheller, E.L., Krebsbach, P.H., Kohn, D.H., 2009. Tissue Engineering: State of the Art in Oral Rehabilitation. Oxford, UK, pp. 368−389.

Schmelzeisen, R., Schimming, R., Sittinger, M., 2003. Making bone: implant insertion into tissue-engineered bone for maxillary sinus floor augmentation—a preliminary report. J. Cranio-Maxillofac. Surg. 31, 34−39.

Seebach, C., Henrich, D., Kaehling, C., Wilhelm, K., Tami, A., Alini, M., et al., 2010. Endothelial progenitor cells and mesenchymal stem cells seeded onto beta—TCP granules enhance early vascularization and bone healing in a critical-sized bone defect in rats. Tissue Eng. A: Tissue Eng. 16, 1961−1970.

Shah, N.J., Hyder, M.N., Quadir, M.A., Dorval Courchesne, N.-M., Seeherman, H.J., Nevins, M., et al., 2014. Adaptive growth factor delivery from a polyelectrolyte coating promotes synergistic bone tissue repair and reconstruction. Proc. Natl. Acad. Sci. U.S.A. 111, 12847.

Shrivats, A.R., Mcdermott, M.C., Hollinger, J.O., 2014. Bone tissue engineering: state of the union. Drug Discov. Today. 19, 781−786.

Spector, M., 2002. Novel cell- scaffold interactions encountered in tissue engineering: contractile behavior of musculoskeletal connective tissue cells. Tissue Eng. 8 (3), 351−357.

Sulaiman, S., Keong, T., Cheng, C., Saim, A., Idrus, R., 2013. Tricalcium phosphate/hydroxyapatite (TCP-HA) bone scaffold as potential candidate for the formation of tissue engineered bone. Ind. J. Med. Res. 137, 1093−1101.

Sun, F., Zhou, H., Lee, J., 2011. Various preparation methods of highly porous hydroxyapatite/polymer nanoscale biocomposites for bone regeneration. Acta Biomaterialia. 7, 3813−3828.

van der Stok, J., Siebelt, M., Sandker, M., Waarsing, J.H., Verhaar, J.A.N., Jahr, H., et al., 2013. Enhanced bone regeneration of cortical segmental bone defects using porous titanium scaffolds incorporated with colloidal gelatin gels for time-and dose-controlled delivery of dual growth factors. Tissue Eng. A. 19, 2605−2614.

van der Worp, H.B., Howells, D.W., Sena, E.S., Porritt, M.J., Rewell, S., Collins, V., et al., 2010. Can animal models of disease reliably inform human studies? PLoS Med. 7, e1000245.

Wang, M.O., Vorwald, C.E., Dreher, M.L., Mott, E.J., Cheng, M.H., Cinar, A., et al., 2015. Evaluating 3D-printed biomaterials as scaffolds for vascularized bone tissue engineering. Adv. Mater. 27, 138−144.

Williams, D.F., 2008. On the mechanisms of biocompatibility. Biomaterials. 29, 2941−2953.

Woodard, J.R., Hilldore, A.J., Lan, S.K., Park, C.J., Morgan, A.W., Eurell, J.A.C., et al., 2007. The mechanical properties and osteoconductivity of hydroxyapatite bone scaffolds with multi-scale porosity. Biomaterials. 28, 45−54.

Yang, H., Gao, L.-N., An, Y., Hu, C.-H., Jin, F., Zhou, J., et al., 2013. Comparison of mesenchymal stem cells derived from gingival tissue and periodontal ligament in different incubation conditions. Biomaterials. 34, 7033−7047.

Yilgor, P., Tuzlakoglu, K., Reis, R.L., Hasirci, N., Hasirci, V., 2009. Incorporation of a sequential BMP-2/BMP-7 delivery system into chitosan-based scaffolds for bone tissue engineering. Biomaterials. 30, 3551−3559.

Zhong, J.-J., 2010. Recent advances in bioreactor engineering. Korean J. Chem. Eng. 27, 1035−1041.

Zhou, H., Weir, M.D., Xu, H.H.K., 2011. Effect of cell seeding density on proliferation and osteodifferentiation of umbilical cord stem cells on calcium phosphate cement-fiber scaffold. Tissue Eng. A. 17, 2603.

Zizelmann, C., Schoen, R., Metzger, M.C., Schmelzeisen, R., Schramm, A., Dott, B., et al., 2007. Bone formation after sinus augmentation with engineered bone. Clin. Oral Implants Res. 18, 69−73.

Periodontal and peri-implant hard tissue regeneration

24

Zahrasadat Paknejad, Maissa Jafari, Pantea Nazeman, Maryam Rezai Rad and Arash Khojasteh
Shahid Beheshti University of Medical Sciences, Tehran, Iran

24.1 Introduction

In recent decades, tooth loss and its complications have turned into a disturbing concern which leads to unacceptable appearance and negative social consequences. Thus numerous treatment approaches for replacement of lost teeth have been developed in order to overcome this paralyzing ailment. Dental implants are one of these therapeutic modalities which have been introduced by Prof. Branemark in 1960s and ever since application of implants by clinicians and researchers have promoted the science of implantology.

Despite of promising achievements in implants, prolonged edentulism causes ridge defects which compromises the implant placement. Peri-implant bone mass has a crucial role in generation of the implant-bone contact. Hence, the process of bone augmentation is mandatory for successful dental implant placement.

Instantly after implant insertion, a cascade of events launches to occur. At the first stage accumulation of blood and extracellular fluid forms a water molecule layer around the implant surface. Following stage which is characterized by extracellular matrix development due to protein adsorption takes place within few hours (Chug et al., 2013). Extracellular matrix is a medium which participates in information transferring via a number of proteins including collagen I, fibronectin, thrombospondin, osteoadherin, bone sialoprotein, and biglycin which play a role in cell adhesion, migration, and differentiation take several days (Puleo and Nanci, 1999; Cooper, 1998; Chug et al., 2013). Differentiated osteoblasts not only contribute to bone formation, but also, inhibit osteoclastic bone resorption via secretion of osteoprotegerin (Baron, 2003). On this account, lack of sufficient bone support and inappropriate bone quality are the most common local reasons of osseointegration failure. Several approaches have been attempted to overcome the insufficient bone volume over the years. In the present chapter, following approaches have been categorized into conventional and tissue engineering approaches.

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00024-4
© 2017 Elsevier Ltd. All rights reserved.

24.2 Conventional approaches

24.2.1 Dental prosthesis and preprosthetic surgery

Prior to evolution of implants in the field of dentistry, lost teeth were replaced by prosthesis including fixed-partial and removable dentures. Application of these remedies requires appropriate bone and mucosal bed to provide sufficient support and stability of the prosthesis. Preprosthetic surgery is the term used for any procedure related to either hard/soft tissue correction or augmentation before prosthetic treatment. Removal of tissue interferences such as bony spikes, mylohyoid ridge, genial tubercle, hyperplastic tuberosity, exostosis, hyperplastic, hypertrophic, and hypermobile soft tissue are recognized as preprosthetic and reconstructive surgeries (Miloro et al., 2004). However, currently, it is recommended to preserve the remaining bone and even increase the bone mass by augmentation techniques instead of reducing bone interferences (Miloro et al., 2004). The greatest advantage of this approach is increasing the bed and support for future prosthesis (Van den Bergh et al., 1998).

24.2.2 Anatomic repositioning

24.2.2.1 Distraction osteoogenesis (gradual repositioning)

Distraction osteoogenesis (DO) is based on bone generation following segmental bone separation in an osteotomy site in order to correct maxillofacial deformities (Fig. 24.1) and has recommended to rectify the atrophic and deficit alveolar ridges in vertical and horizontal dimensions without any morbidity of harvesting site. Alveolar DO allows bone augmentation along with the nearby soft tissue recontouring gradually, providing more predictable outcomes and less relapses (7). Although introductory reports of DO application for ridge augmentation were encouraging, controversies still exist for deciding which of the treatments is the preferable choice for ridge correction. Previously, no difference was demonstrated between ramus-harvested autografts and DO in vertical bone height gain (Kim et al., 2013; Chiapasco et al., 2007). Prospective studies of Chiapasco revealed that although both DO and guided bone regeneration (GBR) techniques benefit vertically

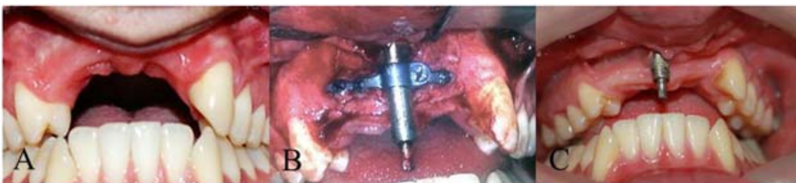


Figure 24.1 View of the patient with premaxillary deficiency. (A) Anterior maxillary defect before DO. (B) The inserted distractor device. (C) Increased alveolar bone height after DO. Figure obtained from Khojasteh, A., Esmaeelinejad, M., Aghdashi, F., 2015. Regenerative techniques in oral and maxillofacial bone grafting. Regenerative Tech. Oral Maxillofac. Bone Grafting. Permissions granted from Intech publisher.

resorbed ridges, DO appeared to be more predictable and the success rate of implants installed in DO augmented ridges were greater than the other group (Chiapasco et al., 2004a, 2004b). However, DO may be prone to several challenges including relapse, tooth injury, neurosensory disturbance which limits its application (Master et al., 2010).

24.2.2.2 Inlay bone grafting (acute repositioning)

Rehabilitation of atrophic bone by interpositional grafts after segmental osteotomy was first introduced in 1976 for reconstruction of anterior mandible and improving the denture retention (Schettler, 1976). Then the "inlay grafting" or "sandwich technique," which implies to positioning the graft materials within a 3- to 5-walled cancellous compartment, has been advocated for bone and ridge augmentation prior to denture fabrication or implant insertion (López-Cedrún, 2011). Although the osteotomy cavity can be filled by various substitutional materials, the bone mass achieved by this technique does not have acceptable volume. Bechara and colleagues conducted a split-mouth design study using hydroxyapatite (HA) and autogenous inlay graft materials for posterior mandible ridge augmentation. Their study showed that more new bone formation observed by autografting compared to HA-received sites (Bechara et al., 2014). Inlay grafting has been appreciated as an alternative treatment to block onlay grafts or DO for bone reconstruction (Fig. 24.2). Felice et al. compared the efficacy and safety of inlay and onlay grafts for vertical augmentation of atrophic mandibles. They reported that inlay grafts achieved approximately identical final vertical augmentation due to less resorption and less bone gain; however, the technique was more prone to complications and required more intricate surgery. Also, implant survival and success rate were similar in both techniques (Felice et al., 2009). Similarly, DO has shown greater complication rate but this procedure gained significantly more bone height when compared to inlay grafting (Bianchi et al., 2008). Besides to difficulty of surgery and other complications of segmental sandwich technique, it is documented with high success rate of augmented bone and well survival of the inserted dental implants. Thus inlay grafting should be considered as an appropriate method for bone rehabilitation (Kamperos et al., 2017).

Figure 24.2 Inlay bone grafting. (A and B) Segmental osteotomy. (C) Graft placement and fixation.

24.2.2.3 Inferior alveolar nerve lateralization

A 2 mm distance is recommended between the alveolar canal and the implant while in posterior segments of severely atrophic mandibles this may not be achieved and inferior alveolar nerve (IAN) encroachment may be a challenge in this case (Morad and Khojasteh, 2013). Several measures are developed to avoid this sequala such as nerve lateralization (Fig. 24.3). In this surgery, IAN is freed from the nerve canal and implants are placed in the jaw, and afterwards the nerve will be placed over the implants (Hashemi, 2010). The major pitfall of this approach is the subsequent neurosensory dysfunction and it was estimated that 95.9% of the patients experience this condition following the surgery (Vetromilla et al., 2014). Accordingly, some modifications are applied to the conventional inferior alveolar nerve lateralization (IANL) to decrease the subsequent neural dysfunction like making a platelet-rich fibrin (PRF) tube around the nerve (Khojasteh et al., 2016) or replacing surgical bur with piezotome (Díaz and Gías, 2013) yet no definite protocol is developed to avoid the neural dysfunction and discomfort.

24.2.3 Bone augmentation techniques

Bone augmentation includes various biological agents, biomaterials, and numerous alternative techniques and is indicated in variety of conditions as socket preservation, sinus augmentation and ridge augmentation. These surgeries may be accompanied with application of several graft materials including autografts, allografts, and xenografts. Autografts are obtained from the same patient receiving the grafts. Reconstruction of extremely atrophic mandible requires a large amount (70−140cc) of bone that could be harvested from extraoral sites, i.e., iliac crest (Khojasteh and Sadeghi, 2016), tibia, femur, and fibula and intraoral sites, i.e., symphis, ascending ramus, maxillary tuberosity (Khojasteh et al., 2016c) and anterior palate (Hassani

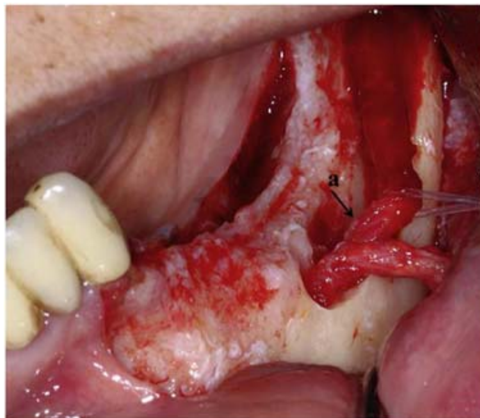


Figure 24.3 Lateralization of the IAN during augmentation or the implant placement (“a” indicates the IAN). Permissions granted from ELSEVIER publisher.

et al., 2008) which they may be harvested for minor augmentation procedures (Khojasteh et al., 2016c; Hassani et al., 2005; Damien and Parsons, 1991; Marx, 1993; Tolstunov, 2009; Motamedian et al., 2016b). Autografts are considered as the gold standard for tissue regeneration owing to their crucial advantages. Inherent biocompatibility and congruous cell antigens of autografts reduce the risk of rejection and immunologic response. Despite these advantages, inadequate quantity of provided bone, necessity of secondary surgery at the donor site, complicated surgery, morbidity, and complications at the donor site such as chronic pain (Chiarello et al., 2013; Damien and Parsons, 1991; Cowley and Anderson, 1983) may hamper their application. Allografts are derived from another individual of the same species, mostly from cadavers. To prevent the negative effects of heterogenicity and immunogenicity of allografts, sterilization and special processing techniques should be performed (Asselmeier et al., 1993). Although processing procedure is essential for preventing the immune rejection, it reduces the biological and biomechanical features of the graft (Vastel et al., 2004; Pelker and Friedlaender, 1987). The antigenicity of allografts could be reduced by freezing, freeze-drying, and demineralization. Although freeze-drying is more potent in eliminating immunogenic response comparing to conventional freezing, the osteoinduction capability and strength are further diminished along with the antigenicity (Ehrler and Vaccaro, 2000). Additional sterilization of freeze-dried grafts further weakens the osteoinductive potential (Sandhu et al., 1999; Nguyen et al., 2007). In contrast to autografts, these bone substitutes do not have any limitation in bone volume. However, the major pitfall of allografts is the transmission of diseases from the donors. Xenografts are attained from species other than the recipient. Bovine bone is one of the most common sources of these grafts. Bio-Oss, a deproteinized bovine bone mineral, is one of the most well-known commercially available products which has been widely used for bone augmentation (Hassani et al., 2009; Jafarian et al., 2008; Khojasteh et al., 2008). Genetic dissimilarity of xenografts and the subsequent immune response requires arduous preparation procedures, including deproteination and defatting of grafts, in addition to sterilization (Bauer and Muschler, 2000). Following these procedures, the grafts exhibit less immunogenicity, less osteoinductivity, and prolonged resorption time in the implantation site (Elves and Salama, 1974). These drawbacks render the use of grafts challenging, and this matter has spawned researchers to develop and apply the synthetic materials as bone substitutes.

24.2.3.1 Socket preservation

Socket compression following extraction is believed to reduce healing time, risk of dry sockets, and formation of tissue undercuts interfering prosthesis insertion (Duane Bennett, 2013). In socket preservation procedures the main goal is to preserve the bone volume by filling the socket with bone material substitutes. Demineralized freeze-dried bone allograft (DFDBA) (Froum et al., 2002; Becker et al., 1994; Wood and Mealey, 2012), freeze-dried bone allograft (FDBA) (Wood and Mealey, 2012), autograft (Becker et al., 1996; Araújo and Lindhe, 2011; Paolantonio et al., 2001), hard tissue replacement polymer (Serino et al., 2003;

Ashman, 1989), xenograft (Barone et al., 2008; Gholami et al., 2012), and HA (Gholami et al., 2012; Rothamel et al., 2008; Artzi et al., 2000) have been used as socket fillers, and expanded polytetrafluoroethylene (ePTFE) and high-density polytetrafluoroethylene (dPTFE) have been used as nonresorbable/bioabsorbable membranes (Zubillaga et al., 2003; Lekovic et al., 1998) and connective tissue barriers (Edel, 1995; Evian and Cutler, 1994), i.e., have been applied as barriers to stabilize the graft fillers to prevent epithelial cells migration and repopulation in the healing site (Rakhmatia et al., 2013). However, any of these materials have not demonstrated any further significant performance to the others (McAllister and Haghighat, 2007; Santis et al., 2011).

24.2.3.2 Sinus augmentation

Sinus floor pneumatization and alveolar ridge resorption cause inadequate bone volume in the upper jaw following tooth extraction. Therefore implantation of root fixtures in this area requires apico-occlusal augmentation of the residual alveolar bone. In case adequate bone quality and bone width are available to provide primary stability of the implants, 4 mm height can be assumed as the "cut off" measurement for sinus lifting indication (Chiapasco and Zaniboni, 2009). Sinus augmentation was first introduced by Boyne and James in 1980, by grafting the autogenous bone (Boyne, 1980; Tatum Jr, 1986; Tatum et al., 1993). Although numerous materials such as autologous bone, DFDBA, Bioglass, calcium sulfate, Bio-Oss, and HA have been used for sinus reconstruction, studies demonstrated that autografts are still the gold standard for sinus augmentation with nonsignificant priority and other substitutes are also compatible for this aim (Scarano et al., 2006; Schmitt et al., 2013; Shayesteh et al., 2008). Coverage of the bone materials by application of resorbable and nonresorbable membranes in the graft site suggested a significant increase in success rate of implants (Pjetursson et al., 2008).

24.2.3.3 Guided bone regeneration

To promote bone formation at the healing site the principles of GBR are practicable, which is defined as placing barrier membranes over a defect for space maintenance and providing the ideal environment for denovo bone formation. Osteogenesis of larger defects occurs mostly in the marginal zone and the central part contains merely a connective tissue with no evidence of bone formation. Thus the combined implementation of grafts and membranes is recommended (McAllister and Haghighat, 2007). Two kinds of membranes have been used in GBR; absorbable and nonresorbable. ePTFE, titanium reinforced ePTFE, dPTFE, or titanium mesh are the most commonly used nonresorbable membranes (da Silva Pereira et al., 2000; Jovanovic and Nevins, 1995; Bartee, 1995; Canullo and Malagnino, 2008). Nevertheless the necessity of second surgery to remove these barriers and greater risk of bone resorption after flap elevation in addition to colonization of oral bacteria due to exposure during healing time have limited their applications (Zitzmann et al., 1997; Rasmusson et al., 1997; Lekovic et al., 1998). Resorbable membranes are divided to

natural and synthetic groups. Collagen is a protagonist of natural membranes which is degraded by enzymatic process. Synthetic barriers including poly(lactic) and poly (glycolic) acid copolymers, subgroups of aliphatic polyesters, can be deteriorated by hydrolysis (Hutmacher et al., 1996; McAllister and Haghighat, 2007). However, the literature has suggested that coapplication of barriers with grafts is accompanied with more successful outcomes. In long-term follow-up prospective studies, it has been demonstrated that survival rate of implants placed in vertical augmented ridges by GBR membranes with or without bone grafts was comparable to the ones implanted in native or horizontally augmented ridges (Simion et al., 2001; Dahlin et al., 1991).

24.2.3.4 Onlay bone grafting

Reconstruction of combined bony defects representing both horizontal and vertical deficiencies necessitates alternative treatment modalities. Combination of GBR with onlay bone grafts harvested from the donor sites is a satisfactory modality for regeneration of small combined defects such as atrophic anterior maxilla (Fig. 24.4) (Khojasteh et al., 2015). In this surgical operation, according to the required amount of bone substitute, the onlay bone graft can be obtained from intraoral sources especially from the mandibular symphysis and ramus (Schwartz-Arad et al., 2005). Onlay block is placed and fixed in a prepared recipient site and followed by classic GBR procedure (Khojasteh et al., 2015). Based on a retrospective study of Schwartz-Arad et al. focused on the surgical success of intraoral autogenous onlay block for alveolar ridge augmentation, the average vertical gain was approximately 5.6 mm from the bottom of the vertical defect prior grafting to the top of the onlay block. In this study there were no relationship between complications or failure rates and type of bone source, length of recipient site, and recipient site areas (Schwartz-Arad et al., 2005). The advantages of this method are that it can be performed under local anesthesia

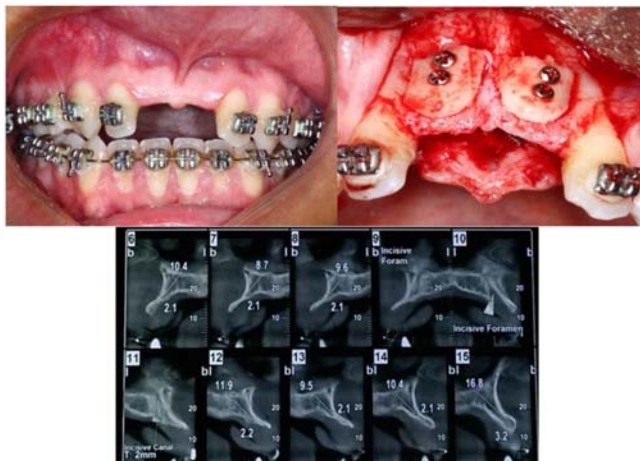


Figure 24.4 Onlay bone grafting and preoperative CBCT of the defect.

and there is no requirement for extraoral harvesting nor subsequent patient discomfort and pain (Khojasteh et al., 2015). However, high failure rate of this technique in reconstruction of large defects such as posterior of mandible is among the outstanding disadvantages (Nissan et al., 2011).

24.3 Tissue engineering

In the recent decades, cell-mediated therapies and tissue engineering have become more appealing approaches. In 1993, Langer and Vacanti defined tissue engineering as "an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain or improve tissue or organ function" (Langer, 1993). Tissue engineering can be considered as a three-parted complex; stem/progenitor cells, signaling mediators, and scaffolds. However, autografts in conventional approaches are still the standard of cure, the indications of tissue engineering in the medical fields have been outspreaded widely. Scaffolds by themselves mostly fail to stimulate bone healing and osteoinduction specifically in large defects. In the lack of bone repair other elements of periodontium precede osteoblasts and preclude bone formation. Utilization of growth factors and stem/progenitor cells compensates the problems caused by using scaffolds alone and benefits bone regeneration (Khojasteh et al., 2012).

Mesenchymal stem cells (MSCs) are adult stem cells obtained from various tissues and their greatest advantage is lack of ethical issues associated with embryonic stem cells (Khojasteh et al., 2016b) and tumorigenesis of induced pluripotent stem cells (Bastami et al., 2016). Stem cell therapy may be applicable in treatment of various conditions, such as local defects like deficient ridges (Khojasteh et al., 2012) or systemic diseases like osteoporosis (Yao and Lane, 2015). In local defects the cells may be delivered to the tissue by two major routes: systemic or local. In systemic delivery the MSCs are injected intravenously and it is hypothesized that the cells migrate to the defect however it is demonstrated that the majority of the cells home in the lungs and fail to participate in regeneration (Barbash et al., 2003). In local delivery, cells are culture expanded ex vivo and seeded on a scaffold and the complex is delivered to the defect. Although this method may be associated with several concerns, such as cell viability on the scaffold (Motamedian et al., 2015) and need for an interventional surgery at the defect (Du et al., 2014), but several studies have supported efficacy of this method in treatment of bone defects by 51.3% bone fill (Behnia et al., 2012). Our previous experiment also verified this assumption and we observed enhanced bone regeneration in mandibular defects when the cells were locally delivered rather than systemic (unpublished data) although systemic delivery may be beneficial for general conditions such as osteoporosis or osteogenesis imperfecta (Yao and Lane, 2015; Horwitz et al., 2002).

Available scaffolds for cell delivery generally fall into three main categories: polymers, ceramics, and composites. Natural bone which is composed of 70% inorganic component (HA) and 30% organic substance (mainly collagen), and ceramic

scaffolds have similar composition to natural bone, hence these materials may be an appropriate option for bone regeneration (Kamitakahara et al., 2008; Park, 1992). To list a few, HA, beta tricalcium phosphate (β-TCP), tetracalcium phosphate, and amorphous calcium phosphate are amid the most frequent research materials (Burg et al., 2000; Lee et al., 2001) and β-TCP has attracted more attention to serve as a scaffold for bone tissue engineering (Kamitakahara et al., 2008). The review on previous literature has also demonstrated enhanced outcomes when cells were delivered by scaffolds and synthetic scaffolds such as β-TCP and HA/TCP have demonstrated promising outcomes (Motamedian et al., 2016c). It was demonstrated that dental pulp stem cells (DPSCs) have the highest proliferation and attachment to β-TCP while FDBA demonstrated to have greater osteoinductive properties (Motamedian et al., 2016c). Hence the choice of right scaffold may be dictated by the clinical situation and requirements.

Finally, implementation of three key factors, scaffold, cell, and growth factors, has been assumed to have the best upshots in terms of tissue engineering as discussed here (Motamedian et al., 2016a). These new trends have been applied for augmentation of bone volume in sinus lifting and ridge augmentation as well as promoting the osseous integration at the implantation site (Khojasteh et al., 2012).

24.3.1 Mesenchymal stem cells in preprosthetic hard tissue engineering

As described earlier, sinus augmentation is routinely performed by virtue of autografts, xenografts, or allografts but due to their limitations, stem cells have been utilized in this condition to enhance the results. In a human study, bone marrow-derived mesenchymal stem cells (BMMSCs) were obtained from iliac crest of patients and culture expanded. The cells were implanted on HA/TCP scaffolds and placed below the sinus membrane to enhance bone formation followed by implant insertion in 3 months (Fig. 24.5). The results demonstrated 41.34% bone formation and 93% implant success rate (Shayesteh et al., 2008).

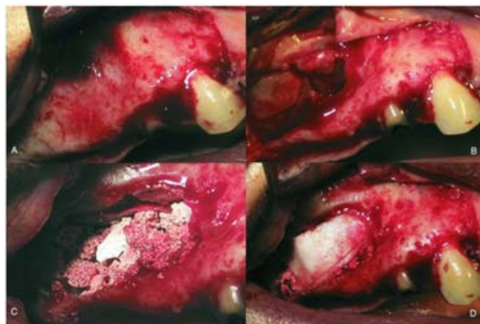


Figure 24.5 (A−D) Sinus lifting procedure with HA/TCP loaded with BMMSCs and collagen membrane coverage. Permissions granted from ELSEVIER publisher.

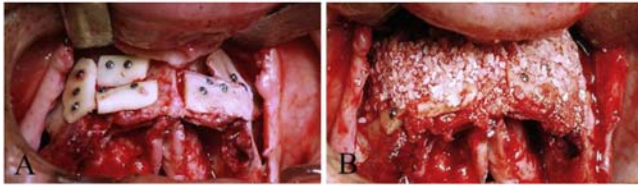


Figure 24.6 (A) Inserting onlay bone blocks over deficient ridge. (B) Covering the gaps with allografts + MSCs. Permissions granted from ELSEVIER publisher.

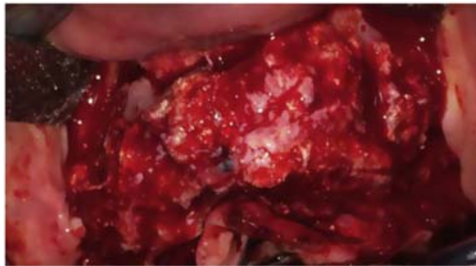


Figure 24.7 Clinical view of the augmented jaw 5 months following the surgery. Permissions granted from ELSEVIER publisher.

In a clinical study, onlay autogenous blocks of iliac crest were inserted over atrophic jaws and the gaps were filled with allografts loaded with buccal fat pad-derived stem cells and secured by a collagen membrane (Fig. 24.6). The cone beam computed tomography results demonstrated greater bone width in cell therapy group (3.94 mm vs 3.01 mm) and also greater bone formation (65.32% vs. 49.21%) at 5-months postoperation comparing to cell-free group and these results were indicative of enhanced bone formation and decreased secondary resorption (Khojasteh and Sadeghi, 2016) (Fig. 24.7).

These studies may reflect enhanced bone regeneration and reduced resorption by coapplication of bone substitution materials and stem cells, however, further studies are required to determine the best cell source and the scaffold to further boost regeneration potential and efficacy.

24.3.2 Mesenchymal stem cells in peri-implant hard tissue engineering

Utilization of graft materials in peri-implant bone regeneration has been studied and results indicated that implants installed in defects which were filled with xenografts have an almost identical degree of osseous integration with ones implanted in native bone (Berglundh and Lindhe, 1997). However, a study by Carmagnola showed that these grafts did not integrate with the native bone tissue and inserted implants did not exhibit osseous integration (Carmagnola et al., 2000, 2008).

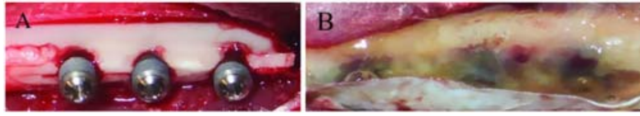


Figure 24.8 (A) Partially inserted implants in the rabbit tibia. (B) Placing bone particle + MSCs with fibrin glue around the implants. Permissions granted from Journal of Oral Implantology.

Despite these controversies, implanting particulate mineralized bone loaded with BMMSCs in combination with fibrin glue at the periphery of microthreaded section of the implants in rabbit tibia has demonstrated significantly increased vertical bone formation, 2.09 mm in the test group comparing to 1.03 mm in the allograft + fibrin glue (control). Also the amount of supracrestal bone formation was significantly greater in the test group comparing to the control, 28.5% versus 4.3%, respectively (Khojasteh et al., 2013b) (Fig. 24.8). Similarly, an injectable tissue engineered bone consisted of dog stem cells, platelet-rich plasma (PRP), and fibrin glue implanted simultaneously with titanium fixtures illustrated more bone-implant contact followed by MSCs/fibrin admixture and fibrin alone after 2, 4, and 8 weeks, respectively (Ito et al., 2006). Moreover, different graft types motivate new bone formation around implants to different degrees. Hassan indicated that immediate placement of dental implants accompanied by autogenous bone graft resulted in higher bone density and less marginal bone loss compared to application of synthetic bioabsorbable polylactic polyglycolic acid polymer for peri-impalnt reconstruction and increasing the osseous integration in a human investigation (Hassan et al., 2008). Furthermore, GBR technique can also promote the success of MSCs application for bone reconstruction around implants (Ribeiro et al., 2012).

Efficiency of MSCs derived from various sources was analyzed by Kim and his group. HA/TCP-loaded BMMSCs with scaffolds had the highest bone-implant contact and new bone formation in a canine model followed by periodontal ligament stem cells and cell-free samples, respectively (Kim et al., 2009). Periosteum-derived and BMMSCs have been compared for their osteogenic potential around dental implants and results showed that they have similar capability for bone reconstruction (Ribeiro et al., 2010). A comparative investigation carried out by Ito et al., compared DPSCs, BMMSCs, and periosteal cells (PCs) for bone regeneration around implants. They placed implants in the healed defects regenerated by the cells in addition to PRP and reported that DPSC exhibited the greatest bone regeneration potential in the implant site followed by BMMSCs and PCs (Ito et al., 2011).

The other potent stem cells for peri-implant bone regeneration are umbilical cord mesenchymal stem cells (UCMSCs). Hao et al. (2014) showed that injection of both UCMSCs and PRF into implant defects was more efficient to fulfill the osseous integration criteria compared to using PRF agent alone. Similar studies reported the potential of adipose-derived stem cells (ASCs) in accelerating bone regeneration and osseous integration (Bressan et al., 2015; Pieri et al., 2010). Further data

respecting implantation of ASCs in peri-implant defects suggested a dose−response relationship of cells; the higher concentration of the loading cells leads to greater new bone formation (Pieri et al., 2010).

24.3.3 Coapplication of mesenchymal stem cells with growth factors

Application of xenografts in combination with growth factors like PRP in bone tissue engineering is a thought-provoking scenario in literature. A study by Yun et al., verified that application of HA, BMMSCs, and PRP showed better results compared to these factors separately (Yun et al., 2014). Despite of preliminary hypothesis indicating that combined use of bone grafts and PRP would facilitate the osseous integration process, some studies revealed that addition of PRP to xenografts decreases the bone healing around dental implants (You et al., 2007; Sánchez et al., 2005). The given outcomes might be a consequent of inappropriate concentration of PRP. Although several studies performed on human and animal models demonstrated the capability of PRP for peri-implant and periodontal reconstruction (Lynch et al., 1989; Nevins et al., 2014, 2013), Weibrich et al. in 2004 reported the dose-dependent efficacy of PRP for regenerative therapies. In their study the optimal PRP concentration was measured at a narrow range approximate to 1,000,000/μl. They suggested that lower doses cause suboptimal response while higher amounts of PRP may have an inhibitory effect (Weibrich et al., 2004).

The other biologic agent which has gained attention for peri-implant bone regeneration is platelet-derived growth factor (PDGF). Two separate investigations described the outperformance of the combined usage of PDGF and bone graft materials to PDGF alone (Simion et al., 2006; Schwarz et al., 2009). Similarly, mixture of PDGF and xenografts applied for healing of dehiscence around implants showed thriving results in terms of bone height and bone-implant contact (Al-Hazmi et al., 2013). Other studies suggested that application of PDGF/insulin-like growth factor-I (IGF) complex resulted in considerable improvement in bone fill and bone-implant contact (Lynch et al., 1991). Similar findings by Howell et al., showed the higher osseous defect fill by 43.2% in PDGF/IGF group comparing to 18.5% in control group (Howell et al., 1997).

Incorporation of gene therapy with bioengineering science has been executed in a study by Chang et al., in which implant osseous integration accelerated by delivering the adenoviral vector encoding PDGF gene instead of conventional protein injection. Their results corroborated the comparable outcomes of this technique to recombinant human PDGF protein transportation in bone repair around implants (Chang et al., 2010). In addition, gene therapy has been utilized for delivering vascular endothelial growth factor (VEGF) and bone morphogenic protein-2 (BMP-2) for peri-implant bone regeneration. It is demonstrated that scaffolds containing VEGF protein and adenovirus expressing BMP-2 had the greatest outcomes (Luo et al., 2012). Similar results were obtained by delivering BMP-2 gene to bone grafts via liposomal vectors in peri-implant bone defects (Park et al., 2007).

Bone morphogenetic proteins, particularly rhBMP-2, are widely known biological agents due to their potential for bone regeneration which is also influenced by concentration, type of carrier, and dosage (Haÿ et al., 2002; Wikesjö et al., 2009; Khojasteh et al., 2013a; Bastami et al., 2017). Kim et al. studied the optimum dose of BMP-2 for bone formation using three different concentrations in a split-mouth study. It was suggested that concentrations of 0.5 and 1 mg/ml BMP-2 had higher bone volume and bone-implant contact (Kim et al., 2015). Exploration the dose-dependent effect of BMP-2 for bone augmentation revealed that the 1.5 mg/ml concentration of BMP-2 exhibited better responses (Fiorellini et al., 2001; Cochran et al., 2000; Boyne et al., 1997). Despite the fact that majority of the studies on BMP-2 demonstrated a favorable outcome in terms of bone healing, but application of this agent with bovine-derived deproteinized bone did not fulfill the expected results (Kao et al., 2012).

24.3.4 Other approaches

In addition to all mentioned investigations that have been conducted on triple components of tissue engineering, in order to improve bone growth at the implant site, several modifications have been applied on implant surface and implantation condition. One of the most well-known alterations of dental implants relates to increasing the surface roughness. Various procedures can be applied for achieving this aim such as acid etching, grit blasting, plasma spray, anodization, and coating (Fig. 24.9). Manual grit blasting by 50 μm particle size alumina powder did not accomplish in bone-implant responses compared to microrobotized blasted implants with 50, 100, and 150 μm particles. Additionally microrobotized implants with

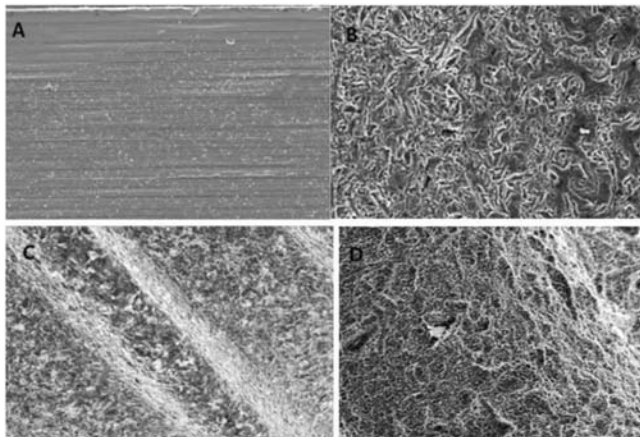


Figure 24.9 Titanium dental implant surfaces observed by scanning electron microscopy. (A) control, (B) acid-etched, (C) grit-blasted, and (D) grit-blasted and acid etched. Permissions granted from Springer publisher.

rougher surface texture, blasted by 50 μm particle size, outperform others when inserted in vivo (Gil et al., 2016).

Such like studies evaluated the influence of combined acid etching and grit blasting of implants on osseous integration and interestingly similar results were obtained indicating higher biomechanical fixation and bone regeneration of two step modified implants compared with each preparation alone (Coelho et al., 2016; Herrero-Climent et al., 2013; Gil et al., 2014; Aparicio et al., 2011). Coarsening the implant surfaces can be achieved by plasma spraying technique in which powders of different materials are heated to high temperatures, then are projected onto roughened implant surfaces (de Jonge et al., 2008) and the 20-year prospective follow-up spotlighted the great success rate of these modified implants (Chappuis et al., 2013). Anodization is the electrochemical procedure to roughen the surfaces in which the implant is immersed in an electrolyte while a current is applied, giving micropores with variable size (Le Guéhennec et al., 2007; Gupta et al., 2010). Ding et al. (2014) fabricated the titanium implants characterized by different nanotube diameters (30, 50, and 80 nm) via anodization and reported that anodized implants with 80 nm nanotubes were more favorable to promote osteogenesis.

Coating strategies also have been used in implant dentistry. Not only increased surface roughness, but also functionalization of implant surfaces via different bioactive materials is among the most beneficial features of these techniques. Coatings can be performed by numerous substances like collagen, sulfated hyaluronan, elastin-like peptide, keratin hydrogel, and arginylglycylaspartic acid peptide (RGD). Surface coverage by these proteins promotes early and rapid osseous integration, however further investigations are required to verify the concrete response (Schliephake et al., 2002; Campbell and Duncan, 2014; Raphel et al., 2016; Schulz et al., 2014).

Besides to proteins, other coating agents are ions such as fluoride, zinc, and magnesium. It is known that fluoride at the micromolar level promotes osteoblast activity and differentiation in addition to bone apposition, also antimicrobial features of this molecule is another provoking characteristic for bone tissue engineering (Farley et al., 1983; Cooper et al., 2006; Wiegand et al., 2007). According to the study of Wang et al. (2015), fluoride-coated titanium implants exhibited slightly higher bone-implant contact rates. Implementation of Zn and Mg ions on implant surface revealed the most rapid osseous integration and prolonged stability compared with implants coated by either ions (Yu et al., 2016).

Collectedly in order to compare the different surface treatment methods a 5-year follow-up study demonstrated that acid-etched implant surfaces lead to a higher success rate in comparison to coated and nonmodified groups (Oliva et al., 2010).

24.4 Conclusion and future direction

Above-mentioned scenario can be conducted to positively influence osseous integration of dental implants, certainly there are further parameters that promote peri-implant successful bone regeneration. The ability to induce and support vascular

infiltration is an essential parameter which needs to be considered in bone tissue engineering. Accordingly, novel techniques are developed to enhance the outcomes such as coculturing BMMSCs and endothelial progenitor cells (Khojasteh et al., 2016a), fabricating a scaffold with sustained release of growth factors such as BMP-2 or VEGF (Bastami et al., 2017) and transfection of MSCs with regulatory miRNA like anti-mir221 (Sadeghi et al., 2016).

References

Al-Hazmi, B.A., Al-Hamdan, K.S., Al-Rasheed, A., Babay, N., Wang, H.-L., Al-Hezaimi, K., 2013. Efficacy of using PDGF and xenograft with or without collagen membrane for bone regeneration around immediate implants with induced dehiscence-type defects: a microcomputed tomographic study in dogs. J. Periodontol. 84, 371−378.

Aparicio, C., Padrós, A., Gil, F.-J., 2011. In vivo evaluation of micro-rough and bioactive titanium dental implants using histometry and pull-out tests. J. Mech. Behav. Biomed. Mater. 4, 1672−1682.

Araújo, M.G., Lindhe, J., 2011. Socket grafting with the use of autologous bone: an experimental study in the dog. Clin. Oral Implants Res. 22, 9−13.

Artzi, Z., Tal, H., Dayan, D., 2000. Porous bovine bone mineral in healing of human extraction sockets. Part 1: histomorphometric evaluations at 9 months. J. Periodontol. 71, 1015−1023.

Ashman, A., 1989. An immediate tooth root replacement: an implant cylinder and synthetic bone combination. J. Oral Implantol. 16, 28−38.

Asselmeier, M.A., Caspari, R.B., Bottenfield, S., 1993. A review of allograft processing and sterilization techniques and their role in transmission of the human immunodeficiency virus. Am. J. Sports Med. 21, 170−175.

Barbash, I.M., Chouraqui, P., Baron, J., Feinberg, M.S., Etzion, S., Tessone, A., Miller, L., Guetta, E., Zipori, D., Kedes, L.H., Kloner, R.A., Leor, J., 2003. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. Circulation. 108, 863−868.

Baron, R., 2003. General principles of bone biology, Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, 5. Wiley-Blackwell, NJ, United States, pp. 1−8.

Barone, A., Aldini, N.N., Fini, M., Giardino, R., Calvo Guirado, J.L., Covani, U., 2008. Xenograft versus extraction alone for ridge preservation after tooth removal: a clinical and histomorphometric study. J. Periodontol. 79, 1370−1377.

Bartee, B.K., 1995. The use of high-density polytetrafluoroethylene membrane to treat osseous defects: clinical reports. Implant Dent. 4, 21−31.

Bastami, F., Nazeman, P., Moslemi, H., Rezai Rad, M., Sharifi, K., Khojasteh, A., 2016. Induced pluripotent stem cells as a new getaway for bone tissue engineering: a systematic review. Cell Prolifer. Available from: http://dx.doi.org/10.1111/cpr.12321.

Bastami, F., Paknejad, Z., Jafari, M., Salehi, M., Rad, M.R., Khojasteh, A., 2017. Fabrication of a three-dimensional β-tricalcium-phosphate/gelatin containing chitosan-based nanoparticles for sustained release of bone morphogenetic protein-2: implication for bone tissue engineering. Mater. Sci. Eng. C. 72, 481−491.

Bauer, T.W., Muschler, G.F., 2000. Bone graft materials: an overview of the basic science. Clin. Orthopaed. Related Res. 371, 10−27.

Bechara, K., Dottore, A.M., Kawakami, P.Y., Cassoni, A., Giro, G., Rodrigues, J.A., Chambrone, L., Piattelli, A., Iezzi, G., Shibli, J.A., 2014. Interpositional graft associated with alveolar osteotomy for posterior mandible ridge augmentation (sandwich osteotomy) using hydroxyapatite or autogenous bone: histological evaluation. POSEIDO. 2 (4), 233.

Becker, W., Becker, B.E., Caffesse, R., 1994. A comparison of demineralized freeze-dried bone and autologous bone to induce bone formation in human extraction sockets. J. Periodontol. 65, 1128–1133.

Becker, W., Urist, M., Becker, B.E., Jackson, W., Party, D.A., Bartold, M., Vincenzzi, G., de Georges, D., Niederwanger, M., 1996. Clinical and histologic observations of sites implanted with intraoral autologous bone grafts or allografts. 15 human case reports. J. Periodontol. 67, 1025–1033.

Behnia, H., Khojasteh, A., Soleimani, M., Tehranchi, A., Atashi, A., 2012. Repair of alveolar cleft defect with mesenchymal stem cells and platelet derived growth factors: a preliminary report. J. Craniomaxillofac. Surg. 40, 2–7.

Berglundh, T., Lindhe, J., 1997. Healing around implants placed in bone defects treated with Bio-Oss®. An experimental study in the dog. Clin. Oral Implants Res. 8, 117–124.

Bianchi, A., Felice, P., Lizio, G., Marchetti, C., 2008. Alveolar distraction osteogenesis versus inlay bone grafting in posterior mandibular atrophy: a prospective study. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol. 105, 282–292.

Boyne, P.J., 1980. Grafting of maxillary sinus floor with autogenous marrow and bone. J. Oral Surg. 38, 613–616.

Boyne, P.J., Marx, R.E., Nevins, M., Triplett, G., Lazaro, E., Lilly, L.C., Alder, M., Nummikoski, P., 1997. A feasibility study evaluating rhBMP-2/absorbable collagen sponge for maxillary sinus floor augmentation. Int. J. Periodontics Restorative Dent. 17, 11–25.

Bressan, E., Botticelli, D., Sivolella, S., Bengazi, F., Guazzo, R., Sbricoli, L., Ricci, S., Ferroni, L., Gardin, C., Velez, J.U., 2015. Adipose-derived stem cells as a tool for dental implant osseointegration: an experimental study in the dog. Int. J. Mol. Cell. Med. 4, 197.

Burg, K.J., Porter, S., Kellam, J.F., 2000. Biomaterial developments for bone tissue engineering. Biomaterials. 21, 2347–2359.

Campbell, D.I., Duncan, W.J., 2014. The effect of a keratin hydrogel coating on osseointegration: an histological comparison of coated and non-coated dental titanium implants in an ovine model. J. Maxillofac. Oral Surg. 13, 159–164.

Canullo, L., Malagnino, V.A., 2008. Vertical ridge augmentation around implants by e-PTFE titanium-reinforced membrane and bovine bone matrix: a 24-to 54-month study of 10 consecutive cases. Int. J. Oral Maxillofac. Implants. 23 (5), 858–866.

Carmagnola, D., Abati, S., Celestino, S., Chiapasco, M., Bosshardt, D., Lang, N.P., 2008. Oral implants placed in bone defects treated with Bio-Oss®, Ostim®-Paste or PerioGlas: an experimental study in the rabbit tibiae. Clin. Oral Implants Res. 19, 1246–1253.

Carmagnola, D., Berglundh, T., Araujo, M., Albrektsson, T., Lindhe, J., 2000. Bone healing around implants placed in a jaw defect augmented with Bio-Oss®. J. Clin. Periodontol. 27, 799–805.

Chang, P.-C., Seol, Y.-J., Cirelli, J.A., Pellegrini, G., Jin, Q., Franco, L.M., Goldstein, S.A., Chandler, L.A., Sosnowski, B., Giannobile, W.V., 2010. PDGF-B gene therapy accelerates bone engineering and oral implant osseointegration. Gene Ther. 17, 95–104.

Chappuis, V., Buser, R., Brägger, U., Bornstein, M.M., Salvi, G.E., Buser, D., 2013. Long-term outcomes of dental implants with a titanium plasma-sprayed surface: a 20-year

prospective case series study in partially edentulous patients. Clin. Implant Dent. Related Res. 15, 780−790.

Chiapasco, M., Consolo, U., Bianchi, A., Ronchi, P., 2004a. Alveolar distraction osteogenesis for the correction of vertically deficient edentulous ridges: a multicenter prospective study on humans. Int. J. Oral Maxillofac. Implants. 19 (3), 399−407.

Chiapasco, M., Romeo, E., Casentini, P., Rimondini, L., 2004b. Alveolar distraction osteogenesis vs. vertical guided bone regeneration for the correction of vertically deficient edentulous ridges: a 1−3-year prospective study on humans. Clin. Oral Implants Res. 15, 82−95.

Chiapasco, M., Zaniboni, M., 2009. Methods to treat the edentulous posterior maxilla: implants with sinus grafting. J. Oral Maxillofac. Surg. 67, 867−871.

Chiapasco, M., Zaniboni, M., Rimondini, L., 2007. Autogenous onlay bone grafts vs. alveolar distraction osteogenesis for the correction of vertically deficient edentulous ridges: a 2−4-year prospective study on humans. Clin. Oral Implants Res. 18, 432−440.

Chiarello, E., Cadossi, M., Tedesco, G., Capra, P., Calamelli, C., Shehu, A., Giannini, S., 2013. Autograft, allograft and bone substitutes in reconstructive orthopedic surgery. Aging Clin. Exp. Res. 25, 101−103.

Chug, A., Shukla, S., Mahesh, L., Jadwani, S., 2013. Osseointegration—molecular events at the bone−implant interface: a review. J. Oral Maxillofac. Surg. Med. Pathol. 25, 1−4.

Cochran, D.L., Jones, A.A., Lilly, L.C., Fiorellini, J.P., Howell, H., 2000. Evaluation of recombinant human bone morphogenetic protein-2 in oral applications including the use of endosseous implants: 3-year results of a pilot study in humans. J. Periodontol. 71, 1241−1257.

Coelho, P.G., Gil, L.F., Neiva, R., Jimbo, R., Tovar, N., Lilin, T., Bonfante, E.A., 2016. Microrobotized blasting improves the bone-to-textured implant response. A preclinical in vivo biomechanical study. J. Mech. Behav. Biomed. Mater. 56, 175−182.

Cooper, L.F., 1998. Biologic determinants of bone formation for osseointegration: clues for future clinical improvements. J. Prosthetic Dent. 80, 439−449.

Cooper, L.F., Zhou, Y., Takebe, J., Guo, J., Abron, A., Holmen, A., Ellingsen, J.E., 2006. Fluoride modification effects on osteoblast behavior and bone formation at TiO_2 grit-blasted cp titanium endosseous implants. Biomaterials. 27, 926−936.

Cowley, S., Anderson, L., 1983. Hernias through donor sites for iliac-bone grafts. J. Bone Joint Surg. Am. 65, 1023−1025.

da Silva Pereira, S.L., Sallum, A.W., Casati, M.Z., Caffesse, R.G., Weng, D., Nociti, F.H., Sallum, E.A., 2000. Comparison of bioabsorbable and non-resorbable membranes in the treatment of dehiscence-type defects. A histomorphometric study in dogs. J. Periodontol. 71, 1306−1314.

Dahlin, C., Lekholm, U., Linde, A., 1991. Membrane-induced bone augmentation at titanium implants. A report on ten fixtures followed from 1 to 3 years after loading. Int. J. Periodontics Restorative Dent. 11, 273.

Damien, C.J., Parsons, J.R., 1991. Bone graft and bone graft substitutes: a review of current technology and applications. J. Appl.Biomater. 2, 187−208.

de Jonge, L.T., Leeuwenburgh, S.C., Wolke, J.G., Jansen, J.A., 2008. Organic−inorganic surface modifications for titanium implant surfaces. Pharmaceut. Res. 25, 2357−2369.

Díaz, J.F., Gías, L.N., 2013. Rehabilitation of edentulous posterior atrophic mandible: inferior alveolar nerve lateralization by piezotome and immediate implant placement. Int. J. Oral Maxillofac. Surg. 42, 521−526.

Ding, X., Zhou, L., Wang, J., Zhao, Q., Lin, X., Gao, Y., Li, S., Wu, J., Rong, M., Guo, Z., 2014. The effects of hierarchical micro/nanosurfaces decorated with TiO_2 nanotubes on

the bioactivity of titanium implants in vitro and in vivo. Int. J. Nanomed. 10, 6955−6973.

Du, J., Shan, Z., Ma, P., Wang, S., Fan, Z., 2014. Allogeneic bone marrow mesenchymal stem cell transplantation for periodontal regeneration. J. Dent. Res. 93, 183−188.

Duane Bennett, I., 2013. Ridge dimensional changes: a comparative study of socket compression after dental extraction with no compression. Faculty of the School of Dentistry in Partial Fulfillment of the Requirements for the Degree of Master of Science in Dentistry, Indiana University.

Edel, A., 1995. The use of a connective tissue graft for closure over an immediate implant covered with an occlusive membrane. Clin. Oral Implants Res. 6, 60−65.

Ehrler, D.M., Vaccaro, A.R., 2000. The use of allograft bone in lumbar spine surgery. Clin. Orthopaed. Related Res. 371, 38−45.

Elves, M., Salama, R., 1974. A study of the development of cytotoxic antibodies produced in recipients of xenografts (heterografts) of iliac bone. Bone Joint J. 56, 331−339.

Evian, C.I., Cutler, S., 1994. Autogenous gingival grafts as epithelial barriers for immediate implants: case reports. J. Periodontol. 65, 201−210.

Farley, J.R., Wergedal, J.E., Baylink, D.J., 1983. Fluoride directly stimulates proliferation and alkaline phosphatase activity of bone-forming cells. Science. 222, 330−333.

Felice, P., Pistilli, R., Lizio, G., Pellegrino, G., Nisii, A., Marchetti, C., 2009. Inlay versus onlay iliac bone grafting in atrophic posterior mandible: a prospective controlled clinical trial for the comparison of two techniques. Clin. Implant Dent. Related Res. 11 (Suppl 1), e69−e82.

Fiorellini, J.P., Buser, D., Riley, E., Howell, T.H., 2001. Effect on bone healing of bone morphogenetic protein placed in combination with endosseous implants: a pilot study in beagle dogs. Int. J. Periodontics Restorative Dent. 21 (1), 41−47.

Froum, S., Cho, S.-C., Rosenberg, E., Rohrer, M., Tarnow, D., 2002. Histological comparison of healing extraction sockets implanted with bioactive glass or demineralized freeze-dried bone allograft: a pilot study. J. Periodontol. 73, 94−102.

Gholami, G.A., Najafi, B., Mashhadiabbas, F., Goetz, W., Najafi, S., 2012. Clinical, histologic and histomorphometric evaluation of socket preservation using a synthetic nanocrystalline hydroxyapatite in comparison with a bovine xenograft: a randomized clinical trial. Clin. Oral Implants Res. 23, 1198−1204.

Gil, F.J., Manzanares, N., Badet, A., Aparicio, C., Ginebra, M.-P., 2014. Biomimetic treatment on dental implants for short-term bone regeneration. Clin. Oral Investig. 18, 59−66.

Gil, L.F., Marin, C., Teixeira, H., Marão, H.F., Tovar, N., Khan, R., Bonfante, E.A., Janal, M., Coelho, P.G., 2016. The effect of controlled microrobotized blasting on implant surface texturing and early osseointegration. J. Biomater. Appl. 30, 900−907.

Gupta, A., Dhanraj, M., Sivagami, G., 2010. Status of surface treatment in endosseous implant: a literary overview. Indian J. Dent. Res. 21, 433.

Hao, P.-J., Wang, Z.-G., Xu, Q.-C., Xu, S., Li, Z.-R., Yang, P.-S., Liu, Z.-H., 2014. Effect of umbilical cord mesenchymal stem cell in peri-implant bone defect after immediate implant: an experiment study in beagle dogs. Int. J. Clin. Exp. Pathol. 7, 8271.

Hashemi, H., 2010. Neurosensory function following mandibular nerve lateralization for placement of implants. Int. J. Oral Maxillofac. Surg. 39, 452−456.

Hassan, K.S., Kassim, A., Al Ogaly, A.-U.R., 2008. A comparative evaluation of immediate dental implant with autogenous versus synthetic guided bone regeneration. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol. 106, e8−e15.

Hassani, A., Khojasteh, A., Alikhasi, M., 2008. Anterior palate of the maxilla as a donor site for oral and maxillofacial reconstructive procedures. Asian J. Oral Maxillofac. Surg. 20, 135−138.

Hassani, A., Khojasteh, A., Alikhasi, M., Vaziri, H., 2009. Measurement of volume changes of sinus floor augmentation covered with buccal fat pad: a case series study. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol. 107, 369–374.

Hassani, A., Khojasteh, A., Shamsabad, A.N., 2005. The anterior palate as a donor site in maxillofacial bone grafting: a quantitative anatomic study. J. Oral Maxillofac. Surg. 63, 1196–1200.

Haÿ, E., Marie, P., Debiais, F., 2002. Regulation of human cranial osteoblast phenotype by FGF-2, FGFR-2 and BMP-2 signaling. Histol. Histopathol. 17 (3), 877–885.

Herrero-Climent, M., Lázaro, P., Rios, J.V., Lluch, S., Marques, M., Guillem-Martí, J., Gil, F., 2013. Influence of acid-etching after grit-blasted on osseointegration of titanium dental implants: in vitro and in vivo studies. J. Mater. Sci. Mater. Med. 24, 2047–2055.

Horwitz, E.M., Gordon, P.L., Koo, W.K., Marx, J.C., Neel, M.D., Mcnall, R.Y., Muul, L., Hofmann, T., 2002. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. Proc. Natl. Acad. Sci. U.S.A. 99, 8932–8937.

Howell, T.H., Fiorellini, J.P., Paquette, D.W., Offenbacher, S., Giannobile, W.V., Lynch, S.E., 1997. A phase I/II clinical trial to evaluate a combination of recombinant human platelet-derived growth factor-BB and recombinant human insulin-like growth factor-I in patients with periodontal disease. J. Periodontol. 68, 1186–1193.

Hutmacher, D., Hurzeler, M.B., Schliephake, H., 1996. A review of material properties of biodegradable and bioresorbable polymers and devices for GTR and GBR applications. Int. J. Oral Maxillofac. Implants. 11, :667-–678.

Ito, K., Yamada, Y., Naiki, T., Ueda, M., 2006. Simultaneous implant placement and bone regeneration around dental implants using tissue-engineered bone with fibrin glue, mesenchymal stem cells and platelet-rich plasma. Clin. Oral Implants Res. 17, 579–586.

Ito, K., Yamada, Y., Nakamura, S., Ueda, M., 2011. Osteogenic potential of effective bone engineering using dental pulp stem cells, bone marrow stem cells, and periosteal cells for osseointegration of dental implants. Int. J. Oral Maxillofac. Implants. 26 (5), 947–954.

Jafarian, M., Eslaminejad, M.B., Khojasteh, A., Abbas, F.M., Dehghan, M.M., Hassanizadeh, R., Houshmand, B., 2008. Marrow-derived mesenchymal stem cells-directed bone regeneration in the dog mandible: a comparison between biphasic calcium phosphate and natural bone mineral. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontology. 105, e14–e24.

Jovanovic, S.A., Nevins, M., 1995. Bone formation utilizing titanium-reinforced barrier membranes. Int. J. Periodontics Restorative Dent. 15 (1), 56–69.

Kamitakahara, M., Ohtsuki, C., Miyazaki, T., 2008. Review paper: behavior of ceramic biomaterials derived from tricalcium phosphate in physiological condition. J. Biomater. Appl. 23, 197–212.

Kamperos, G., Zografos, I., Tzermpos, F., Iatrou, I., 2017. Segmental sandwich osteotomy of the posterior mandible in pre-implant surgery-A systematic review. Med. Oral Patología Oral Cirugía Bucal. 22, e132.

Kao, D.W., Kubota, A., Nevins, M., Fiorellini, J.P., 2012. The negative effect of combining rhBMP-2 and Bio-Oss on bone formation for maxillary sinus augmentation. Int. J. Periodontics Restorative Dent. 32 (1), 61–67.

Khojasteh, A., Behnia, H., Dashti, S.G., Stevens, M., 2012. Current trends in mesenchymal stem cell application in bone augmentation: a review of the literature. J. Oral Maxillofac. Surg. 70, 972–982.

Khojasteh, A., Behnia, H., Naghdi, N., Esmaeelinejad, M., Alikhassy, Z., Stevens, M., 2013a. Effects of different growth factors and carriers on bone regeneration: a systematic review. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 116, e405−e423.

Khojasteh, A., Eslaminejad, M.B., Nazarian, H., 2008. Mesenchymal stem cells enhance bone regeneration in rat calvarial critical size defects more than platelete-rich plasma. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol. 106, 356−362.

Khojasteh, A., Eslaminejad, M.B., Nazarian, H., Morad, G., Dashti, S.G., Behnia, H., Stevens, M., 2013b. Vertical bone augmentation with simultaneous implant placement using particulate mineralized bone and mesenchymal stem cells: a preliminary study in rabbit. J. Oral Implantol. 39, 3−13.

Khojasteh, A., Esmaeelinejad, M., Aghdashi, F., 2015. Regenerative techniques in oral and maxillofacial bone grafting. Regenerative Tech. Oral Maxillofac. Bone Grafting. Available from: http://dx.doi.org/10.5772/59236.

Khojasteh, A., Hosseinpour, S., Nazeman, P., Dehghan, M., 2016. The effect of a platelet-rich fibrin conduit on neurosensory recovery following inferior alveolar nerve lateralization: a preliminary clinical study. Int. J. Oral Maxillofac. Surg. 45, 1303−1308.

Khojasteh, A., Fahimipour, F., Jafarian, M., Sharifi, D., Jahangir, S., Khayyatan, F., Baghaban Eslaminejad, M., 2016a. Bone engineering in dog mandible: Coculturing mesenchymal stem cells with endothelial progenitor cells in a composite scaffold containing vascular endothelial growth factor. J. Biomed. Mater. Res. B: Appl. Biomater. Available from: http://dx.doi.org/10.1002/jbm.b.33707.

Khojasteh, A., Nazeman, P., Rad, M.R., 2016b. Dental stem cells in oral, maxillofacial and craniofacial regeneration. Dental Stem Cells. Springer, Berlin, Heidelberg.

Khojasteh, A., Nazeman, P., Tolstunov, L., 2016c. Tuberosity-alveolar block as a donor site for localised augmentation of the maxilla: a retrospective clinical study. Br. J. Oral Maxillofac. Surg. 54, 950−955.

Khojasteh, A., Sadeghi, N., 2016. Application of buccal fat pad-derived stem cells in combination with autogenous iliac bone graft in the treatment of maxillomandibular atrophy: a preliminary human study. Int. J. Oral Maxillofac. Surg. 45, 864−871.

Kim, J.-W., Cho, M.-H., Kim, S.-J., Kim, M.-R., 2013. Alveolar distraction osteogenesis versus autogenous onlay bone graft for vertical augmentation of severely atrophied alveolar ridges after 12 years of long-term follow-up. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 116, 540−549.

Kim, N.-H., Lee, S.-H., Ryu, J.-J., Choi, K.-H., Huh, J.-B., 2015. Effects of rhBMP-2 on sandblasted and acid etched titanium implant surfaces on bone regeneration and osseointegration: spilt-mouth designed pilot study. BioMed Res. Int. 2015, 459393.

Kim, S.-H., Kim, K.-H., Seo, B.-M., Koo, K.-T., Kim, T.-I., Seol, Y.-J., Ku, Y., Rhyu, I.-C., Chung, C.-P., Lee, Y.-M., 2009. Alveolar bone regeneration by transplantation of periodontal ligament stem cells and bone marrow stem cells in a canine peri-implant defect model: a pilot study. J. Periodontol. 80, 1815−1823.

Langer, R., 1993. Tissue engineering. Science. 260, 920−926.

Le Guehennec, L., Soueidan, A., Layrolle, P., Amouriq, Y., 2007. Surface treatments of titanium dental implants for rapid osseointegration. Dental Mater. 23, 844−854.

Lee, Y.M., Seol, Y.J., Lim, Y.T., Kim, S., Han, S.B., Rhyu, I.C., Baek, S.H., Heo, S.J., Choi, J.Y., Klokkevold, P.R., 2001. Tissue-engineered growth of bone by marrow cell transplantation using porous calcium metaphosphate matrices. J. Biomed. Mater. Res. 54, 216−223.

Lekovic, V., Camargo, P.M., Klokkevold, P.R., Weinlaender, M., Kenney, E.B., Dimitrijevic, B., Nedic, M., 1998. Preservation of alveolar bone in extraction sockets using bioabsorbable membranes. J. Periodontol. 69, 1044−1049.

López-Cedrún, J.L., 2011. Implant rehabilitation of the edentulous posterior atrophic mandible: the sandwich osteotomy revisited. Int. J. Oral Maxillofac. Implants. 26 (1), 195−202.

Luo, T., Zhang, W., Shi, B., Cheng, X., Zhang, Y., 2012. Enhanced bone regeneration around dental implant with bone morphogenetic protein 2 gene and vascular endothelial growth factor protein delivery. Clin. Oral Implants Res. 23, 467−473.

Lynch, S., Williams, R., Poison, A., Howell, T., Reddy, M., Zappa, U., Antoniades, H., 1989. A combination of platelet-derived and insulin-like growth factors enhances periodontal regeneration. J. Clin. Periodontol. 16, 545−548.

Lynch, S.E., Buser, D., Hernandez, R.A., Weber, H., Stich, H., Fox, C.H., Williams, R.C., 1991. Effects of the platelet-derived growth factor/insulin-like growth factor-I combination on bone regeneration around titanium dental implants. Results of a pilot study in beagle dogs. J. Periodontol. 62, 710−716.

Marx, R., 1993. Philosophy and particulars of autogenous bone grafting. Oral Maxillofac. Surg. Clin. North Am. 5, 599.

Master, D.L., Hanson, P.R., Gosain, A.K., 2010. Complications of mandibular distraction osteogenesis. J. Craniofac. Surg. 21, 1565−1570.

McAllister, B.S., Haghighat, K., 2007. Bone augmentation techniques. J. Periodontol. 78, 377−396.

Miloro, M., Ghali, G., Larsen, P., Waite, P., 2004. Peterson's Principles of Oral and Maxillofacial Surgery. PMPH, USA.

Morad, G., Khojasteh, A., 2013. Cortical tenting technique versus onlay layered technique for vertical augmentation of atrophic posterior mandibles: a split-mouth pilot study. Implant Dent. 22, 566−571.

Motamedian, S.R., Hosseinpour, S., Ahsaie, M.G., Khojasteh, A., 2015. Smart scaffolds in bone tissue engineering: a systematic review of literature. World J. Stem Cells. 7, 657−668.

Motamedian, S.R., Iranparvar, P., Nahvi, G., Khojasteh, A., 2016a. Bone tissue engineering: a literature review. Regeneration Reconstruct. Restoration. 1, 103−120.

Motamedian, S.R., Khojaste, M., Khojasteh, A., 2016b. Success rate of implants placed in autogenous bone blocks versus allogenic bone blocks: a systematic literature review. Annal. Maxillofac. Surg. 6, 78.

Motamedian, S.R., Tabatabaei, F.S., Akhlaghi, F., Torshabi, M., Gholamin, P., Khojasteh, A., 2016c. Response of dental pulp stem cells to synthetic, allograft, and xenograft bone scaffolds. Int. J. Periodontics Restorative Dent. 37, 49−59.

Nevins, M., Kao, R.T., Mcguire, M.K., Mcclain, P.K., Hinrichs, J.E., Mcallister, B.S., Reddy, M.S., Nevins, M.L., Genco, R.J., Lynch, S.E., 2013. Platelet-derived growth factor promotes periodontal regeneration in localized osseous defects: 36-month extension results from a randomized, controlled, double-masked clinical trial. J. Periodontol. 84, 456−464.

Nevins, M.L., Reynolds, M.A., Camelo, M., Schupbach, P., Kim, D.M., Nevins, M., 2014. Recombinant human platelet-derived growth factor BB for reconstruction of human large extraction site defects. Int. J. Periodontics Restorative Dent. 34 (2), 157−163.

Nguyen, H., Morgan, D.A., Forwood, M.R., 2007. Sterilization of allograft bone: effects of gamma irradiation on allograft biology and biomechanics. Cell Tissue Bank. 8, 93−105.

Nissan, J., Ghelfan, O., Mardinger, O., Calderon, S., Chaushu, G., 2011. Efficacy of cancellous block allograft augmentation prior to implant placement in the posterior atrophic mandible. Clin. Implant Dent. Related Res. 13, 279−285.

Oliva, J., Oliva, X., Oliva, J.D., 2010. Five-year success rate of 831 consecutively placed Zirconia dental implants in humans: a comparison of three different rough surfaces. Int. J. Oral Maxillofac. Implants. 25 (2), 336−344.

Paolantonio, M., Dolci, M., Scarano, A., D'archivio, D., Placido, G.D., Tumini, V., Piattelli, A., 2001. Immediate implantation in fresh extraction sockets. A controlled clinical and histological study in man. J. Periodontol. 72, 1560−1571.

Park, J., Lutz, R., Felszeghy, E., Wiltfang, J., Nkenke, E., Neukam, F., Schlegel, K., 2007. The effect on bone regeneration of a liposomal vector to deliver BMP-2 gene to bone grafts in peri-implant bone defects. Biomaterials. 28, 2772−2782.

Park, J.B., 1992. Lakes, Biomaterials: An Introduction. *Plenum Press*, *New York*.

Pelker, R.R., Friedlaender, G.E., 1987. Biomechanical aspects of bone autografts and allografts. Ortho. Clin. North Am. 18, 235−239.

Pieri, F., Lucarelli, E., Corinaldesi, G., Aldini, N.N., Fini, M., Parrilli, A., Dozza, B., Donati, D., Marchetti, C., 2010. Dose-dependent effect of adipose-derived adult stem cells on vertical bone regeneration in rabbit calvarium. Biomaterials. 31, 3527−3535.

Pjetursson, B.E., Tan, W.C., Zwahlen, M., Lang, N.P., 2008. A systematic review of the success of sinus floor elevation and survival of implants inserted in combination with sinus floor elevation. J. Clin. Periodontol. 35, 216−240.

Puleo, D., Nanci, A., 1999. Understanding and controlling the bone−implant interface. Biomaterials. 20, 2311−2321.

Rakhmatia, Y.D., Ayukawa, Y., Furuhashi, A., Koyano, K., 2013. Current barrier membranes: titanium mesh and other membranes for guided bone regeneration in dental applications. J. Prosthodont. Res. 57, 3−14.

Raphel, J., Karlsson, J., Galli, S., Wennerberg, A., Lindsay, C., Haugh, M.G., Pajarinen, J., Goodman, S.B., Jimbo, R., Andersson, M., 2016. Engineered protein coatings to improve the osseointegration of dental and orthopaedic implants. Biomaterials. 83, 269−282.

Rasmusson, L., Sennerby, L., Lundgren, D., Nyman, S., 1997. Morphological and dimensional changes after barrier removal in bone formed beyond the skeletal borders at titanium implants. A kinetic study in the rabbit tibia. Clin. Oral Implants Res. 8, 103−116.

Ribeiro, F., Suaid, F., Ruiz, K., Rodrigues, T., Carvalho, M., Nociti, F., Sallum, E., Casati, M., 2012. Effect of autologous bone marrow-derived cells associated with guided bone regeneration or not in the treatment of peri-implant defects. Int. J. Oral Maxillofac. Surg. 41, 121−127.

Ribeiro, F.V., Suaid, F.F., Ruiz, K.G., Salmon, C.R., Paparotto, T., Nociti JR, F.H., Sallum, E.A., Casati, M.Z., 2010. Periosteum-derived cells as an alternative to bone marrow cells for bone tissue engineering around dental implants. A histomorphometric study in beagle dogs. J. Periodontol. 81, 907−916.

Rothamel, D., Schwarz, F., Herten, M., Engelhardt, E., Donath, K., Kuehn, P., Becker, J., 2008. Dimensional ridge alterations following socket preservation using a nanocrystalline hydroxyapatite paste. A histomorphometrical study in dogs. Int. J. Oral Maxillofac. Surg. 37, 741−747.

Sadeghi, M., Bakhshandeh, B., Dehghan, M.M., Mehrnia, M.R., Khojasteh, A., 2016. Functional synergy of anti-mir221 and nanohydroxyapatite scaffold in bone tissue engineering of rat skull. J. Mater. Sci. Mater. Med. 27, 132.

Sánchez, A.R., Sheridan, P.J., Eckert, S.E., Weaver, A.L., 2005. Regenerative potential of platelet-rich plasma added to xenogenic bone grafts in peri-implant defects: a histomorphometric analysis in dogs. J. Periodontol. 76, 1637−1644.

Sandhu, H.S., Grewal, H.S., Parvataneni, H., 1999. Bone grafting for spinal fusion. Orthopedic Clin. 30, 685−698.

Santis, E.D., Botticelli, D., Pantani, F., Pereira, F.P., Beolchini, M., Lang, N.P., 2011. Bone regeneration at implants placed into extraction sockets of maxillary incisors in dogs. Clin. Oral Implants Res. 22, 430−437.

Scarano, A., Degidi, M., Iezzi, G., Pecora, G., Piattelli, M., Orsini, G., Caputi, S., Perrotti, V., Mangano, C., Piattelli, A., 2006. Maxillary sinus augmentation with different biomaterials: a comparative histologic and histomorphometric study in man. Implant Dent. 15, 197–207.

Schettler, D., 1976. Sandwich technic with cartilage transplant for raising the alveolar process in the lower jaw. Fortschritte der Kiefer-und Gesichts-Chirurgie. 20, 61.

Schliephake, H., Scharnweber, D., Dard, M., Rößler, S., Sewing, A., Meyer, J., Hoogestraat, D., 2002. Effect of RGD peptide coating of titanium implants on periimplant bone formation in the alveolar crest. Clin. Oral Implants Res. 13, 312–319.

Schmitt, C.M., Doering, H., Schmidt, T., Lutz, R., Neukam, F.W., Schlegel, K.A., 2013. Histological results after maxillary sinus augmentation with Straumann® BoneCeramic, Bio-Oss®, Puros®, and autologous bone. A randomized controlled clinical trial. Clin. Oral Implants Res. 24, 576–585.

Schulz, M.C., Korn, P., Stadlinger, B., Range, U., Möller, S., Becher, J., Schnabelrauch, M., 2014. Coating with artificial matrices from collagen and sulfated hyaluronan influences the osseointegration of dental implants. J. Mater. Sci.: Mater. Med. 25, 247–258.

Schwartz-Arad, D., Levin, L., Sigal, L., 2005. Surgical success of intraoral autogenous block onlay bone grafting for alveolar ridge augmentation. Implant Dent. 14, 131–138.

Schwarz, F., Sager, M., Ferrari, D., Mihatovic, I., Becker, J., 2009. Influence of recombinant human platelet-derived growth factor on lateral ridge augmentation using biphasic calcium phosphate and guided bone regeneration: a histomorphometric study in dogs. J. Periodontol. 80, 1315–1323.

Serino, G., Biancu, S., Iezzi, G., Piattelli, A., 2003. Ridge preservation following tooth extraction using a polylactide and polyglycolide sponge as space filler: a clinical and histological study in humans. Clin. Oral Implants Res. 14, 651–658.

Shayesteh, Y.S., Khojasteh, A., Soleimani, M., Alikhasi, M., Khoshzaban, A., Ahmadbeigi, N., 2008. Sinus augmentation using human mesenchymal stem cells loaded into a β-tricalcium phosphate/hydroxyapatite scaffold. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol. 106, 203–209.

Simion, M., Jovanovic, S.A., Tinti, C., Benfenati, S.P., 2001. Long-term evaluation of osseointegrated implants inserted at the time or after vertical ridge augmentation. Clin. Oral Implants Res. 12, 35–45.

Simion, M., Rocchietta, I., Kim, D., Nevins, M., Fiorellini, J., 2006. Vertical ridge augmentation by means of deproteinized bovine bone block and recombinant human platelet-derived growth factor-BB: a histologic study in a dog model. Int. J. Periodontics Restorative Dent. 26 (5), 415–423.

Tatum JR, H., 1986. Maxillary and sinus implant reconstructions. Dental Clin. North Am. 30, 207–229.

Tatum, O.J., Lebowitz, M., Tatum, C., Al, E., 1993. Sinus augmentation: Rationale, development, long-term results. N Y State Dent. J. 59, 43–48.

Tolstunov, L., 2009. Maxillary tuberosity block bone graft: innovative technique and case report. J. Oral Maxillofac. Surg. 67, 1723–1729.

van den Bergh, J., Ten Bruggenkate, C., Tuinzing, D., 1998. Preimplant surgery of the bony tissues. J. Prosthetic Dent. 80, 175–183.

Vastel, L., Meunier, A., Siney, H., Sedel, L., Courpied, J.-P., 2004. Effect of different sterilization processing methods on the mechanical properties of human cancellous bone allografts. Biomaterials. 25, 2105–2110.

Vetromilla, B., Moura, L., Sonego, C., Torriani, M., Chagas, O., 2014. Complications associated with inferior alveolar nerve repositioning for dental implant placement: a systematic review. Int. J. Oral Maxillofac. Surg. 43, 1360–1366.

Wang, X.-J., Liu, H.-Y., Ren, X., Sun, H.-Y., Zhu, L.-Y., Ying, X.-X., Hu, S.-H., 2015. Effects of fluoride-ion-implanted titanium surface on the cytocompatibility in vitro and osseointegatation in vivo for dental implant applications. Colloids Surf. B: Biointerfaces. 136, 752–760.

Weibrich, G., Hansen, T., Kleis, W., Buch, R., Hitzler, W., 2004. Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. Bone. 34, 665–671.

Wiegand, A., Buchalla, W., Attin, T., 2007. Review on fluoride-releasing restorative materials—fluoride release and uptake characteristics, antibacterial activity and influence on caries formation. Dent. Mater. 23, 343–362.

Wikesjö, U., Qahash, M., Huang, Y.H., Xiropaidis, A., Polimeni, G., Susin, C., 2009. Bone morphogenetic proteins for periodontal and alveolar indications; biological observations–clinical implications. Orthodontics Craniofac. Res. 12, 263–270.

Wood, R.A., Mealey, B.L., 2012. Histologic comparison of healing after tooth extraction with ridge preservation using mineralized versus demineralized freeze-dried bone allograft. J. Periodontol. 83, 329–336.

Yao, W., Lane, N.E., 2015. Targeted delivery of mesenchymal stem cells to the bone. Bone. 70, 62–65.

You, T.-M., Choi, B.-H., Li, J., Jung, J.-H., Lee, H.-J., Lee, S.-H., Jeong, S.-M., 2007. The effect of platelet-rich plasma on bone healing around implants placed in bone defects treated with Bio-Oss: a pilot study in the dog tibia. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol. 103, e8–e12.

Yu, Y., Jin, G., Xue, Y., Wang, D., Liu, X., Sun, J., 2016. Multifunctions of dual Zn/Mg ion co-implanted titanium on osteogenesis, angiogenesis and bacteria inhibition for dental implants. Acta Biomater. 49, 590–603.

Yun, J.H., Han, S.H., Choi, S.H., Lee, M.H., Lee, S.J., Song, S.U., Oh, N., 2014. Effects of bone marrow-derived mesenchymal stem cells and platelet-rich plasma on bone regeneration for osseointegration of dental implants: preliminary study in canine three-wall intrabony defects. J. Biomed. Mater. Res. B: Appl. Biomater. 102, 1021–1030.

Zitzmann, N.U., Naef, R., Schärer, P., 1997. Resorbable versus nonresorbable membranes in combination with Bio-Oss for guided bone regeneration. Int. J. Oral Maxillofac. Implants. 12 (6), 844–852.

Zubillaga, G., Hagen, S.V., Simon, B.I., Deasy, M.J., 2003. Changes in alveolar bone height and width following post-extraction ridge augmentation using a fixed bioabsorbable membrane and demineralized freeze-dried bone osteoinductive graft. J. Periodontol. 74, 965–975.

Regeneration concerns in craniofacial cartilage and bone defects

25

Aileen Crawford[1] *and Katie Bardsley*[2]
[1]University of Sheffield, Sheffield, United Kingdom, [2]University of Keele, Staffordshire, United Kingdom

25.1 Introduction

The human face is highly individualized and is important in providing a person's sense of identity. It is the individual differences in appearance that distinguish one human being from another and facial expression is an essential part of communication. In the United Kingdom alone, over 540,000 people in the United Kingdom are estimated to acquire a disfiguring condition to their face, hands, or body (www.changingfaces.org.uk). Craniofacial disfigurement can be devastating for the patient resulting in both physical problems (e.g., loss of facial features, facial expression, or sense of smell) and psychosocial problems (Newell and Marks, 2000; Rhotena Murphy and Ridner, 2013). People with disfigurements can experience severe negative reactions in social situations leading to high levels of anxiety, social isolation, loss of self-esteem and self-confidence, and difficulties in gaining employment. Facial disfigurement can occur through a variety of causes including trauma, burns, tumors and their surgical resection, chronic disease, and congenital abnormalities such as cleft lip and/or cleft palate.

Current maxillofacial reconstruction is individualized and aims to restore the craniofacial features to optimize functional and esthetic outcomes. Surgeons employ a number of techniques to rebuild facial tissues. Removable, customized biomaterial prostheses (e.g., silicone elastomers) are still a mainstay for esthetic restoration of features such as ears or nose (Wagenblast et al., 2008; Ariani et al., 2013; Ciocca et al., 2010). In addition, wearing of prosthetic features may be essential until the wound bed has healed sufficiently for reconstructive surgery to rebuild the facial features can begin or while waiting to ensure no reoccurrence of a tumor. For patients this process can be complicated and disconcerting, while prosthetic facial features can give important acceptable esthetic appearances, particularly for ears, there are disadvantages. The prostheses cannot provide a "functional" tissue restoration and can be much heavier than the natural tissue, color-matching the prosthesis to a patient's skin tone can be difficult and the prostheses often have limited lifespan and as consequence they must be repeatedly fabricated (Visser et al., 2008).

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00025-6
© 2017 Elsevier Ltd. All rights reserved.

In terms of surgical reconstruction, autologous skin flap coverings over a supporting autologous or allogeneic tissue graft or biocompatible synthetic biomaterial (or titanium support for large bone voids) are routinely used to reconstruct the craniofacial bones and provide the underlying support tissue in nasal reconstruction. Autologous bone grafts where bone tissue is harvested from the patient are considered the "gold standard" for bone repair. Autologous bone has osteoconductive properties (due to the physical supporting/scaffold effect of the bone extracellular matrix (ECM)), it also has osteoinductive properties (which are a result of various osteogenic growth factors, such as bone morphogenic proteins (BMPs), in the ECM of the graft) and osteogenic properties (as the graft contains osteoprogenitor cells in the tissue). In addition, autologous bone grafts also do not provoke an immune rejection of the graft (Chang et al., 2009). The repair of large bone defects remains particularly challenging. Bone tissue needs adequate blood supply for tissue survival and this can be compromised at a wound site, particularly after chemotherapy and radiotherapy for cancer. An autologous bone graft has the advantage that it can be harvested with its vascularization intact, which can be reattached to vessels at the site of injury to reduce the chance of tissue necrosis after implantation into wound bed. While generally giving the best clinical results (Frolich et al., 2008) autologous bone has drawbacks including long-term postoperative pain and/or donor site morbidity (Meijer, 2007). The donor bone is often taken from the iliac crest, ribs, or tibia and there is a limit to the volume of bone which can be taken by autograft which is of particular concern in young children (Goulet et al., 1997).

Allogenic bone and xenografts, where bone is taken from a cadaver or animal source respectively, have been used to circumvent the issues seen when using autografts, particularly enabling higher volumes of bone to be harvested and therefore are useful for repair of large voids or multidefects. Bone allografts require a high degree of processing before they can be used to reduce immunogenicity and ensure sterility (Mohr et al., 2016). However, this processing removes the cellular components (and hence osteogenic inductive properties) and decreases their osteoinductive potential (Finkemeier, 2002; Epple et al., 2005) which can lead to slower rates of revascularization and a higher rate of reabsorption (Frohlich et al., 2008). Allografts also have the potential disadvantage to cause immunogenic responses and possible viral transmission between patients (Chang et al., 2009; Damien Parsons, 1991; Delloye et al., 2007).

Bone-graft substitutes composed of synthetic or natural materials are been developed as alternatives to autologous, xenogenic, and allogenic bone grafts. These bone-graft substitutes encompass a wide range of materials including collagen and collagen-hydroxyapatite (HA)-based, HA and HA-calcium orthophosphate-based composites, metals and bioglass ceramics, and cements (Finkemeier, 2002; Giannoudis et al., 2005; Hurrell-Gillingham et al., 2003; Hatton et al., 2006). However, although biomaterial bone-graft substitutes have good osteoconductive properties, their osteoinductive properties are limited. Calcium phosphates are commonly used as biomaterials for bone repair due to their osteogenic potential and biocompatibility. While HA $[Ca_{10}/(PO_4)_6(OH)_2]$ is the main component in bone and has a high compressive strength, it is a brittle material which has low rates of

resorption (Stevens, 2008). Therefore HA is often utilized as a composite with tricalcium phosphate, or polymers such as collagen (reviewed in Dorozhikin, 2009; Boher et al., 2012; Wagoner Johnson and Herscher, 2011). These composites are advantageous as they are a balance between strength, from the HA, and higher resorption rates, from tricalcium phosphate or polymers. Bioactive glasses, such as 4555 Bioglass are reported to stimulate bone regeneration to a greater degree than HA-calcium phosphate ceramics (extensively reviewed in Jones, 2013). However, bioactive glasses have not been used as bone-graft substitutes to the same extent as HA-calcium phosphate ceramics.

25.2 Tissue engineering bone grafts

Despite bone tissue being the most transplanted tissue after blood (Gupte and Mia, 2012), the regeneration of large and/or complex bone defects remains clinically challenging. Owing to the limited supply of autologous bone and the limited osteoinductive properties of allografts and bone-graft substitutes there is a strong clinical need to develop new bone-graft substitutes for regeneration of these large bone defects. Tissue engineering and regenerative medicine technologies are promising technologies to produce bone-graft substitutes to address this clinical need. Tissue engineering is an interdisciplinary field, requiring both engineering and life science fields to create substitute tissues and organs which maintain, restore, or improve function (Langer and Vacanti, 1993; Scheller et al., 2009). This field is rapidly and continuously evolving to include more novel approaches using a combination of: a supporting three-dimensional (3D) scaffold to serve as a supporting template for tissue formation, living cells to produce the bone tissue and growth factors (e.g., BMPs and transforming growth factor β (TGF β) isoforms) which stimulate osteogenesis (Helios et al., 2009; Fisher et al., 2013; Oppenheimer et al., 2012). Tissue engineering of a bone graft using patient-derived cells and an optimized biomaterial has the advantage of enabling an autologous, natural tissue to be produced that can promote bone regeneration with fewer side-effects to the patient. Additionally, the availability of high-resolution medical imaging and computer-aided design and manufacture give the potential to fabricate patient-customized, 3D scaffolds for tissue regeneration of complex, critical sized, and bone defects.

Scaffold materials for bone regeneration must be (1) biocompatible, enabling cell adhesion and proliferation, and supporting normal cellular function without significant local or systemic toxicity; (2) biodegradable (ideally with scaffold degradation occurring at the same rate as that of new tissue formation); (3) representative of the physical, and mechanical properties representative of the target tissue to withstand implantation; and (4) an internal architecture/porosity to facilitate cell differentiation and facilitate diffusion of essential nutrients and oxygen for cell survival (Williams, 2008; Bose et al., 2012; Murphy et al., 2010). Ideally a scaffold should not be just osteoconductive (i.e., just a support for cell attachment and tissue formation) but also designed to be osteoinductive (i.e., actively promote tissue

formation through recruitment of progenitor cells and/or stimulate osteogenic differentiation and ECM formation).

Within the bone tissue engineering field the majority of biomaterials used to form scaffolds are often based on the bone-graft substitutes described above can be classified as bioactive ceramics and glasses, hydrogels, metals, such as titanium and natural and synthetic polymers. The ideal scaffold should mimic the structural and biochemical information of the native tissue ECM as closely as possible. The ECM is the unique, tissue-specific, noncellular substance composed of structural and functional molecules which is secreted by the tissue cells and is the "adhesive" which holds the cells together and provides the tissues mechanical properties (Badylak et al., 2012). Apart from being a physical scaffold for tissue formation the ECM also provides a number of biochemical cues provided by the chemistry and macro, micro, and nanoarchitecture of the ECM and its sequestered biological factors such as growth modulating and migratory factors which together are critical in instructing and modifying cell behaviors such as differentiation and proliferation and maintenance of cell phenotype and tissue integrity (Lotolf, and Hubbell, 2005; Watt and Huck, 2013).

25.2.1 Scaffolds for bone and cartilage tissue engineering

Biodegradable natural and synthetic polymer scaffolds have been widely used for bone tissue engineering (comprehensively reviewed in Hsu et al., 2016; Jafari et al., 2017). The natural polymers commonly used include collagen (collagen I nanofibres are a natural component of bone ECM), gelatin, and silk proteins. Other natural polymers used for bone tissue engineering include alginate, chitosan, hyaluronan, and cellulose (Holzwarth and Ma, 2011). Natural polymers have been chosen due to their excellent biocompatibility and inherent biological activity with peptide sequences which promote cell adhesion. Also, apart from alginate, most natural polymers can be readily processed to provide sponge-like structures or nano- and microfibrous scaffolds (Holzwarth and Ma, 2011). However, the natural polymers have the disadvantages of limited supply and difficulty in maintaining consistency in quality and relatively poor compressive mechanical properties and uncontrollable biodegradation (Hsu et al., 2016). Synthetic polymers have the advantages over the natural polymers in that they can be fabricated with better mechanical properties, tuneable degradation rates, and consistent product quality. Commonly used synthetic polymers are polyglycolic acid (PGA), poly L-lactic acid (PLLA), and polycaprolactone and their copolymers. Synthetic polymers such as PLLA have FDA approval for other applications (Yu et al., 2010) and been used clinically for many years in the form of plates, suture materials, and screws and pins in orthopedic and craniofacial surgery (Ahmed et al., 2008). However, these synthetic polymers have lower osteoconductive properties compared to natural polymers; hence, they have often been used to fabricate composite scaffolds with natural polymers (to increase cell adhesion), HA and calcium phosphate-based ceramics or bioglasses to increase bioreactivity and produce a hybrid scaffold with enhanced mechanical properties (Hsu et al., 2016; Holzwarth, 2010). HA and HA/β

tricalcium phosphate-based ceramics are the most frequent class of ceramics used to produce composite scaffolds used in tissue engineering due to their biomimetic and osteoconductive properties (Davies et al., 2010; Li et al., 2011).

As the understanding of bone structure and the cellular mechanisms of osteoblastic differentiation of MSCs and bone ECM formation and wound healing has increased, various approaches have been used to increase the bioactivity of scaffolds used in tissue engineering. In fabrication of scaffold materials for bone tissue engineering the size of the pores and the degree of interconnectivity is crucial both for cell ingress through the scaffold, cell differentiation, and nutrient exchange. The optimum pore size for osteogenic differentiation is well known to be around 300−500 μm (Kuhne et al., 1994). However, while this allows for good nutrient exchange, increasing the pore size in ceramic-based scaffolds causes the material become more brittle (Stevens, 2008).

One approach to increasing scaffold bioactivity is the development and fabrication of synthetic and natural nanomaterials and composites to mimic the structural nanofeatures of the collagenous ECM matrix and the nanocrystalline HA structure of bone to promote osteogenesis and ECM formation (Holzwarth and Ma, 2011; Wang et al., 2016). Another, extensive approach has been the incorporation and release of various osteoinductive and angiogenic bioactive factors into scaffolds. The osteoinductive growth factors most commonly used include the BMPs, particularly BMP 2 and BMP 7, TGF β family, fibroblast growth factors, platelet-derived growth factors, and insulin-like growth factor-1 (Porter et al., 2009; Bessa et al., 2008). In terms of angiogenic factors, vascular endothelial growth factor is one of the most important growth factors for vascular development and angiogenesis (Hu and Olsen, 2016).

Since the discovery of the osteogenic effect of BMPs was first reported by Urist in 1965, two BMPs, BMP 2 and BMP 7 have gained FDA approval for clinical use in spinal fusion and long-bone fractures. Biomaterial carriers such as collagen gels, devitalized bone powder and HA-based bone substitutes soaked in BMPs have been used clinically to regenerate bone in preclinical studies in various animal models. Clinically, carriers (collagen, or bone-graft substitutes) soaked in BMP 2 and/or 7 have been used for spinal fusion, fractures of the long bones and repair of maxillary floor augmentation (Begam et al., 2017).

The various osteogenic and angiogenic growth factors have been incorporated into scaffolds and bone graft substitutes by various means and soaking the scaffolds in growth factor solutions or encapsulation in polymer vesicles has been a common approach (Amini et al., 2012). The rate of release of the biological factors is crucial to successful tissue engineering of cartilage or bone. Soaking scaffold materials in various growth factors or encapsulating them in polymeric materials has been used by many investigators. However, this can lead to a very rapid initial release of growth factors (well known in pharmacology as a “burst release”) followed by a period (over hours or days) of a slower rate of passive release due to diffusion. During the “burst” release phase the regenerating cells and tissues can be exposed to supraphysiological concentrations of growth factors which could have detrimental biological effects. Currently, there is ongoing research to

fabricate controlled drug/biological factor release systems. This research encompasses chemical covalent coupling of growth factors to scaffolds, coupling growth factors to protease-sensitive linkers so that the cells can release the factors, encapsulating growth factors in stimulus-sensitive (e.g., pH sensitive) micro or nanoparticles or by using growth factor gene-activated matrices so that the growth factor gene or a modified RNA transcript is transiently transfected into cells subsequently bound to the scaffold (Thambi et al., 2016; Arcos and Regi, 2013; Park et al., 2016; Metzar et al., 2016; Yang et al., 2017).

25.2.2 Cell sources for bone and cartilage tissue engineering

This review will focus on those adult cell sources which have high clinical potential for personalized craniofacial reconstruction. There is widespread interest in the field of regenerative medicine and tissue engineering in using mesenchymal stem cells (MSCs) to produced tissue engineered for tissue regeneration in the body. This also applies to bone tissue engineering both for craniofacial and orthopaedic indications. Seeding clinically sized scaffolds requires large numbers of cells and MSCs are an attractive cell source due to their well-known proliferative capacity. Adult stem cells are isolated from mature tissues and enable the harvesting of an autologous stem cell source circumventing both the ethical objections and potential immune responses seen with embryonic stem cells.

MSCs isolated from the bone marrow (BM) are commonly used for bone tissue engineering. BM-MScs are isolated from a BM biopsy and have good proliferative capacity (Bruder et al., 1997). The bone biopsy is an invasive technique and the procedure can cause postoperation pain and morbidity at the donor site (Bain, 2003). However, this risk is lower than that when an autologous bone graft is taken (Hernigou et al., 2014). BM-derived MSCs have been used clinically for regeneration of craniofacial bone defects (Warnke et al., 2004; Chatterjea et al., 2010).

Adipose tissue-derived (ADSC) MSCs are an alternative source of MSCs that been shown to have osteogenic and chondrogenic potential (De Francesco et al., 2015; Naderi et al., 2016). The potential for using ADSC-MSCs is very attractive. Harvesting the ADSC-MSCs is easier than for harvesting BM with less potential donor site morbidity and ADSC-MSCs can be obtained from adipose tissue at 100−1000 times the level of BM-MSCs obtainable from a bone biopsy (Kapur et al., 2015) which makes ADSC-MSCs attractive for translational studies. However, the osteogenic capacity of ASC-MSCs is a matter of contention. Some reports have shown ASC-MSCs to have a lower osteogenic and chondrogenic capacity than BM-MSCs (Im et al., 2005; Mehlhorn et al., 2006). While other research (De Ugarte et al., 2003; Schubert et al., 2011) shows ADSC-MSCs to have a similar or better osteogenesis capacity as BM-MSCs. There are limited clinical studies using autologous ADSC-MSCs mainly for the reconstruction of soft tissues (Naderi et al., 2016) and spinal fusion Yoshimura et al. (2008).

More recently dental pulp stem cells (DPSCs) have been investigated as a potential cell source for craniofacial bone tissue engineering in the preclinical setting (Sharp, 2016; Aurrekoetxea et al., 2015). Unlike MSCs, DPSCs are derived from

the neural crest and therefore of the same embryological origins as some of the bones and cartilages of the face and anterior skull (Berendsen and Olsen, 2015). Preclinical research to date has indicated that DPSCs have good osteogenic capacity and can be differentiated into osteoblasts and form mineralized tissue (Aurrekoetxea et al., 2015).

25.3 Craniofacial tissue engineering approaches

25.3.1 Tissue-engineered bone grafts

Bone is formed in the body from stem cells by two pathways: (1) intramembranous ossification in which MSCs differentiate directly into osteoblasts which then lay down the extracellular bone matrix and (2) endochondral ossification in which the MSCs differentiate in chondrocytes which form a hyaline cartilage anlagen (template) which by complex biological process is remodeled into mineralized bone (Berendsen and Olsen, 2015). Most bones of the body are formed via endochondral ossification; however, the facial bones and those enclosing the skull are formed by intramembranous ossification (Berendsen and Olsen, 2015). Endochondral ossification is an important mechanism for the healing of bone fractures in which the soft tissue callus composed of hyaline cartilage undergoes hypertrophy to hypertrophic cartilage followed by ossification into woven bone.

To date the majority of research in bone tissue engineering research has centered on the use of stem cells and their differentiation down an osteogenic pathway to recapitulate bone formation by the intramembranous ossification route. One of the key challenges to forming tissue engineered bone via the intramembranous route is maintaining the viability of the forming tissue in vitro due to the problem of maintaining good gaseous and nutrient exchange. This has been partially overcome by using fabricating scaffolds to contain small blood vessel-sized channels, to reflect the structure of blood vessels, incorporation of angiogenic factors to promote angiogenesis in vivo, coseeding the scaffolds with endothelial cell progenitors (with or without angiogenic factors such as VEGEF) which will form capillary-like structures within the scaffold, and the use of bioreactors to provide a dynamic culture environment to maximize nutrient exchange and tissue formation (Zhao et al., 2016; Kirkpatrick et al., 2011). Another approach which is under investigation is to implant the developing tissue engineered subcutaneously or intramuscularly in a patient so that the patient becomes their own bioreactor. This approach has the advantage of reducing the potential risk of tissue microbial contamination which is present in prolonged bioreactor culture. Using the patient as a bioreactor has been used successfully in a case study to produce a customized vascular mandibular bone graft (Warnke et al., 2004).

To date, there has been relatively little research in recapitulating the endochondral ossification pathway to regenerate bone, although interest in this area is growing. In this approach a tissue-engineered hypertrophic cartilage “template” is produced and implanted into the bone defect. The hypothesis behind this approach is that once

implanted, the hypertrophic cartilage will become be invaded by blood vessels from the surrounding host tissue followed by cartilage mineralization and remodeling of the cartilage into bone. A tissue-engineered cartilage graft should have advantages over a tissue-engineered bone graft produced via intramembranous ossification. Osteoblasts and bone tissue require high nutrient exchange rates and oxygen levels within the tissue and because of these requirements, cannot withstand the hypoxic conditions usually found in a wound bed (Malda et al., 2003). It is therefore, more difficult for these grafts to survive once implanted into a defect unless a functionally adequate blood supply can be immediately established to the tissue. In contrast, cartilage is an avascular tissue that is better able to survive the low oxygen tensions found within the wound bed (Hirao et al., 2006). Importantly, hypertrophic chondrocytes secrete biological factors to promote angiogenesis (such as VEGF) osteogenesis and ECM remodeling which are crucial for the vascularization of the cartilage and remodeling into bone (Ortega et al., 2010).

To recapitulate the endochondral pathway to tissue engineer a hypertrophic cartilage a hyaline cartilage must first be formed (Berendsen and Olsen, 2015). The hyaline chondrocytes are then stimulated to undergo terminal differentiation to form hypertrophic chondrocytes which remodel the ECM to hypertrophic cartilage which can then be implanted into a bone defect. The hyaline and hypertrophic cartilage can be distinguished by a temporal gene expression profile and determining alkaline phosphatase and collagen X in the cartilage. Alkaline phosphatase and collagen X are required for cartilage mineralization and are classical markers of hypertrophic cartilage (Gawlitta et al., 2010). Most research into tissue engineering hypertrophic cartilage has been carried out using BM-MSCs which are known to differentiate into hyaline chondrocytes under chondrogenic culture conditions and subsequently differentiate further into hypertrophic chondrocytes (Hellingman et al., 2010). Chondrogenic priming of BM-MSC seeded on collagen scaffolds formed trabecular-like bone on subcutaneous implantation (Scotti et al., 2013). Also, BM-MSCs encapsulated in a shaped hydrogel structure formed layer of mineralized tissue in the periphery of the constructs after in vitro chondrogenic priming followed by subcutaneous implantation (Sheey et al., 2015). Another clinically relevant source for tissue engineering hypertrophic cartilage is hyaline chondrocytes isolated from nasal, costal, or articular cartilage. Using chondrocytes for engineering hypertrophic cartilage is advantageous over MSCs as chondrocytes have been shown to produce tissue engineered cartilage with higher levels of ECM with better mechanical properties compared to MSCs (Berstein et al., 2010). Use of hyaline chondrocytes for regenerative medicine and tissue engineering hyaline cartilage has been studied extensively for the clinical repair of articular cartilage and is comprehensively reviewed in Huang et al. (2016). In terms of tissue engineering hypertrophic cartilage for craniofacial reconstruction, nasal chondrocytes isolated from a biopsy of the nasal septum and rib cartilages have potential to undergo hypertrophic differentiation. Bardsley et al. (2017) have shown that nasal chondrocytes seeded onto PGA scaffolds and cultured in chondrogenic medium could form a nonmineralized hypertrophic cartilage-like tissue in vitro which showed a temporal gene profile of hypertrophic chondrocyte differentiation and alkaline phosphatase and collagen X protein expression. On in vivo

implantation into a cranial defect in the rat the nasal chondrocyte-engineered hypertrophic cartilage remodeled into bone (Bardsley et al., 2017). Pippenger et al. reported that did not mineralize following subcutaneous implantation, but did show some mineralization following osteogenic preculture and implantation within ceramic scaffolds (Pippenger et al., 2015). To date, hypertrophic tissue-engineered cartilage has not been used clinically but research to date suggests that this tissue has a high potential to lead to the production of a new clinical bone-graft substitute.

25.3.2 Tissue-engineered cartilage grafts for cartilage craniofacial features

Reconstruction of the cartilage-based features of the face (nose and ears) is currently achieved by using implant-retained silicon prostheses (Hofstede et al., 2016) or surgical reconstruction using autografts comprising skin and cartilage (Nordman et al., 2016). The costal cartilage is often used to sculpt a suitable shaped framework for nasal or auricular cartilage construction however, pain at the donor site from costochondritis can occur. Alternatively, shaped material implants are used to give preformed shapes which can be covered by soft tissue particularly in the case of ear reconstruction. However, problems can arise caused by extrusion of the implants due to a "foreign body" reaction (Rettinger, 2007) although there is ongoing research in the field to develop more suitable implant materials which have the elastic properties which match those of the native tissue and stimulate appropriate cellular interaction to promote integration of the implant with the host tissue (Monibi and Cook, 2016). Although both tissues are cartilage in nature, the nasal cartilages are composed of hyaline cartilage whereas the auricular cartilages have an elastic cartilage phenotype. Cartilage tissue engineering and regenerative cell therapies have been widely researched in the area of articular cartilage regeneration and have been extensively reviewed elsewhere (Huang et al., 2016; Lee et al., 2014; Nayyer et al., 2014). Articular cartilage is a hyaline cartilage which covers the ends of the long bones in joints and functions as a "biological shock absorber" absorbing the forces generated during joint motion. Cell-based tissue engineering therapies such as chondrocyte implantation and the matrix-associated chondrocyte implantation have been used clinically in repair of articular cartilage for some years (Huang et al., 2016). Development of tissue-engineered nasal or auricular cartilages has lagged behind that of articular cartilage and taken similar approaches to tissue engineering articular cartilage. There are various reports describing in vitro auricular cartilage (Nayyer et al., 2012, 2014) and nasal cartilage tissue engineering (Watson and Reuther, 2014; Pleumeekers et al., 2015; San-Marina et al., 2016). These in vitro studies have used autologous chondrocytes, BM-MSCs, or ADSC-MSCs. Recently a population of nasoseptal chondrocytes with progenitor features was found which hold good potential for tissue engineering nasal cartilage due to their proliferative potential (Elsaesser et al., 2016). One of the challenges faced in using MSCs to produce tissue-engineered nasal and auricular cartilages is that the neo-cartilage tissue formed in the laboratory (and particularly that formed form

MSCs) was found to be of an unstable phenotype and on in vivo implantation resulted in resorption of the neo-cartilage (Nayyer et al., 2012). Additionally, the cartilage neo-tissues do not have the mechanical stiffness approaching that of the native tissues. The stability of the cartilage neo-tissues is enhanced by prolonging the period of in vitro culture (to 8–12 weeks) before implantation in in vivo (Lui et al., 2017). This procedure gave a more mature cartilage on subcutaneous in vivo implantation and was also found to reduce potential inflammatory reactions induced by scaffold materials. This type of approach would also enable the patient to be their own bioreactor as discussed above. After a period of in vitro culture an autologous tissue-engineered nasal or auricular cartilage could be further matured subcutaneously until the tissue-engineered tissue was sufficiently mature with the appropriate mechanical properties to be transplanted for reconstruction of the nose or ears. As discussed above cartilage is particularly suited to maturation by subcutaneous implantation as it is better able to survive the low oxygen tensions found within the wound bed. Currently there are only two clinical studies using tissue-engineered nasal cartilage for reconstruction of alar cartilage defects. Fulco et al. (2014) described the first-in-human trial with an engineered cartilage graft formed from nasal chondrocytes cultured on an FDA approved collagen membrane. Gentile et al. (2016) used autologous nasal chondrocytes encapsulated in a fibrin gel formed using autologous platelet rich plasma.

25.4 Conclusions/future trends

The current “gold standard” for repairing defects in craniofacial bone and cartilage structures is autograft bone. However, large tissue defects may require more tissue than the available autologous tissues. Hence there is a real clinical need to find alternative strategies to regenerate craniofacial cartilage and bone defects. Tissue engineering technologies have tremendous potential to provide engineered cartilage and bone tissues for transplantation. However, there are still hurdles to be overcome before this technology will enter into more mainstream craniofacial reconstructive surgery. The ideal biocompatible, bioactive scaffolds for cartilage, and bone regeneration still need to be developed for use in man. These scaffolds should be able to direct the appropriate differentiation and tissue formation by MSCs or primary cells and also promote stability of the immature engineered tissue without causing any inflammatory response in the host tissues. Translation into the clinic requires: appropriate regulatory approvals, approved clean rooms in which to prepare the tissues, development of scaled up bioreactor systems to maintain nutrient transfer through the developing engineered tissue before implantation into a tissue defect or possible subcutaneous implantation a more prolonged period to enable tissue maturation before surgical transplantation into a craniofacial defect. Although challenges remain to translate tissue-engineered cartilage and bone into the clinical setting, the early clinical trials and case studies have shown that tissue engineering can offer cartilage and bone grafts for successful regeneration of craniofacial defects.

References

Ahmed, N., Lyles, J., Panchal, J., Deschamps-Braley, J., 2008. Outcomes and complications based on experience with resorbable plates in paediatric craniosynostosis patients. J. Craniofac. Surg. 19, 855–860.

Amini, A.R., Laurencin, C.T., Nukavarapu, S.P., 2012. Bone tissue engineering: recent advances and challenges. Crit. Rev. Biomed. Eng. 40, 363–408.

Ariani, N., Visser, A., van Oort, R.P., et al., 2013. Current state of craniofacial prosthetic rehabilitation. Int. J. Prosthodont. 26 (1), 57–67.

Arcos, D., Regi, M.V., 2013. Bioceramics for drug delivery. Acta Biomater. 61, 890–911.

Aurrekoetxea, M., Garcia-Gallastegui, P., Irastoraza, I., Luzuriaga, J., et al., 2015. Dental pulp stem cells as a multifaceted tool for bioengineering and the regeneration of cranio-maxillofacial tissues. Front. Physiol. 6, Article 289.

Badylak, S.F., Weiss, D.J., Caplan, A., Macchiarini, P., 2012. Engineered whole organs and complex tissues. Lancet. 379 (9819), 943–952.

Bain, B.J., 2003. Bone marrow biopsy morbidity and mortality. Br. J. Haematol. 121 (6), 949–951.

Bardsley, K., Kwarciak, A., Christine Freeman, C., Brook, I.M., et al., 2017. Repair of bone defects *in vivo* using tissue engineered hypertrophic cartilage grafts produced from nasal chondrocytes. Biomaterials. 112, 313–323.

Begam, H., Nandi, S.K., Kundu, B., Chanda, A., 2017. Strategies for delivering bone morphogenic protein for bone healing. Mater. Sci. Eng. C. 70, 856–869.

Bernstein, P., Sticht, C., Jacobi, A., Liebers, C., et al., 2010. Expression pattern differences between osteoarthritic chondrocytes and mesenchymal stem cells during chondrogenic differentiation. Osteoarthrit. Cartil. 18, 1596–1607.

Berendsen, A.D., Olsen, B.R., 2015. Bone. Bone. 80, 14–18.

Bessa, P.C., Casal, M., Reiss, R.L., 2008. Bone morphogenic proteins in tissue engineering: the road from laboratory to clinic, part II (BMP delivery). J. Tissue Eng. Regen. Med. 2 (2-3), 81–96.

Boher, M., Galea, L., Doebelin, N., 2012. Calcium phosphate bone graft substitutes: failures and hopes. J. Eur. Ceram. Soc. 32, 2663–2663.

Bose, S., Roy, M., Bandyopadhyay, A., 2012. Recent advances in bone tissue engineering scaffolds. Trends Biotechnol. 30, 545–554.

Bruder, S.P., Jaiswal, N., Haynesworth, S.E., 1997. Growth kinetics, self-renewal and the osteogenic potential of purified human mesenchymal during extensive subcultivation and following cryopreservation. J. Cell. Biochem. 64 (2), 278–294.

Chang, S.C.N., Tai, C.L., Chung, H.Y., Lin, T.M., Jeng, L.B., 2009. Bone marrow mesenchymal stem cells form ectopic woven bone in vivo through endochondral bone formation. Artificial Organs. 33, 301–308.

Chatterjea, A., Meijer, G., van Blltterswijk, C., de Boer, C., 2010. Clinical application of human mesenchymal stromal cells for bone tissue engineering. Stem Cells Int. 2010, 215625.

Ciocca, L., Fantini, M., Marchetti, C., Scotti, R., et al., 2010. Immediate facial rehabilitation in cancer patients using CAD-CAM and rapid prototyping technology: a pilot study. Support. Care Cancer. 18 (6), 723–728.

Damien, C.J., Parsons, J.R., 1991. Bone-graft and bone-graft substitutes—a review of current technology and applications. J. Appl. Biomater. 2, 187–208.

Davies, J.E., Matta, R., Mendes, V.C., De Carvalho, Perri, 2010. Development, characterisation and clinical use of a biodegradable composite scaffold for bone tissue engineering in oro-maxillofacial surgery. Organogenesis. 6 (3), 161–166.

De Francesco, M.D., Ricci, G., D'Andrea, F., Nicoletti, G.F., Ferraro, G.A., 2015. Human adipose stem cells from bench to bedside. Tissue Eng. B. 21 (6), 572–584.

De Ugarte, D.A., Morizono, K., Elbarbary, A., Alfonso, Z., et al., 2003. Comparison of multi-lineage cells from human adipose tissue and bone marrow. Cells Tissues Organs. 174, 101–109.

Delloye, C., Cornu, O., Druez, V., Barbier, O., 2007. Bone allografts. J. Bone Joint Surg. (Br.). 89-B, 574–580.

Dorozhkin, S.V., 2009. Calcium orthophosphate-based biocomposites and hybrid biomaterials. J. Mater. Sci. 44, 2343–2387.

Elsaesser, A.F., Schwartz, S., Joos, H., Koerber, L., et al., 2016. Characterisation of a migrative subpopulation of adult human nasoseptal chondrocytes with progenitor cell features and their potential for in vivo cartilage regeneration studies. Cell Biosci. 6 (11), doi:10.1186/s13578-016-0078-6 (ahead of print).

Epple, B.L., Pietrzak, W.S., Blanton, M.W., 2005. Allograft and alloplastic bone substitutes. A review of science and technology for the craniofacial surgeon. J. Craniofact. Surg. 16, 901–989.

Finkemeier, C.G., 2002. Bone grafting and bone graft substitutes. J. Bone Joint Surg. Am. 84 (3), 454–464.

Fisher, D.M., Wong, J.M.L., Crowley, C., Kahn, W.S., Wasim, S., 2013. Preclinical and clinical studies on the use of growth factors for bone repair: a systematic review. Curr. Stem Cell Res. Ther. 8, 260–268.

Frohlich, M., Grayson, W.L., Wan, L., Marolt, D., et al., 2008. Tissue engineered bone grafts: biological requirements, tissue culture and clinical relevance. Curr. Stem Cell Res. Ther. 3, 254–264.

Fulco, I., Haug, M.D., Barbero, A., Wixmerten, A., et al., 2014. Engineered autologous cartilage tissue for nasal reconstruction after tumour resection: an observational first-in-human trial. Lancet. 384, 337–346.

Gawlitta, D., Farrell, E., Malda, J., Creemers, L.B., et al., 2010. Modulating endochondral ossification of multipotent stromal cells for bone regeneration. Tissue Eng. B. 16, 385–395.

Gentile, P., Scioli, M., Bielli, A., Orlandini, A., Cervelli, V., 2016. Reconstruction of alar nasal cartilage defects using a tissue engineering technique based on a combined used of autologous chondrocyte micrografts and platelet-rich plasma: preliminary clinical and instrumental evaluation. Plast Reconstr. Surg. Glob. Open. 4, e 1027.

Giannoudis, P.V., Dinopoulos, H., Tsiridis, E., 2005. Bone substitutes: an update. Injury. 36 (Suppl. 3), S1–S3.

Goulet, J.A., Senunas, L.E., DeSilva, G.L., Greenfield, M., 1997. Autogenous iliac crest bone graft—complications and functional assessment. Clin. Orthopaed. Related Res. 339, 76–81.

Gupte, M.J., Ma, P.X., 2012. Nanofibrous scaffolds for dental and craniofacial applications. J. Dent. Res. 91, 227–234.

Hatton, P.V., Hurrell-Gillingham, K., Brook, I.M., 2006. Biocompatibility of glass-ionomer cements. J. Dent. 34, 598–601.

Helios, M., Ripamonti, U., Ferretti, C., Kerawala, C., et al., 2009. The basic science of bone induction. Br. J. Oral Maxillofac. Surg. 47, 511–514.

Hellingman, C.A., Koevoet, W., Kops, N., et al., 2010. Fibroblast growth factor receptors I n *in vitro* and *in vivo* chondrogenesis: relating tissue engineering using adult mesenchymal stem cells to embryonic development. Tissue Eng. A. 16, 545–556.

Hernigou, P., Desroches, A., Queinnec, S., Lachaniette, C.H.F., 2014. Morbidity of graft harvesting versus bone marrow aspiration in cell regenerative therapy. Int. Orthopaed. (SICOT). 38, 1855–1860.

Hirao, M., Tamai, N., Tsumaki, N., Yoshikawa, H., Myoui, A., 2006. Oxygen tension regulates chondrocyte differentiation and function during endochondral ossification. J. Biol. Chem. 281, 31079−33109.

Hofstede, T.M., Rhonda, F., Jacob, D.D.S., Montgomery, P., Wesley, P., 2016. Surgical preparation of nasal defects to enhance the implant-retained facial prostheses: a case report. Head Neck. 39, E4−E11.

Huang, B.J., Hu, J.C., Athanasiou, K.A., 2016. Cell-based tissue engineering strategies used in the clinical repair of articular cartilage. Biomaterials. 98, 1−22.

Hurrell-Gillingham, K., Reaney, I.M., Miller, C.A., Crawford, A., Hatton, P.V., 2003. Devitrification of ionomer glass and its effect on the *in vitro* biocompatibility of glass-ionomer cements. Biomaterials. 24 (18), 3153−3160.

Holzwarth, J.M., Ma, P.X., 2011. Biomimetic nanofibrous scaffolds for bone tissue engineering. Biomaterials. 32 (9622−9628), 92−93.

Hsu, S.-H., Hung, K.-C., Chen, C.-W., 2016. Biodegradable polymer scaffolds. J. Mater. Chem. B. 4, 7493−7505.

Hu, K., Olsen, B.R., 2016. The roles of vascular endothelial growth factor in bone repair and regeneration. Bone. 91, 30−38, 2.

Im, G.I., Shin, Y.W., Lee, K.B., 2005. Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells?. Osteoarthrit. Cartilage. 13, 845−853.

Jafari, M., Paknejad, Z., Rad, M.R., Motamedian, S.R., et al., 2017. Polymeric scaffolds in tissue engineering: a literature review. J. Biomed. Mater. Res. B-Appl. Biomater. 105 (2), 431−459.

Jones, J.J., 2013. Review of bioactive glass: from Hench to hybrids. Acta Biomater. 9, 4457−4486.

Kapur, S.K., Dos-Anjos Vilaboa, S., Llull, R., Katz, A.J., 2015. Adipose tissue and stem/progenitor cells: discovery and development. Clin. Plast. Surg. 42, 155−167.

Kirkpatrick, C.J., Fuchs, S., Unger, R.E., 2011. Co-culture systems for vascularization—learning from nature. Adv. Drug Deliv. Rev. 63 (4-5), 291−299, Special issue SI.

Kuhne, J.H., Bart, L.R., Frisch, B., Hammer, C., et al., 1994. Bone-formation in coralline hydroxyapatite—effects of pore-size studied in rabbits. Acta Orthop. Scand. 65, 246−252, 1994.

Langer, R., Vacanti, J.P., 1993. Tissue engineering. Science. 260, 920−926.

Lee, J.K., Responte, D.D., Cissell, D.D., Hu, J.C., et al., 2014. Clinical translation of stem cells: insight for cartilage therapies. Crit. Rev. Biotechnol. 34 (1), 89−100.

Li, J., Zhang, L., LV, S., Li, N., et al., 2011. Fabrication of individual scaffolds based on a patient-specific alveolar bone defect model. J. Biotechnol. 151 (1), 87−93.

Lotolf, M.P., Hubbell, J.A., 2005. Synthetic biomaterials as instructive microenvironments for morphogenesis in tissue engineering. Nature Biotech. 23, 47−55.

Lui, Y., Li, D., Yin, Z.Q., Luo, X.S., et al., 2017. Prolonged *in vitro* culture precultivation alleviates post-implantation inflammation and promotes stable subcutaneous cartilage formation in a goat model. Biomed. Biomater. 12 (1), doi:10.1088/1748-605X/12/1/015006. (Epub ahead of print).

Malda, J., Martens, De, Tramper, J., Van Blitterswijk, C.A., Riesle, J., 2003. Cartilage tissue engineering: controversy in the effect of oxygen. Crit. Rev. Biotech. 23, 175−194.

Mehlhorn, A.T., Niemeyer, P., Kaiser, S., Finkenzeller, G., et al., 2006. Differential expression pattern of extracellular matrix molecules during chondrogenesis of mesenchymal stem cells from bone marrow and adipose tissue. Tissue Eng. 12, 2853−2862.

Meijer, G.J., De Bruijn, J.D., Koole, R., Van Blitterswijk, C.A., 2007. Cell-based bone tissue engineering. PLoS Med. 4, 260−264.

Metzar, S., Blanche, U., Lienemann, P.S., Karlsson, M., et al., 2016. Cell-mediated proteolytic release of growth factors from poly(ethylene glycol) matrices. Macromol. Sci. 16 (11), 1703−1713.

Mohr, J., Germain, M., Winters, M., Fraser, S., et al., 2016. Disinfection of human musculoskeletal allografts in tissue banking: a systematic review. Cell Tissue Bank. 17 (4), 573−584.

Monibi, F.A., Cook, J.L., 2016. Tissue engineered matrix bioscaffolds: emerging applications in cartilage and meniscus repair. Tissue Eng. Bdoi:10.1089/ten.TEB.2016.0431 (E-pub ahead of print).

Murphy, C.M., Haugh, M.G., O'Brian, F.J., 2010. The effect of mean pore size on cell attachment, proliferation and migration in collagen-glycosaminoglycan scaffolds for bone tissue engineering. Biomaterials. 31, 461−466.

Nayyer, L., Birchall, M., Seifalian, A.M., Jell, G., 2014. Design and development of nanocomposite scaffolds for auricular reconstruction. Nanomed. Nanaotechnol. Biol. Med. 10, 235−246.

Naderi, N., Combellack, E.J., Griffin, M., Sedaghati, T., et al., 2016. The regenerative role of adipose-derived stem cells (ADSCs) in plastic and reconstructive surgery. Int. Wound J. 14, 112−124.

Nordman, M., Veit, J., Rotter, N., Hoffman, T.K., Schithauer, M.O., 2016. Autologous composite grafts from the caum conchae for reconstruction of multilayer nasal defects. HNO. 64 (5), 288−291.

Nayyer, L., Patel, K.H., Esmaeili, A., Rippel, R.A., et al., 2012. Tissue engineering: revolution and challenge in auricular cartilage reconstruction. Plastic Recon. Surg. 129 (5), 1123−1137.

Newell, R., Marks, I., 2000. Phobic nature of social difficulty in facially disfigured people. Br. J. Psychiatr. 176, 177−181.

Oppenheimer, A.J., Mesa, J., Buchman, S.R., 2012. Current and emerging basic science concepts in bone biology: implications in craniofacial surgery. J. Craniofac. Sur. 23, 30−36.

Ortega, N., Wang, K., Ferrara, N., Werb, Z., et al., 2010. Complementary interplay between matrix metalloproteinase-9, vascular endothelial growth factor and osteoclast function drives endochondral bone formation. Dis. Models Mech. 3 (3-4), 224−230.

Park, J.S., Yi, S.W., Kim, H.J., Kim, S.M., Park, K.H., 2016. Regulation of cell signalling factors using PLGA nanoparticles coated/loaded with genes and proteins for osteogenesis of human mesenchymal cells. ACS Appl. Mater. Interfaces. 8 (44), 30387−30397.

Pippenger, B.E., Ventura, M., Pelttari, K., Feliciano, S., et al., 2015. Bone forming capacity of adult human nasal chondrocytes. J. Cell Mol. Med. 19 (6), 1390−1399.

Pleumeekers, M.M., Nimeskem, L., Koevoet, W.L.M., Karperien, M., et al., 2015. Cartilage regeneration in the head and neck area: combination of ear or nasal chondrocytes and mesenchymal stem cells improves cartilage production. Plastic Recon. Surg. 136 (6), 762E−774E.

Porter, J.R., Ruckh, T.T., Popat, K.C., 2009. Bone tissue engineering: a review in bone biomimetics and drug delivery strategies. Biotechnol. Prog. 25 (6), 1539−1560.

Rettinger, G., 2007. Risks and complications in rhinoplasty. GMS Curr. Top. Otorhinolaryngol. Head Neck Surg.6, Doc 08.

Rhotena, B.A., Murphy, B., Ridner, S.H., 2013. Body image in patients with head and neck cancer: a review of the literature. Oral Oncol. 49 (8), 753−760.

San-Marina, S., Sharma, A., Voss, S., Janus, S.G., et al., 2016. Assessment of scaffolding properties for chondrogenic differentiation of adipose-derived mesenchymal stem cells

in nasal reconstruction. JAMA Facial Plast. Surg.doi:10.1001/jamafacial.2016.1200 (Epub ahead of print).
Scheller, E.L., Krebsbach, P.H., Kohn, D.H., 2009. Tissue engineering: state of the art in oral rehabilitation. J. Oral Rehabil. 36, 368−389.
Scotti, C., Piccinini, E., Takizawa, H., Toderov, A., et al., 2013. Engineering of a functional bone organ through endochondral ossification. Proc. Natl. Acad. Sci. U.S.A. 110 (10), 3997−4002.
Sheey, E.J., Mesallati, T., Kelly, L., Vinardell, T., et al., 2015. Tissue engineering whole bones through endochondral ossification: regenerating the distal phalanx. Biores. Open Access. 4 (1), 229−241.
Schubert, T., Xhema, D., Veriter, S., Schubert, M., et al., 2011. The enhanced performance of bone allografts using osteogenic-differentiated adipose-derived mesenchymal stem cells. Biomaterials. 32, 8880−8891.
Sharpe, P.T., 2016. Dental mesenchymal stem cells. Development. 143, 2273−2280.
Stevens, M.M., 2008. Biomaterials for bone tissue engineering. Mater. Today. 11, 18−25.
Thambi, T., Phan, V.G.H., Lee, D.S., 2016. Stimuli-sensitive injectable hydrogels based on polysaccharides and their biomedical applications. Macromol. Rapid Commun. 37 (23), 1881−1896, Sp issue 1.
Visser, A., Raghoebar, G.M., van Oort, R.P., Vissink, A., et al., 2008. Fate of implant-retained craniofacial prostheses: life span and aftercare. Int. J. Oral Maxillofac. Implants. 23 (1), 89−98.
Wagenblast, J., Mehran, B., Helbig, M., Arnoldner, C., et al., 2008. Craniofacial reconstruction with bone-anchored epithesis in head and neck cancer patients—a valid way back to self-perception and social reintegration. Anticancer Res. 28 (4C), 2349−2352.
Wagoner Johnson, A.J., Herscher, B.A., 2011. A review of the mechanical behaviour of CaP and CaP/polymer composites for applications in bone replacement and repair. Acta Biomater. 7, 16−30.
Wang, Q.F., Yan, J.H., Yang, J., Li, B.Y., 2016. Nanomaterials promise better bone repair. Mater. Today. 19 (8), 451−463.
Warnke, P.H., Springer, I.N.G., Wiltfang, J., Acil, Y., et al., 2004. Growth and transplantation of a custom vascularised bone graft in a man. Lancet. 364, 766−770.
Watson, D., Reuther, M.S., 2014. Tissue-engineered cartilage for facial surgery. Curr. Opin. Otol. Head Neck Surg. 22 (4), 3000−3306.
Watt, F.M., Huck, W.T.S., 2013. Role of the extracellular matrix in regulating stem cell fate. Natl. Rev. Mol. Cell Biol. 14, 467−473.
Williams, D.F., 2008. On the mechanisms of biocompatibility. Biomaterials. 29, 2941−2953.
Yoshimura, K., Sato, K., Aoi, N., Kurita, M., et al., 2008. Cell-assisted lipotransfer for cosmetic breast augmentation: supportive use of adipose-derived stem/stromal cells. Aesthetic Plast. Surg. 32, 48−55.
www.changingfaces.org.uk/Health-Care-Professionals/Intoduction-to-patient-needs/Statistics.
Yang, H.Y., Niu, L.N., Sun, J.L., Huang, X.Q., et al., 2017. Biodegradable mesoporous delivery system for biomineralisation precursors. Int. J. Nanomed. 12, 839−854.
Yu, N.C.Y., Schindler, A., Little, D.G., Ruys, A.J., 2010. Biodegradable poly(alpha-hydroxyacid) polymer scaffolds for bone tissue engineering. J. Biomater. Mater. Res. B. 93B (91), 285−295.
Zhao, J.J., Griffin, M., Cai, J., Li, S.X., et al., 2016. Bioreactors for tissue engineering: an update. Biochem. Eng. J. 109, 268−281.

This page intentionally left blank

Craniofacial surgery, orthodontics, and tissue engineering

26

Mina D. Fahmy[1], *Brinda Shah*[1], *Jasmine Faldu*[2], *Tarek El-Bialy*[3], *Hossein E. Jazayeri*[2] and *Lobat Tayebi*[1]
[1]Marquette University School of Dentistry, Milwaukee, WI, United States, [2]University of Pennsylvania School of Dental Medicine, Philadelphia, PA, United States, [3]University of Alberta Faculty of Medicine and Dentistry, Edmonton, AB, Canada

26.1 Introduction

Tissue engineering is an evolving therapeutic strategy that can be used to build tissues and organs de novo. This principle is usually applied when traditional methods of regeneration (for example, using autografts or allografts) fail or cannot be implemented. Tissue engineering is an emerging field because it has shown to significantly improve the restoration and maintenance of tissue and organ function. Specifically, in the dental field, tissue engineering is aiming to repair the periodontal ligament (PDL), the cementum, and the alveolar bone. Artificial replacements and transplants have shown success thus far; however, results show a heavy need for maintenance, cost, and sensitivity relative to tissue engineering (Taba et al., 2005). Regardless of their reported success, these methods of bone/tissue grafting/implants have been reported to have various degrees of failures. These failures are either due to immune reaction, rejection, inflammation, or imbalance between the functional loading to these implanted tissues/materials and their internal physical and biological characteristics' ability to withstand such loading. The field has evolved to revolutionize healthcare in various specialties of care, such as cardiothoracic surgery, nephrology, orthopedic surgery, otolaryngology, and dental surgery, alike. Methods of tissue reconstruction are being expanded each year, making not only the most optimal and advanced techniques possible for delivery but also the material and cell type that best suits the human body. A wide variety of factors play a role in successful reconstruction, including, but not limited to: scaffold characteristics, such as biodegradability, biocompatibility, mechanical properties, and porosity. Other factors involve clinical outcomes dependent on tissue dynamics, including increased surrounding cell density, neovasculature, and the expression of proteins and biomarkers that enhance holistic repair of tissue architecture. This chapter will shed light on regeneration within the scope of orthodontics and cover existing tactics in craniofacial reconstructive therapy and the newest progress in this patient-specific treatment modality (Jazayeri et al., 2016; Kang et al., 2016).

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00026-8
© 2017 Elsevier Ltd. All rights reserved.

26.2 Scaffolds

Craniofacial tissue engineering involves utilization/seeding of human tissue related cells, preferably from the craniofacial area, onto a biocompatible scaffold. The proliferation of these cells is normally followed by migration and differentiation into various needed tissues. It is important to select scaffolds with characteristics that allow for the production of tissues with specific geometry and size. Basic requirements for craniofacial skeletal scaffolds are the ability to: (1) adapt to three-dimensional (3D) anatomical defects, (2) provide temporary load bearing until the tissue forms, and (3) enhance tissue regeneration through biofactor delivery. The scaffold must be mechanically durable to withstand the high compressive force of craniofacial bone. Similarly, it must be bioactive to incorporate cell proliferation and differentiation, ultimately leading to new tissue formation. The pores designed throughout the scaffold facilitate nutrient and cell migration to properly mitigate the therapy and recreate the environment of native tissue. Scaffolds vary in material type, and the incorporation of cells and bioactive agents need to allow for the regeneration of the craniofacial osteology. For future use in clinical orthodontics the scaffold needs to be easily manufactured, cost-effective, and highly reproducible with the same bioengineering specifications in order to be marketed and commercialized (Young et al., 2002; Wang et al., 2005).

26.3 Stem cells

There are two sources from which stem cells are derived: adult stem cells and embryonic stem cells (Seo et al., 2004a). Adult stem cells, also known as progenitor cells, are undifferentiated cells that can undergo self-renewal and differentiate to any cell type in the body. In addition, adult stem cells divide in order to replenish the dying/apoptotic cells and tissues. Many of these cells are found in the oral region and are used during alveolar bone formation. Embryonic stem cells are created through culturing cells during the early phases of embryonic development into a specific, desired goal (Seo et al., 2004a). Undifferentiated biological cells that have the capacity to differentiate into specialized cells that form specific tissues or self-renew to foster more multipotent cells are known as stem cells. These cell types are categorized in mammals as embryonic stem cells or adult stem cells, which is dependent on their origin. Adult stem cells are present in humans postnatally and include a variety of tissues such as blood, neural, and skin (Mao et al., 2006). These adult stem cells are mesenchymal cells (MSCs), which have incredible regenerative capacity and can be found in dental tissues as well as bone marrow, adipose tissue, skeletal muscle, and several other anatomical sites (Sakaguchi et al., 2005; Erices et al., 2000; Gonzalez et al., 2009; Zaky and Cancedda, 2009). The orofacial region alone is home to six different subsets of stem cells recently discovered by researchers. Dr. Songtao Shi from the University of Pennsylvania has

discovered the presence of stem cells in various orofacial tissues, including dental pulp, human exfoliated deciduous teeth, PDL, and gingiva derived mesenchyme (Gronthos et al., 2000, 2002; Miura et al., 2003; Seo et al., 2004b). Other researchers have also studied progenitor cells from the dental follicle and bone marrow mesenchymal stem cells present in mandible. The multipotent property of MSCs makes them a critical player in tissue engineering possibilities.

Since the orofacial region is extremely intricate and complex, traditional therapeutic approaches of autologous bone grafting have several limitations including the lack of available graft material and donor site morbidity. With the rise and discovery of new stem cell populations derived from MSCs, the application of these cells in the oromaxillofacial complex are vast (Tevlin et al., 2014). This region of the body is unique in that when fabricating the tissue from stem cells on a scaffold, it is critical to realize the various vectors of force at play. Creating a scaffold for the mandible or maxilla, which experiences different vertical, horizontal, and shear forces, requires the scaffold to adapt with initial plasticity (Zaky and Cancedda, 2009). The scaffold creates a framework and sound structural foundation for the cells to grow and develop the necessary extracellular matrix and other required elements for proper form and function. Years ago, this concept of tissue engineering for craniofacial structures was practically unknown; however, the field has made many advances within the last decade. There has been an active effort to extract different stem cell populations from various craniofacial tissues in order to purify them and apply them to tissue engineering studies focused on the facial region. For example, a study has been conducted using a single population of MSCs to develop a temporomandibular joint (TMJ) with layers of bone and integration of cartilage to mimic a human TMJ (Alsberg et al., 2001). Another study illustrated the capability of dental pulp stem cells developing dentin-like crystalline structures from mineral deposits present during cell growth (Gronthos et al., 2000; About et al., 2000; Couble et al., 2000). Furthermore, a study conducted by Seo et al. that utilized PDL stem cells was able to mimic native PDL that interfaced with structures similar to Sharpey's fibers (Seo et al., 2004b). Stem cells from human exfoliated deciduous teeth (SHED) have also given rise to incredible tissue engineering opportunities. Within this population of cells, there is immense capacity for proliferation. Several osteoblastic markers were expressed on the cells that were cultured, such as bone sialoprotein, Runx2, alkaline phosphatase, matrix extracellular phosphoglycoprotein, and DSPP, which suggests that these cells can be harvested and used within scaffolds to fabricate craniofacial structures that mimic dentin-like structures (Miura et al., 2003).

The evidence presented in a snapshot of only a few studies illustrates the clinical applications of stem cells within the dental field are vast with endless applications. Although stem cells derived from mesenchymal origin and specific craniofacial anatomical regions opens the door to future advancements in tissue engineering that can change the direction of dentistry, there are still many challenges remaining with regard to the clinical application of the stem cells (Almela et al., 2016).

26.4 Alveolar bone defects, current, and future repairing techniques

Alveolar bone defects may result from trauma, tumors, developmental defects such as cleft palate, or periodontal disease, where bone destruction involves alteration of the height and morphology of alveolar bone. The four main types of alveolar bone destruction due to periodontitis include horizontal defects, vertical or angular defects, fenestrations, and dehiscence (Teng et al., 2000). Horizontal bone loss results in uniformly decreased height of the affected alveolar bone. This uniform change in height occurs because this type of bone loss results in a consistent amount of bone resorption. Vertical defects include bone loss resulting in a nonuniform decrease in height, generally occurring adjacent to a tooth and taking the shape of a triangle. A fenestration is depicted as an opening of bone loss on the facial or lingual aspect, resulting in exposure of root surfaces that usually come into contact with the gingiva. Fenestrations are different from dehiscence, because they are surrounded by alveolar bone along the coronal aspect. A dehiscence mostly causes alveolar bone loss on the facial aspect, resulting in the exposure of an oval dental root defect (Teng et al., 2000).

In periodontal disease due to bacterial invasion the first response to a bacterial infection is an inflammatory reaction, which causes the stimulation of the innate immune system. The immune response targets specific antigens by releasing cytokines, leukocytes, and other agents resulting in the inflammation of gingival tissue. The spread of the inflammation from the gingival tissue to the connective tissue and subsequently to the alveolar bone directly underneath is the main cause of bone loss. The spread of inflammation does not cease until the source of infection and plaque is completely removed (Kolokythas et al., 2010).

Factors that determine the exact pattern and type of alveolar bone defects include the bone thickness, anatomical location, and anatomy of the tooth. Each tooth contains a variable alveolar thickness and blood supply; therefore the pattern in which bone loss occurs differs from case to case. For example, a dehiscence usually occurs in areas with a thin bone plate. In contrast, a fenestration typically occurs in areas where the bone is fairly thick and large. The location and anatomy of the tooth are also factors that play a critical role in the overall pattern of the defect. For example, incisors tend to have thicker bone, while canines tend to have thinner bone. The contact between two teeth produces yet another variable to consider, as the environment can create various shapes of bone resorption (Schwartz et al., 1997). Clinicians must keep these characteristics in mind when dealing with different types of alveolar bone defects, especially because each patient can present with unique bone destruction patterns. In order to facilitate proper treatment approaches, it is imperative to distinguish between these patterns.

There are many forms of treatment for alveolar bone defects. Two techniques that have been combined and utilized most frequently with successful results are bone grafting and guided bone regeneration (GBR). Other specific regenerative

methods that have been utilized for alveolar bone reconstruction are growth factor (GF) application, cell therapy, and the combination of both of these methods. A key goal for every reconstruction technique is to restore the anatomy of the alveolar bone to its normal position. The necessity of reconstruction of the alveolar defect in cleft palate patients is crucial, as it will support a prosthesis that can function under multiple potential forces generated, in addition to providing adequate bone for the implant to be placed (van Hout et al., 2011).

As each alveolar bone defect is unique, each case presents itself with a unique possible regeneration method. The selection of the donor site depends on location, size, and anatomy of the alveolar defect. The main sites used for harvesting bone grafts include the iliac crest, tibia, rib, mandibular symphysis, and the ramus buccal shelf. The intraoral donor sites have increased in popularity due to time efficiency and easy access during surgery, as well as leading to less bone resorption overall compared to the use of distant sites listed above (Persson et al., 1998). The reason why autologous bone grafting has been such a popular method of bone regeneration for alveolar bone defects is due to the viability of osteoblasts and progenitor cells that are directly used for bone formation. A disadvantage of using autografts exclusively is that a very small amount of bone tissue can be harvested, and molding the tissue into the desired shape is extremely difficult. Owing to these setbacks, autografts are combined with the use of GFs and bone tissue engineering in order to provide even more successful outcomes (Semb, 2012).

A very important regenerative method for alveolar bone reconstruction is the use of GFs. GFs play a crucial role in cell growth, proliferation, cell differentiation, and repair. Their characteristics include being fairly large, hydrophilic, and stored in vesicles released by exocytosis. Because of their hydrophilic nature, GFs are impermeable to a cell membrane; therefore they act on high-affinity, cell membrane receptors in order to perform their desired functions. GFs can either target one specific line of a cell population, or transduce to multiple cell populations (Haraji et al., 2012). The entire regeneration process using GFs can be grouped into three stages: inflammation, proliferation, and remodeling. The inflammatory stage, as mentioned above, occurs directly after infection or injury occurs. This is when clotting occurs, and immune cells are signaled to perform the desired duties of keeping the injury clean. In the proliferative stage, fibroblasts produce a granulation tissue over the area of injury to seal the gap and prevent any incoming foreign material. Lastly, in the remodeling stage, which is the longest, tissue is regenerated and has strong mechanical properties. GFs can be used in all three of these stages (Haraji et al., 2012). The most popular GFs used in dentistry are the bone morphogenetic proteins (BMPs), which are highly functional cytokines that are involved directly in bone formation and regulation of the entire developmental process of the bone. There are over 20 identified BMPs with various functions in humans, related to the formation of bone and cartilage. The BMP ligand binds to the specific receptor on the surface of a cell. From there, the binding triggers a certain intracellular signaling pathway to be activated. Thus the cascade causes gene transcription to occur in the nucleus, where initiation of new bone generation is started (Dunn et al., 2005). Another very popular GF used

in modern dentistry is the growth and differentiation factor (GDF), which has very similar properties to BMPs. The importance of GDFs is that they significantly increase cell adhesion in the first stages of regeneration, and also increase the amount of cartilage cells during the cell proliferation stage of bone regeneration, which takes place in the later stages of the overall process (Morotome et al., 1998; Schliephake, 2002). The use of GFs has been a very promising approach recently for new bone formation, as it has created faster and enhanced results due to the direct delivery of GFs to the site of the graft. In addition, since GFs have a 3D protein structure, it provides stability and protection during the bone replacement process. In addition, the use of GFs has also been tested to lower the occurrence of dry sockets, the infection of the tooth extraction site, as well as pain and the healing processes for patients (Haraji et al., 2012).

Owing to the soluble characteristics of BMPs and other GFs, they have a higher rate of degradation when being delivered to the site of injury. Therefore there is an insufficient amount of bioavailability at the site. Additionally, as GFs are proteins, this indicates potential consequences, such as denaturation due to pH and temperature as well as competition with other enzymes and proteins (van Hout et al., 2011). Due to this setback, a proper delivery system of the GF is necessary for an adequate amount of protein concentrations to reach the site of injury because regenerative cells need to be able to thrive and perform their action during all the stages of bone regeneration. Furthermore, not only is a proper delivery system needed, but also an effective and accurate release to the desired target, because the growth of bone or cartilage tissue in a nondesired area can have detrimental and unhealthy outcomes. Some complications include hematomas, carcinogenicity, and teratogenic effects (van Hout et al., 2011).

Certain GFs work better during particular stages of the regeneration process. For example, some are expressed heavily in the inflammatory stage, while others are only regulated during the osteogenic phases towards the end of the bone regeneration. When higher concentrations and prolonged delivery of GFs in proper doses are occurring, studies have shown that osteogenic regeneration is more effective. The timing at which the GFs are released depends on the healing time of the specific patient being treated, as well as the overall type, location, and anatomy of the infection or injury that is being treated (Giannobile, 1996). Three crucial parts of bone regeneration are osteogenesis, osteoinduction, and osteoconduction. Osteogenesis is defined as new bone formation by cells within an autograft. Osteoinduction is when the process of osteogenesis is activated, or when immature cells are stimulated to become preosteoblasts. Lastly, osteoconduction is the creation of a new haversian system and blood vessels are oriented to support and surround the bone that has been regenerated (van Hout et al., 2011).

Recent studies have shown that the use of cell therapy, or stem cells, can be used to regenerate bone formation and repair the mandible. Stem cells are unspecialized cells that can develop or differentiate into a variety of various cell lines. Normally, the bone that is found in the mandible is spongy and soft, however, the regenerated bone is hard, compact, and highly vascularized, while still maintaining the normal jaw function. A reason for the formation of compact bone is due to stem

cells not following the signals of the spongy tissue that they are surrounded by. Despite regenerating a different bone type in the jaw, the new bone has shown to be positively impactful (Krause et al., 2001). The positive results of using dental pulp stem cells have shown indication for treatment of oral cancer.

Recent studies have also shown that gene therapy has had a profound impact on the regeneration of the PDL and surrounding alveolar bone. These methods can be beneficial because normally, a very high concentration of GFs are needed in order for any given tissue to regenerate, specifically in large defects. Therefore gene therapy allows for the insertion of genes into specific cells with a matrix in order to stimulate a certain targeted effect. The overall goal of gene therapy is to replace a defective mutant allele with a functional one. Another goal of gene therapy is to create a more favorable host response (Jin et al., 2004). With these available and future treatment approaches, alveolar bone defects may have promising regeneration outcomes in the future.

26.5 Periodontal ligament tissue engineering

The PDL is a fibrous connective tissue that connects the tooth to the alveolar bone. PDL contains neural and vascular portions, which are covered by the epithelium above. The connection allows the teeth to have support during their normal functions. The connective tissue is attached to the tooth through the cementum on one side and to the alveolar bone of the jaw on the other. On average the width of PDL is approximately 0.2 mm, with slight variation depending on the patient and the circumstance present. Although the PDL is characterized as being extremely fibrous, it is a very cellular structure that has many important functions to allow the masticatory apparatus to function properly (Beertsen et al., 1997).

The primary function of the PDL is the support system it provides when attaching the tooth to the alveolar bone. The principal fibers are what specifically connect the root cementum to the alveolar bone. The PDL also has the ability to function as a shock-absorber by pushing intravascular fluid tissue out of the PDL space. The PDL also plays a role in remodeling, because the ligament has the ability to create cells that can either turn into or absorb various types of tissues that make up the bone, cementum, and the ligament (Shimono et al., 2003). In addition, the PDL has a sensory function due to the vast number of nerve endings that exist within it (Shimono et al., 2003), which are receptors for pain and pressure. Lastly, the PDL has a nutritive function as it allows for the sustainability of various cells. The PDL is a highly vascularized structure because it contains a large amount of blood supply that maintains and supplies the surrounding cells. The main blood supply to the PDL comes from the superior and inferior alveolar arteries, which are derived from the apical, intraalveolar, and gingival blood vessels (Panagiotopoulou et al., 2011).

The development of the PDL can occur before or after tooth eruption, and it takes place in a few steps. First the fibroblasts that are directly next to the

cementum become aligned in the oblique direction of the tooth. Shortly after, the first collagen fiber bundles of the PDL become visible. The fibers from the cementum and the alveolar bone continually grow and elongate until they eventually connect and fuse. After the oblique and apical fibers are fully grown and formed, the complete structure of the PDL is formed. When the root is still developing, the surrounding dental follicle becomes a thin sheet of connective tissue that will undergo conformation and eventually become the PDL. The cells derived from the dental follicle can also serve to produce various parts of the entire apparatus such as the cementum tissue, alveolar bone, and the ligament itself (Shimono et al., 2003).

The PDL fibers are composed of principal periodontal fibers, which are made of type I collagen fibrils. Depending on their location around the tooth, they possess different names, such as oblique, periapical, or horizontal fibers. The PDL also consists of oxytalan fibers, which are a major component of the extracellular matrix and elastic fibers. Oxytalan fibers tend to have a larger diameter at the side of the alveolar bone compared to the cementum side. Eventually, these fibers from both sides continue unwinding and unraveling into smaller fibers until a connection is made. This framework of interconnected fibers becomes thicker fiber bundles over time for support, and keep the tooth anchored to the alveolar bone (McCulloch et al., 2000). Owing to the fact that teeth tend to drift towards the midline over their lifetime, a tooth tends to be a little displaced mesially. To compensate for this movement the PDL must be able to remodel itself so that it can adjust to the new alveolar bone and cementum parameters. PDLs are composed of unique variety of cells which all function to produce and maintain the three main tissues in this area: the cementum, alveolar bone, and the PDL itself. The main cells that compose the PDL include synthetic cells, resorptive cells, progenitor cells, epithelial cells, and connective tissue cells (Nyman et al., 1982).

Synthetic cells are constantly making new ribosomes, and they increase the function of the rough endoplasmic reticulum and the golgi apparatus. There are three major types of synthetic cells. The first type is called osteoblasts, which cover the surface of the alveolar bone by producing the bone. The second type is called fibroblasts, which make up about 65% of the overall cells in a PDL. These cells are responsible for the synthesis of collagen, or connective tissue, as well as removing collagen fibers during the remodeling portion of the ligament. The last type of synthetic cells are cementoblasts, which make collagen and protein polysaccharides that make up the cementum (Zeichner-David, 2006). Resorptive cells are also divided into three main types. The first type is osteoclasts, which function to resorb bones. Resorption occurs in two main stages: removal of minerals at the margins of the bone and then disintegration and removal of organic and inorganic matrix. There are also collagen-resorbing fibroblasts, which function during the remodeling of the ligament. The cementoclasts are the last type of resorptive cells. They are found on the surface of the cementum, but do not play a role during the remodeling phase of the ligament. Another important cell type that makes up the PDL are called progenitor cells. These cells are extremely important because they are considered undifferentiated mesenchymal cells that can differentiate into various types of cells through mitotic division and replace the dying cells (Kanzaki et al., 2001).

In order for the regeneration of periodontal tissue to be successful, four basic criteria need to be met: correct signaling molecules, cells, blood supply, and scaffolding all need to be able to reach the area of the defect. When the PDL is damaged, if all four components are correctly stimulating and coordinating with one another to form the bioengineering process, then the healing process and generation of tissues can be successful. If this regeneration process can be achieved with the advancements in technology, it can be applied to oral healthcare including orthodontic applications and craniofacial reconstruction, as the PDL is one of the most important features of craniofacial health (Caffesse and Becker, 1991).

The cells involved are crucial because they are the foundation and necessity of new tissue formation and growth. Various signaling molecules, such as GFs, modulate the activity of the cells to allow them to differentiate into many different lines of cells and also regulate this process. In addition, these signaling molecules create the extracellular matrix, which nourishes the growing tissue and ligament. The blood supply is what provides the nutritional element for the developing tissues, as well as allowing the cells to reach homeostasis. Lastly the scaffold provides a template for the regeneration of the tissue (Zeichner-David, 2006).

The most important morphogenetic signaling molecules in this process include BMPs, which lead to the production of cementum and stimulation of cell recruitment during early wound healing in order to regenerate the PDL. Application of these signaling molecules leads to an increase in bone deposition and cementum formation in the areas of periodontal defects and abnormalities (Huang et al., 2005). The mesenchymal and epithelial stem cells are derived from the neural crest and have emerged from the dental follicle during the formation of the root. The scaffolds consist of mainly collagen and fibronectin cells that provide the overall framework for this bioengineering process to achieve the proper environment for the PDL (Chamila Prageeth Pandula et al., 2014). However, despite all four components functioning accurately, a major limiting factor is the potential presence of periodontal pathogens due to infection of the wound or the development of plaque on the teeth. With proper control around the site, the regeneration process of the tissue can still be successful despite periodontitis or other oral infections and inflammation.

Stem cells are a major component in the tissue engineering process. Stem cells have the potential of being differentiated into any specific type of cell that meets certain requirements and properties. The two specific types of stem cells that are used during PDL bioengineering are MSCs and epithelium-originated dental stem cells. MSCs are used to identify osteogenic precursors in the bone marrow, and can form in cartilage, bone, and connective tissue. Epithelium dental stem cells are used to form enamel in teeth (Singh et al., 2012). In order for proper reconstruction of the PDL, an interaction between mesenchymal and epithelial cells must occur. In addition, the stages of osteogenesis, cementogenesis, and connective tissue must occur in the proper order for the ligament to be regenerated correctly. The use of stimulatory and selective methods of bioengineering have been used to regenerate the PDL (Chamila Prageeth Pandula et al., 2014). One method used is a combination of guided tissue regeneration and cell repopulation. The epithelial cells are

used to restrict the growth of the periodontal defect, so that the actual PDL tissues and cells have more space to grow in a more adaptable environment. Some of the materials used as barriers include collagen barriers and ceramic barriers, because these both have the ability to be degraded with enzymatic function. The basic format of the regeneration of the PDL starts off by combining epithelial stem cells with MSCs until they form an attachment, which is an essential step. The stems cells are then recombined and cocultured until they contain the proper properties for their desired locations. GFs are then added to the scaffold, or template, to essentially create the PDL (Dowell et al., 1991).

Specifically, for the regeneration of the PDL, the involvement of mesenchymal progenitor cells, which come from the dental follicle, are essential. PDL progenitor cells slightly differ from stem cells in that they are more specific, meaning they already have a predetermined target cell that they will differentiate into. Additionally, angiogenic factors play a major role in the bioengineering of the PDL. As discussed above, the PDL is an extremely vascularized component in which blood supply is crucial for adequate nourishment and oxygen intake of the developing tissue. The complication that arises is routing the blood supply to the root surface of the tooth, because it is avascular (Takayama et al., 1997).

The scaffold plays a vital role for the tissue engineering of the PDL due to its important structure and template. The scaffold combines a nanofibrous network of pores with microspheres in order for there to be a controlled and effective release of regenerative factors. This element of the scaffold allows for a very high cell surface ratio in order for a higher number of cells to attach, thereby increasing cell-to-cell communication and interaction. With the increase in cell recruiting and signaling the regeneration process is more efficient (Porter et al., 2000).

One major application that depends heavily on the subsequent remodeling of the PDL is orthodontics. In orthodontics a presumably controlled force is applied upon the teeth to move them in a predetermined direction, and hence, the remodeling of the PDL and alveolar bone takes place. Orthodontics result in a very complex bone remodeling response and the cells responsible for this process are osteoclasts, macrophages, and osteoblasts (Taba et al., 2005). In normal function the PDL is very dynamic and is constantly remodeling as a result of this characteristic. The cementum and bone are responsible for this change—they form and reform with the normal forces of mastication and occlusion. This bite function produces tension and compression, which is transmitted through the fibers of the PDL. Tension causes bone deposition while compression causes bone resorption. The PDL and the oral structures maintain this balance in normal function in order to keep teeth in proper position and alignment (McCulloch et al., 2000).

With orthodontic force and movement, there are local changes in vascularity, cellular response, and the extracellular matrix, leading to release and changes in neurotransmitters, cytokines, GFs, and many other substances that lead to a shift in the oral environment. The objective of orthodontics is to carefully manipulate forces to guide the dentition into a new position that creates equilibrium and preferable esthetics for the patient. With light and constant forces on the teeth, the dentition can be realigned and positioned in a predictable and optimal manner. This

movement is a product of alveolar bone resorption on the compression side of the PDL and bone deposition on the tension side of the PDL. The meticulous way in which the teeth are displaced within the PDL determines the direction in which the teeth will move and the position in which they will end up in. The appliances used in orthodontics to create this predictable movement and direction of teeth include orthodontic brackets and wires or removable orthodontic appliances, such as corrective retainers. Owing to the advancements in bioengineering of the PDL, this could lead to even more predictable tooth movement and alignment in the field of orthodontics. This emerging field of regeneration and bioengineering may create a functional and esthetic human dentition for people with alveolar bone or PDL defects that wish to improve their self-image, functioning bite, and long-term health and wellness (Meeran, 2013).

26.6 Low-intensity pulsed ultrasound

Tissue engineering has been defined as the reconstruction of tissues, in vitro, for use as grafts to replace body parts that have been damaged or diseased (Izumi et al., 2004). Although this definition encompasses a wide variety of applications, in clinical practice, this definition becomes an alternative term to repair or replace different varieties of tissues. Regardless of this difference in definitions, tissue engineering may be explained by techniques that are performed to improve tissue and organ functions. Many techniques have been described to increase cellular and tissue performance, as well as tissue repair and regeneration. Low-intensity pulsed ultrasound (LIPUS) has been proven to be an important technique in cell metabolism as well as tissue repair/regeneration (Harle et al., 2001).

This section will highlight the contemporary evidence that supports the stimulatory effects of LIPUS and the possibility that it can be used as an application to enhance repair/regeneration or tissue engineering of hard as well as soft tissues in the oral and craniofacial structures.

26.6.1 Mechanical action of low-intensity pulsed ultrasound

Ultrasound is not ionizing radiation as it has been perceived by many clinicians, rather, it is best described as mechanical waves that have frequencies above those of the human hearing frequencies. It has been applied in medicine as a therapeutic, operative, and diagnostic tool. The intensity of the therapeutic ultrasound can range between 30 mW/cm^2 and 70 W/cm^2, while the intensity of the operative ultrasound (also known as shock waves) can range between 0.05 and 27,000 W/cm^2 (Ritchie et al., 2013, Li et al., 2006). In addition, the intensity of the diagnostic ultrasound can range between 5 and 50 mW/cm^2 in order to avoid excessive heating of the exposed tissues and organs (Heckman et al., 1994). Many studies have reported on the stimulatory effect of LIPUS on different cells, tissues, and organs and most of these reports have used the LIPUS parameters as follows: pulse frequency of

1.5 MHz, pulse repetition frequency of 1 kHz, and average intensity of 30 mW/cm^2 of the LIPUS transducer's surface area. Moreover, it is widely accepted that LIPUS has no known side effects. LIPUS application has been reported to have no thermal effects on the exposed tissues. It has been proven that LIPUS can be used as a noninvasive technique to enhance the healing of fractured bone (Padilla et al., 2014; Tsai et al., 1992). Also, it has been reported that LIPUS treatment can have a stimulatory effect on many different cell types such as oral cells (Mostafa et al., 2009; Takeuchi et al., 2008; Hu et al., 2014; Ikeda et al., 2009; Ne et al., 1999; Dalla-Bona et al., 2006; Inubushi et al., 2008; Rego et al., 2010; Scheven et al., 2009), bone and cartilage cells (Iwabuchi et al., 2014; Mukai et al., 2005; Schumann et al., 2006; Takeuchi et al., 2008; Leung et al., 2004; Naruse et al., 2000), muscular cells (Nagata et al., 2013), MSCs (Lim et al., 2013), as well as synovial membrane cells (Nakamura et al., 2010). Owing to the previous evidence, many studies have been conducted to study the possibility of using LIPUS in dental and dentofacial tissue engineering. The mechanical stresses induced by LIPUS and its propagation in cancellous bone has been recently studied (Vafaeian et al., 2014). It is evident from the current literature that every tissue has its own optimum LIPUS stimulatory intensity and frequencies. It has been reported (Tsai et al., 1992) that the best LIPUS parameters for stimulating bone formation/healing are 0.5 mW/cm^2, while the intensity of 1 W/cm^2 showed deleterious effects on bone healing. It has also been reported that LIPUS is the preferred method of mechanical stimulation to tissues and is the "preferred bioreactor" (Nakamura et al., 2011) as it enhances new blood vessel formation, which is important for the process of integrating new tissues to the original ones (El-Bialy et al., 2014; Cowan and Storey, 2003; Faour et al., 2001; Young and Dyson, 1990; Romano et al., 2009).

It has been hypothesized that LIPUS transmits mechanical signals into the exposed cell through an integrin that may act as a mechanoreceptor on the cell membrane (Kokubu et al., 1999); however, the exact mechanism is still unclear. Another mechanism by which LIPUS can potentially stimulate tissues and cells is the nonthermal effect, like acoustic streaming that may modify the local environment of a cell, leading to altered concentration of the gradients around the extracellular membrane (Chapman et al., 1980; Mortimer and Dyson, 1988).

26.6.2 Clinical use of low-intensity pulsed ultrasound in orthodontics

It has been recently shown that LIPUS can minimize orthodontically induced tooth-root resorption while enhancing tooth movement during orthodontic treatment in dogs and in clinical trials (Al-Daghreer et al., 2014; Raza et al., 2015).

LIPUS has the ability to play a vital role in dental applications across many different disciplines. For instance the prevention of root resorption and the enhancement of tooth movement simultaneously, the possibility for regeneration of dentoalveolar fractures for a single tooth application, or as an adjunctive treatment

to periodontal surgery. There may even be a future for LIPUS as a tool used with dental pulp tissue engineering. Although some of the aforementioned speculations are still hypothetically postulated, the current basic science publications support these hypotheses or future applications. Future research is encouraged in order to test the required optimum LIPUS parameters in each application.

26.6.3 Low-intensity pulsed ultrasound conclusions

The available literature supports the following conclusions:

1. LIPUS application is a noninvasive and safe therapeutic technique in tissue repair, as well as tissue engineering.
2. LIPUS can minimize orthodontically induced tooth-root resorption and at the same time can enhance tooth movement.
3. There are potential uses of LIPUS as an adjunctive to periodontal and oral maxillofacial surgery, as well as possibility of dental pulp tissue engineering.

26.7 Future developments

Craniofacial healthcare is an immensely growing field in dentistry. Though many limitations are presently evident, the future of craniofacial reconstruction has been very promising due to the development of craniofacial surgery and orthodontics over the past few years. With careful treatment planning and expansion, craniofacial defects and abnormalities have been restored and reconstructed with orthodontics, surgery, and bioengineering (Mah and Hatcher, 2003). Additionally, surgical planning, craniofacial orthodontics, and tissue engineering have been greatly enhanced with the development of computer and information technology for the purposes of imaging and recording.

Though autologous bone grafting has been the standard procedure for craniofacial surgery, a future alternative is stem cell therapy using BMPs in order to form bone in areas of deficiency. These proteins are the future of stem cell therapy in that they can regulate how osteoprogenitor cells differentiate into their respective osteoblast forms. Therefore these proteins can target different areas of the cell differentiation process and lead to specific end forms by regulating molecular signaling in several molecular pathways in the craniofacial regions. This is quite advanced because there has to be a coordinated way for the brain and craniofacial structures to interconnect and regulate this pathway (Tapadia et al., 2005). This method will require extensive future study and experimentation before being regularly used within the realm of craniofacial reconstruction. BMPs can be used to reconstruct the cranial vault, hard palate, and mandible in ways that are more controlled, regulated, and efficient than other surgical approaches (Chenard et al., 2012).

There is a critical need for three dimensions in orthodontic patient records including 3D models, direct or indirect impressions and records of the dentition,

and digital aids. Imaging and modeling would improve the field by creating an accurate representation of the patient's facial structures. Achieving the "anatomic truth"—in other words, obtaining a 3D model of the anatomy of the human dentition and oral structures is essential to the orthodontic patient record (Kau and Richmond, 2011). Currently, orthodontists use a multitude of two-dimensional images to create a 3D anatomical display of the region of interest. In the future, obtaining a digital 3D model of the oral structures, the dentition, and any other regions of interest would greatly enhance treatment plans, outcomes, and overall cost. Directly creating a replica of the craniofacial anatomy will allow the orthodontist and team to visualize and predict the changes that may occur to the patient's dentition over time with the specific treatment at hand, and therefore will result in a more accurate timeline of events and changes that will occur to the patient's hard and soft tissue (Carlos Quintero et al., 1999).

Some of the newer applications based on these 3D models include treatments such as Invisalign and OrthoCAD to enhance the esthetics and function of craniofacial structures with greater precision, less time, and lower costs (Jyothikiran et al., 2013). Technologies such as the Cone Beam Computerized Tomography (CBCT) show great potential in recording a 3D model in surgery and orthodontics by taking 2D images in slices of a patient's anatomical structures from different angles and transforming them into an accurate 3D record for better understanding and access to soft and hard tissue anatomy. As computer processing is enhanced, more powerful CBCTs will create more accurate images similar to the more expensive CT scanners (Vannier and Jiang, 2014).

Aside from surgical approaches, significant advancements in biotechnology, computer simulation, and surgical planning have occurred over the past decade allowing for greater biomedical applications. These technologies have led to improvements in imaging, prototyping, and simulation for craniofacial tissue. Additionally, 3D reconstruction software has been created to compile a multitude of images of human tissue for a 3D display to aid in surgical techniques (Schendel et al., 2012).

One key application for craniofacial surgery includes anatomical databases, which incorporate 3D and structural relationships of human tissue. Computers are used to improve visualization of anatomical detail and to accurately display images from different perspectives via computer-aided design (CAD). This process transforms virtual images into a real 3D model to reproduce the fine details of a patient's anatomy through computer-aided manufacturing (CAM), which uses a processing machine connected to a computer software database (Maki et al., 2003). Fig. 26.1 illustrates the process of creating a 3D image from the live region (Schendel et al., 2012).

This technology is exceptional in this realm of dentistry because it can assist surgeons during procedures in ways that have never been done before. Some of the benefits of this advancement in computers include treatment planning that can occur during the surgical procedure as more data becomes available. This is significant because it can help all of the surgeons contribute to the surgical planning through a comprehensive understanding of the craniofacial tissue and anatomy

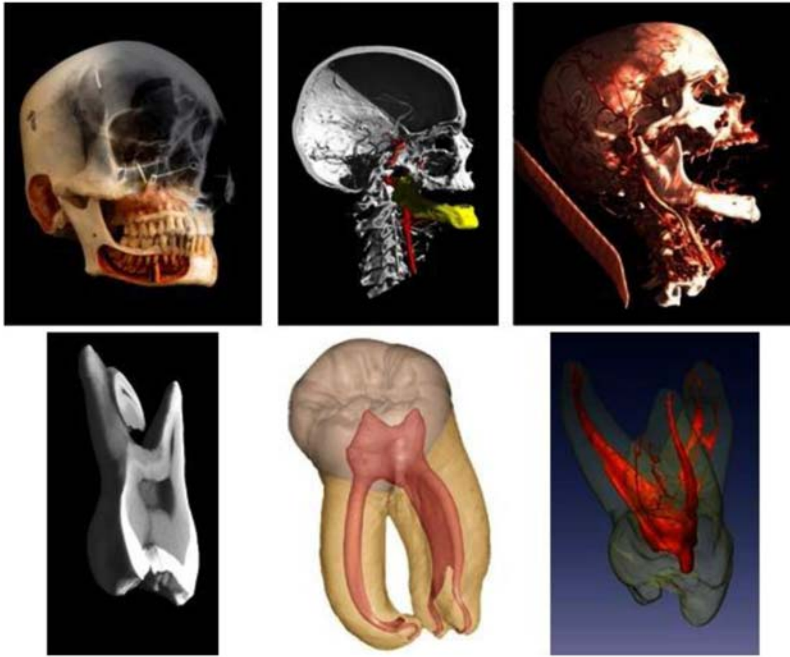


Figure 26.1 The process of 3D virtual imaging of a craniofacial defect (Schendel et al., 2012).

using reconstruction software and 3D modeling (Schendel et al., 2012). Another benefit of 3D imaging is the opportunity for patient-specific care. By creating a physical model through a prototyping machine, data are compiled for the specific patient in order to enhance the process of diagnosing, and treatment planning, with increased accuracy. This prototyping essentially creates an electronic patient database with the CAD−CAM systems that then produces a patient-specific anatomic reconstruction, which is further studied by physicians and surgeons for patient treatments and procedure for optimal care catered to individuals (Maki et al., 2003). This new capability of computer-aided treatment planning, both before the surgical procedure and with the option to revisit the treatment plan during the treatment, leads to more promising results for the patient with fewer risks and greater control over the surgical procedure.

One major future application of 3D modeling is patient-specific implants. These customized implants are enhanced by optimal material characteristics, proper implant design, and precise surgical technique. With the emergence of imaging and processing technologies, surgeons have been able to reproduce the defect into 3D models to visualize both before and during surgical implant procedures, allowing fabrication of custom implants that are ideal for the area of interest. These custom implants adapt more precisely, reduce overall procedure time, and lead to optimal results compared to their generic counterparts (Parthasarathy, 2014). Custom

implants can significantly aid in craniofacial reconstruction with less chance of pain and infection while also decreasing recovery time postsurgery. Additionally, the 3D aid gives the graphic team the opportunity to design an implant that is esthetically fitting for the patient and the case. With the emergence of more durable materials and the CAD/CAM technology, custom implants can be made more accurately, in reasonable time with reasonable cost (Parthasarathy, 2014).

In conclusion the evolving and expanding nature of technology shows promising results for the future of dentistry, surgery, and orthodontics. Craniofacial defects and abnormalities can be treated with better outcomes due to advancements in craniofacial surgery, orthodontics, computer imaging, and 3D simulation of the areas of interest. These technologies will continue to enhance the provider's performance as these systems allow for risk-free and low-cost procedures and outcomes. Transforming two-dimensional imaging to the third dimension will forever change practice in the healthcare field.

References

About, I., Bottero, M.J., de Denato, P., Camps, J., Franquin, J.C., et al., 2000. Human dentin production in vitro. Exp. Cell Res. 258, 33–41.

Al-Daghreer, S., Doschak, M., Sloan, A.J., Major, P.W., Heo, G., Scurtescu, C., et al., 2014. Effect of low-intensity pulsed ultrasound on orthodontically induced root resorption in beagle dogs. Ultras. Med. Biol. 40, 1187–1196.

Almela, T., Brook, I.M., Moharamzadeh, K., 2016. The significance of cell-related challenges in the clinical application of tissue engineering. J. Biomed. Mater. Res. A. 104 (12), 3157–3163.

Alsberg, E., Hill, E.E., Mooney, D.J., 2001. Craniofacial tissue engineering. Crit. Rev. Oral. Biol. Med. 12, 64–75.

Beertsen, W., Mcculloch, C.A.G., Sodek, J., 1997. The periodontal ligament: a unique, multifunctional connective tissue. Periodontology 2000. 13, 20–40.

Caffesse, R., Becker, W., 1991. Principles and techniques of guided tissue regeneration. Dent. Clin. North Am. 35, 479–494.

Carlos Quintero, J., Trosien, A., Hatcher, D., Kapila, S., 1999. Craniofacial imaging in orthodontics: historical perspective, current status, and future developments. Angle Orthodont. 69, 491–506.

Chamila Prageeth Pandula, P.K., Samaranayake, L.P., Jin, L.J., Zhang, C., 2014. Periodontal ligament stem cells: an update and perspectives. J. Invest. Clin. Dent. 5, 81–90.

Chapman, I., Macnally, N., Tucker, S., 1980. Ultrasound-induced changes in rates of influx and efflux of potassium ions in rat thymocytes in vitro. Ultras. Med. Biol. 6, 47–49.

Chenard, K.E., Teven, C.M., He, T.-C., Reid, R.R., 2012. Bone morphogenetic proteins in craniofacial surgery: current techniques, clinical experiences, and the future of personalized stem cell therapy. BioMed Res. Int. 2012, 601549.

Couble, M.L., Farges, J.C., Bleicher, F., Perrat-Mabillon, B., Boudeulle, M., Magloire, H., 2000. Odontoblast differentiation of human dental pulp cells in explant cultures. Calcif. Tissue Int. 66, 129–138.

Cowan, K.J., Storey, K.B., 2003. Mitogen-activated protein kinases: new signaling pathways functioning in cellular responses to environmental stress. J. Exp. Biol. 206, 1107–1115.

Dalla-Bona, D.A., Tanaka, E., Oka, H., Yamano, E., Kawai, N., Miyauchi, M., et al., 2006. Effects of ultrasound on cementoblast metabolism in vitro. Ultras. Med. Biol. 32, 943–948.

Dowell, P., Moran, J., Quteish, D., 1991. Guided tissue regeneration. Br. Dent. J. 171, 125–127.

Dunn, C.A., Jin, Q., Taba, M., Franceschi, R.T., Rutherford, R.B., Giannobile, W.V., 2005. BMP gene delivery for alveolar bone engineering at dental implant defects. Mol. Ther. 11, 294–299.

El-Bialy, T., Alhadlaq, A., Wong, B., Kucharski, C., 2014. Ultrasound effect on neural differentiation of gingival stem/progenitor cells. Annal. Biomed. Eng. 42, 1406–1412.

Erices, A., Conget, P., Minguell, J.J., 2000. Mesenchymal progenitor cells in human umbilical cord blood. Br. J. Haematol. 109, 235–242.

Faour, W.H., He, Y., He, Q.W., De Ladurantaye, M., Quintero, M., Mancini, A., et al., 2001. Prostaglandin E(2) regulates the level and stability of cyclooxygenase-2 mRNA through activation of p38 mitogen-activated protein kinase in interleukin-1 beta-treated human synovial fibroblasts. J. Biol. Chem. 276, 31720–31731.

Giannobile, W., 1996. Periodontal tissue engineering by growth factors. Bone. 19, S23–S37.

Gonzalez, R., Griparic, L., Vargas, V., Burgee, K., Santacruz, P., Anderson, R., et al., 2009. A putative mesenchymal stem cells population isolated from adult human testes. Biochem. Biophys. Res. Commun. 385, 570–575.

Gronthos, S., Brahim, J., Li, W., Fisher, L.W., Cherman, N., Boyde, A., et al., 2002. Stem cell properties of human dental pulp stem cells. J. Dent. Res. 81, 531–535.

Gronthos, S., Mankani, M., Brahim, J., Robey, P.G., Shi, S., 2000. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc. Natl. Acad. Sci. U.S.A. 97, 13625–13630.

Haraji, A., Lassemi, E., Motamedi, M.H.K., Alavi, M., Adibnejad, S., 2012. Effect of plasma rich in growth factors on alveolar osteitis. Natl. J. Maxillofac. Surg. 3, 38.

Harle, J., Salih, V., Mayia, F., Knowles, J.C., Olsen, I., 2001. Effects of ultrasound on the growth and function of bone and periodontal ligament cells in vitro. Ultras. Med. Biol. 27, 579–586.

Heckman, J.D., Ryaby, J.P., Mccabe, J., Frey, J.J., Kilcoyne, R.F., 1994. Acceleration of tibial fracture-healing by non-invasive, low-intensity pulsed ultrasound. J. Bone Joint Surg. Am. 76, 26–34.

Hu, B., Zhang, Y., Zhou, J., Li, J., Deng, F., Wang, Z., et al., 2014. Low-intensity pulsed ultrasound stimulation facilitates osteogenic differentiation of human periodontal ligament cells. PLoS One. 9, e95168.

Huang, K.-K., Shen, C., Chiang, C.-Y., Hsieh, Y.-D., Fu, E., 2005. Effects of bone morphogenetic protein-6 on periodontal wound healing in a fenestration defect of rats. J. Periodontal Res. 40, 1–10.

Ikeda, E., Morita, R., Nakao, K., Ishida, K., Nakamura, T., Takano-Yamamoto, T., et al., 2009. Fully functional bioengineered tooth replacement as an organ replacement therapy. Proc. Natl. Acad. Sci. 106, 13475–13480.

Inubushi, T., Tanaka, E., Rego, E.B., Kitagawa, M., Kawazoe, A., Ohta, A., et al., 2008. Effects of ultrasound on the proliferation and differentiation of cementoblast lineage cells. J. Periodontol. 79, 1984–1990.

Iwabuchi, Y., Tanimoto, K., Tanne, Y., Inubushi, T., Kamiya, T., Kunimatsu, R., et al., 2014. Effects of low-intensity pulsed ultrasound on the expression of cyclooxygenase-2 in mandibular condylar chondrocytes. J. Oral Facial Pain Headache. 28 (3), 261–268.

Izumi, K., Song, J., Feinberg, S.E., 2004. Development of a tissue-engineered human oral mucosa: from the bench to the bed side. Cells Tissues Organs. 176, 134–152.

Jazayeri, H.E., Fahmy, M.D., Razavi, M., Stein, B.E., Nowman, A., Masri, R.M., et al., 2016. Dental applications of natural-origin polymers in hard and soft tissue engineering. J. Prosthodontics. 25 (6), 510−517.

Jin, Q., Anusaksathien, O., Webb, S.A., Printz, M.A., Giannobile, W.V., 2004. Engineering of tooth-supporting structures by delivery of PDGF gene therapy vectors. Mol. Ther. 9, 519−526.

Jyothikiran, H., Shanthara, J., Subbiah, P., Thomas, M., 2013. Craniofacial imaging in orthodontics—past present and future. Int. J. Orthodontics. 25, 21−26.

Kang, H.W., Lee, S.J., Ko, I.K., Kengla, C., Yoo, J.J., Atala, A., 2016. A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. Nat. Biotechnol. 34, 312−319.

Kanzaki, H., Chiba, M., Shimizu, Y., Mitani, H., 2001. Dual regulation of osteoclast differentiation by periodontal ligament cells through RANKL stimulation and OPG inhibition. J. Dent. Res. 80, 887−891.

Kau, C.H., Richmond, S., 2011. Three-Dimensional Imaging for Orthodontics and Maxillofacial Surgery. John Wiley & Sons, NJ, United States.

Kokubu, T., Matsui, N., Fujioka, H., Tsunoda, M., Mizuno, K., 1999. Low intensity pulsed ultrasound exposure increases prostaglandin E2 production via the induction of cyclooxygenase-2 mRNA in mouse osteoblasts. Biochem. Biophys. Res. Commun. 256, 284−287.

Kolokythas, A., Olech, E., Miloro, M., 2010. Alveolar osteitis: a comprehensive review of concepts and controversies. Int. J. Dent.istry. 2010, Article ID: 249073.

Krause, D.S., Theise, N.D., Collector, M.I., Henegariu, O., Hwang, S., Gardner, R., et al., 2001. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell. 105, 369−377.

Leung, K.S., Cheung, W.H., Zhang, C., Lee, K.M., Lo, H.K., 2004. Low intensity pulsed ultrasound stimulates osteogenic activity of human periosteal cells. Clin. Orthop. Relat. Res. 418, 253−259.

Li, F., Wang, Z., Du, Y., Ma, P., Bai, J., Wu, F., et al., 2006. Study on therapeutic dosimetry of HIFU ablation tissue. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi. 23, 839−843.

Lim, K., Kim, J., Seonwoo, H., Park, S.H., Choung, P.-H., Chung, J.H., 2013. In vitro effects of low-intensity pulsed ultrasound stimulation on the osteogenic differentiation of human alveolar bone-derived mesenchymal stem cells for tooth tissue engineering. BioMed Res. Int. 2013, Article ID: 269724.

Mah, J., Hatcher, D., 2003. Current status and future needs in craniofacial imaging. Orthodontics Craniofac. Res. 6, 10−16.

Maki, K., Inou, N., Takanishi, A., Miller, A.J., 2003. Computer-assisted simulations in orthodontic diagnosis and the application of a new cone beam X-ray computed tomography. Orthodontics Craniofac. Res. 6, 95−101.

Mao, J.J., Giannobile, W.V., Helms, J.A., Hollister, S.J., Krebsbach, P.H., Longaker, M. T., et al., 2006. Craniofacial tissue engineering by stem cells. J. Dent. Res. 85, 966−979.

McCulloch, C.A.G., Lekic, P., Mckee, M.D., 2000. Role of physical forces in regulating the form and function of the periodontal ligament. Periodontology 2000. 24, 56−72.

Meeran, N.A., 2013. Cellular response within the periodontal ligament on application of orthodontic forces. J. Indian Soc. Periodontol. 17, 16.

Miura, M., Gronthos, S., Zhao, M., Lu, B., Fisher, L.W., Robey, P.G., et al., 2003. SHED: stem cells from human exfoliated deciduous teeth. Proc. Natl. Acad. Sci. U.S.A. 100, 5807−5812.

Morotome, Y., Goseki-Sone, M., Ishikawa, I., Oida, S., 1998. Gene expression of growth and differentiation factors-5,-6, and-7 in developing bovine tooth at the root forming stage. Biochem. Biophys. Res. Commun. 244, 85−90.

Mortimer, A., Dyson, M., 1988. The effect of therapeutic ultrasound on calcium uptake in fibroblasts. Ultras. Med. Biol. 14, 499−506.

Mostafa, N.Z., Uludag, H., Dederich, D.N., Doschak, M.R., El-Bialy, T.H., 2009. Anabolic effects of low-intensity pulsed ultrasound on human gingival fibroblasts. Arch. Oral Biol. 54, 743−748.

Mukai, S., Ito, H., Nakagawa, Y., Akiyama, H., Miyamoto, M., Nakamura, T., 2005. Transforming growth factor-beta1 mediates the effects of low-intensity pulsed ultrasound in chondrocytes. Ultras. Med. Biol. 31, 1713−1721.

Nagata, K., Nakamura, T., Fujihara, S., Tanaka, E., 2013. Ultrasound modulates the inflammatory response and promotes muscle regeneration in injured muscles. Annal. Biomed. Eng. 41, 1095−1105.

Nakamura, T., Fujihara, S., Katsura, T., Yamamoto, K., Inubushi, T., Tanimoto, K., et al., 2010. Effects of low-intensity pulsed ultrasound on the expression and activity of hyaluronan synthase and hyaluronidase in IL-1β-stimulated synovial cells. Annal. Biomed. Eng. 38, 3363−3370.

Nakamura, T., Fujihara, S., Yamamoto-Nagata, K., Katsura, T., Inubushi, T., Tanaka, E., 2011. Low-intensity pulsed ultrasound reduces the inflammatory activity of synovitis. Annal. Biomed. Eng. 39, 2964−2971.

Naruse, K., Mikuni-Takagaki, Y., Azuma, Y., Ito, M., Oota, T., Kameyama, K.-Z., et al., 2000. Anabolic response of mouse bone-marrow-derived stromal cell clone ST2 cells to low-intensity pulsed ultrasound. Biochem. Biophys. Res. Commun. 268, 216−220.

Ne, R.F., Witherspoon, D.E., Gutmann, J.L., 1999. Tooth resorption. Quintessence Int.-English Ed. 30, 9−26.

Nyman, S., Gottlow, J., Karring, T., Lindhe, J., 1982. The regenerative potential of the periodontal ligament. J. Clin. Periodontol. 9, 257−265.

Padilla, F., Puts, R., Vico, L., Raum, K., 2014. Stimulation of bone repair with ultrasound: a review of the possible mechanic effects. Ultrasonics. 54, 1125−1145.

Panagiotopoulou, O., Kupczik, K., Cobb, S.N., 2011. The mechanical function of the periodontal ligament in the macaque mandible: a validation and sensitivity study using finite element analysis. J. Anat. 218, 75−86.

Parthasarathy, J., 2014. 3D modeling, custom implants and its future perspectives in craniofacial surgery. Annal. Maxillofac. Surg. 4, 9.

Persson, R.E., Rollender, L.G., Laurell, L., Persson, G.R., 1998. Horizontal alveolar bone loss and vertical bone defects in an adult patient population. J. Periodontol. 69, 348−356.

Porter, B., Oldham, J., He, S.-L., Zobitz, M., Payne, R., An, K.-N., et al., 2000. Mechanical properties of a biodegradable bone regeneration scaffold. J. Biomech. Eng. 122, 286−288.

Raza, H., Major, P., Dederich, D., El-Bialy, T., 2015. Effect of low-intensity pulsed ultrasound on orthodontically induced root resorption caused by torque: A prospective, double-blind, controlled clinical trial. The Angle Orthodontist. 86 (4), 550−557.

Rego, E.B., Inubushi, T., Kawazoe, A., Tanimoto, K., Miyauchi, M., Tanaka, E., et al., 2010. Ultrasound stimulation induces PGE 2 synthesis promoting cementoblastic differentiation through EP2/EP4 receptor pathway. Ultras. Med. Biol. 36, 907−915.

Ritchie, R., Collin, J., Coussios, C., Leslie, T., 2013. Attenuation and de-focusing during high-intensity focused ultrasound therapy through peri-nephric fat. Ultras. Med. Biol. 39, 1785−1793.

Romano, C.L., Romano, D., Logoluso, N., 2009. Low-intensity pulsed ultrasound for the treatment of bone delayed union or nonunion: a review. Ultras. Med. Biol. 35, 529–536.

Sakaguchi, Y., Sekiya, I., Yagishita, K., Muneta, T., 2005. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. Arthritis Rheum. 52, 2521–2529.

Schendel, S.A., Hazan-Molina, H., Rachmiel, A., Aizenbud, D., 2012. The future in craniofacial surgery: computer-assisted planning. Rambam Maimonides Med. J. 3 (2), e0012.

Scheven, B., Man, J., Millard, J., Cooper, P., Lea, S., Walmsley, A., et al., 2009. VEGF and odontoblast-like cells: stimulation by low frequency ultrasound. Arch. Oral Biol. 54, 185–191.

Schliephake, H., 2002. Bone growth factors in maxillofacial skeletal reconstruction. Int. J. Oral Maxillofac. Surg. 31, 469–484.

Schumann, D., Kujat, R., Zellner, J., Angele, M., Nerlich, M., Mayr, E., et al., 2006. Treatment of human mesenchymal stem cells with pulsed low intensity ultrasound enhances the chondrogenic phenotype in vitro. Biorheology. 43, 431–443.

Schwartz, Z.V.I., Goultschin, J., Dean, D.D., Boyan, B.D., 1997. Mechanisms of alveolar bone destruction in periodontitis. Periodontology 2000. 14, 158–172.

Semb, G., 2012. Alveolar Bone Grafting. Cleft Lip and Palate. Karger Publishers, Berlin, Germany.

Seo, B.-M., Miura, M., Gronthos, S., Bartold, P.M., Batouli, S., Brahim, J., et al., 2004a. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet. 364, 149–155.

Seo, B.M., Miura, M., Gronthos, S., Bartold, P.M., Batouli, S., Brahim, J., et al., 2004b. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet. 364, 149–155.

Shimono, M., Ishikawa, T., Ishikawa, H., Matsuzaki, H., Hashimoto, S., Muramatsu, T., et al., 2003. Regulatory mechanisms of periodontal regeneration. Microsc. Res. Tech. 60, 491–502.

Singh, K., Mishra, N., Kumar, L., Agarwal, K.K., Agarwal, B., 2012. Role of stem cells in tooth bioengineering. J. Oral Biol. Craniofac. Res. 2, 41–45.

Taba, M., Jin, Q., Sugai, J., Giannobile, W., 2005. Current concepts in periodontal bioengineering. Orthodontics Craniofac. Res. 8, 292–302.

Takayama, S., Murakami, S., Miki, Y., Ikezawa, K., Tasaka, S., Terashima, A., et al., 1997. Effects of basic fibroblast growth factor on human periodontal ligament cells. J. Periodontal. Res. 32, 667–675.

Takeuchi, R., Ryo, A., Komitsu, N., Mikuni-Takagaki, Y., Fukui, A., Takagi, Y., et al., 2008. Low-intensity pulsed ultrasound activates the phosphatidylinositol 3 kinase/Akt pathway and stimulates the growth of chondrocytes in three-dimensional cultures: a basic science study. Arthr. Res. Ther. 10, 1.

Tapadia, M.D., Cordero, D.R., Helms, J.A., 2005. It's all in your head: new insights into craniofacial development and deformation. J. Anat. 207, 461–477.

Teng, Y.-T.A., Nguyen, H., Gao, X., Kong, Y.-Y., Gorczynski, R.M., Singh, B., et al., 2000. Functional human T-cell immunity and osteoprotegerin ligand control alveolar bone destruction in periodontal infection. J. Clin. Investig. 106, R59–R67.

Tevlin, R., Mcardle, A., Atashroo, D., Walmsley, G.G., Senarath-Yapa, K., Zielins, E.R., et al., 2014. Biomaterials for craniofacial bone engineering. J. Dent. Res. 93, 1187–1195.

Tsai, C.L., Chang, W.H., Liu, T.K., 1992. Preliminary studies of duration and intensity of ultrasonic treatments on fracture repair. Chin. J. Physiol. 35, 21–26.

Vafaeian, B., El-Rich, M., El-Bialy, T., Adeeb, S., 2014. The finite element method for micro-scale modeling of ultrasound propagation in cancellous bone. Ultrasonics. 54, 1663−1676.

van Hout, W.M., Van Der Molen, A.B.M., Breugem, C.C., Koole, R., Van Cann, E.M., 2011. Reconstruction of the alveolar cleft: can growth factor-aided tissue engineering replace autologous bone grafting? A literature review and systematic review of results obtained with bone morphogenetic protein-2. Clin. Oral Invest. 15, 297−303.

Vannier, M.W., Jiang, B., 2014. The future of cone beam computed tomography and 3D imaging in orthodontics. Cone Beam Computed Tomography in Orthodontics: Indications, Insights, and Innovations. Wiley-Blackwell, NJ, USA, pp. 103−112.

Wang, Y.W., Wu, Q., Chen, J., Chen, G.Q., 2005. Evaluation of three-dimensional scaffolds made of blends of hydroxyapatite and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) for bone reconstruction. Biomaterials. 26, 899−904.

Young, C., Terada, S., Vacanti, J., Honda, M., Bartlett, J., Yelick, P., 2002. Tissue engineering of complex tooth structures on biodegradable polymer scaffolds. J. Dent. Res. 81, 695−700.

Young, S., Dyson, M., 1990. The effect of therapeutic ultrasound on angiogenesis. Ultras. Med. Biol. 16, 261−269.

Zaky, S.H., Cancedda, R., 2009. Engineering craniofacial structures: facing the challenge. J. Dent. Res. 88, 1077−1091.

Zeichner-David, M., 2006. Regeneration of periodontal tissues: cementogenesis revisited. Periodontology 2000. 41, 196−217.

This page intentionally left blank

Tooth tissue engineering

27

*Samaneh Hosseini**, *Shahrbanoo Jahangir** and
*Mohamadreza Baghaban Eslaminejad***
Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

27.1 Introduction

A tooth, although small, is composed of three mineralized tissues—enamel, dentin, and cementum. Each contains exclusive biochemical and structural properties. It is a unique organ similar to bone and hair in terms of structural properties and developmental process. The tooth is a mineralized hard tissue-like bone, but with a different developmental mechanism. Bone develop without epithelial contribution, whereas reciprocal interactions between the oral epithelium and underlying mesenchyme lead to tooth development (Zhang et al., 2005). This process is similar to hair development. However, unlike hair, permanent teeth do not have the capacity to shed and undergo repeated regrowth. Lost or damaged teeth following caries, periodontal disease, or trauma can lead to physical and mental suffering that compromises quality of life and self-esteem (Pihlstrom et al., 2005). Dental implants are a traditional approach to restore missing teeth. However, implant-retained dental restorations can develop biological and biomechanical complications which can affect the survival and success of these restorations (Jung et al., 2012). Recently, researchers in the field of tissue engineering (TE) have attempted to regenerate dental tissues and entire teeth (Yadav et al., 2016). Conceptually, this approach intends to generate a tooth by mimicking its natural growth process. Numerous TE approaches have been developed that replace damaged teeth. Cell sources, scaffolds, and bioactive factors for TE have been extensively investigated and resulted in successful formation of complex teeth structures in preclinical settings (Galler et al., 2011; Gupte and Ma, 2012; Rosa et al., 2013). However, the generation of a complete human tooth has not been accomplished; additional challenges remain to be elucidated. Here, we first describe the biological structure of a tooth and its developmental process. An understanding of the mechanisms that underlie the development of an organ or tissue is necessary for tooth engineering. This chapter also covers the three basic requirements, cell sources, scaffolds, and growth factors, for tooth TE which can eventually result in novel discoveries in this field. Primary achievements in complete tooth engineering are recapitulated and the paramount questions that remain to be addressed are mentioned.

* Cofirst author.
** Corresponding author: eslami@royaninstitute.org

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00027-X
© 2017 Elsevier Ltd. All rights reserved.

27.2 Biological structure of teeth

Deciduous (primary) and permanent (secondary) teeth comprise two generations of human dentition (Fig. 27.1A). The deciduous teeth refer to the 20 primary teeth that erupt in the oral cavity between the ages of 6 months and 2.5 years. They are eventually lost under the normal physiologic process called shedding or exfoliation in order to make room for permanent teeth (Nanci, 2013). The adult human naturally possesses 32 permanent teeth that begin to appear in the oral cavity at approximately 6 years of age (Nanci, 2013). There are four different types of functionally specialized quadrants. Each quadrant consists of eight teeth—two incisors, one canine, two premolars, and three permanent molars. The third molars or wisdom teeth generally erupt at around 18 years of age, however surgical extractions are often performed to prevent displacement of other teeth.

Structurally, each tooth has three parts that consist of a crown, a constricted neck at the gum, and one or more roots (Fig. 27.1B). The inner part of each tooth is composed of innervated soft connective tissue called the dental pulp. It consists of collagen fibers, fibroblasts, mesenchymal stem cells (MSCs), and blood vessels. Vascular angiogenesis is a fundamental phase for tooth development and healing pulpal injury (Demarco et al., 2011). The root canal extends to an opening (apical foramen) at the tip of each root that enables blood vessels, lymphatics, and nerves to reach the pulp cavity (L.Mescher, 2013). Nerve-endings only produce a sensation of pain, although they are particularly sensitive to heat and cold (Jain et al., 2013). Three calcified tissues cover the pulp—the enamel, cementum, and dentin.

Dentin is a calcified layer which constitutes the bulk of a tooth and surrounds an internal pulp cavity. Although greatly similar to bone, which is mostly composed of type I collagen in a random arrangement and carbonated apatite, dentin matrix does not undergo remodeling (Goldberg et al., 2011). Dentin is composed of dentinal tubules whose numbers and diameters determine its strength (Schilke et al., 2000).

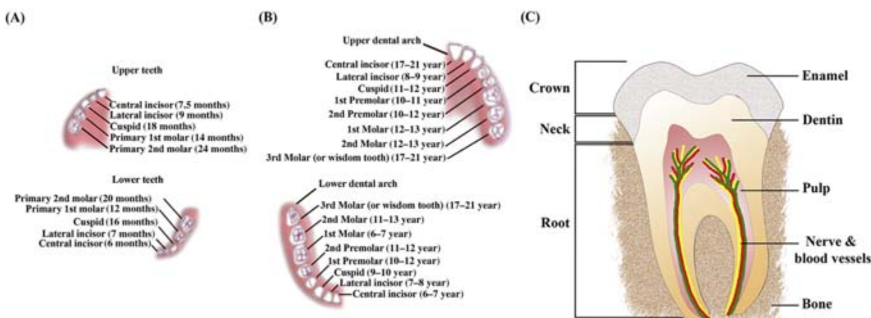


Figure 27.1 Schematic representation of maxillary (upper) and mandibular (lower) dental arches show human (A) deciduous and (B) permanent teeth. The age of eruption of deciduous and permanent teeth is given in months and years, respectively. (C) Adult human tooth morphology defined by its main parts—crown, neck, and root.

In addition, dentin possesses dentin forming cells (odontoblasts) which narrow and extend from the peripheral pulp into dentin (odontoblastic process) as their cytoplasmic processes are seen inside dentin tubules (Arana-Chavez and Massa, 2004).

Enamel is an acellular, nonliving substance deposited by cells in the gum on the outside of the crown. Enamel is formed by a two-step mineralization process that consists of secretory and transition stages, followed by a maturation step (Honda et al., 2015). During first stage ameloblasts secrete soft gel-like enamel-matrix proteins and the amount of appositional growth determines the final thickness of the enamel layer (Simmer et al., 2010). Cementum comprises the part of the periodontium that connects the tooth to the alveolar bone through the periodontal ligament and forms a fibrous joint between the tooth and its alveolus socket. The periodontal ligaments with both the cementum and the alveolar bone form the specialized supporting tissues that tether the teeth tightly against the jaw (L.Mescher, 2013).

27.3 Developmental process of tooth formation

Tooth development is a highly complicated process which directs cells to proper locations and eventually results in the proper tooth shape and number. The tooth results from reciprocal interactions between epithelial and mesenchymal cells of the first pharyngeal arch (Lumsden, 1988). The dental epithelium and mesenchymal cells are derived from the ectoderm and cranial neural crest, respectively. Odontogenesis is a continuous process that can be divided into several distinct developmental stages—initiation, bud, cap, and bell (Fig. 27.2) (Jheon et al., 2013). Dental epithelium at the site of the future teeth first increases in thickness to laterally restrict the tooth-forming area and forms the dental lamina. Local thickening of the epithelium leads to the formation of a tooth placode within the dental lamina (Jussila and Thesleff, 2012). The small cluster of placodal cells, as an early signaling center, express the signaling molecules to regulate tooth bud formation (Balic and Thesleff, 2015). The oral epithelial cells proliferate and undergo invagination into the mesenchymal region to form a tooth bud. Proliferation of epithelial cells in the bud prolongs unequally in its different parts to generate the cap and subsequent bell-shaped structures, which are called the enamel organ and enclose the dental papilla mesenchyme.

From the cap stage the epithelial cells separate into four different types—inner enamel epithelium (IEE), outer enamel epithelium (OEE), the stratum intermedium, and the stellate reticulum (Balint Joseph Orban et al., 1972). The IEE eventually gives rise to enamel-forming ameloblasts. Upon this stage, primary enamel knot forms within IEE. The enamel knot is a reservoir of signaling pathway-related genes and signaling molecules that allow tooth morphogenesis to progress toward the bell stage and regulate the tooth's shape (Jussila and Thesleff, 2012). Signals released by the enamel knot stimulate the growth of flanking basal epithelium to form cervical loops which eventually commit to the roots.

During the bell stage, growth and folding of the tooth germ determines the crown shape based on specific-cusp patterning. In single-cusped teeth the primary

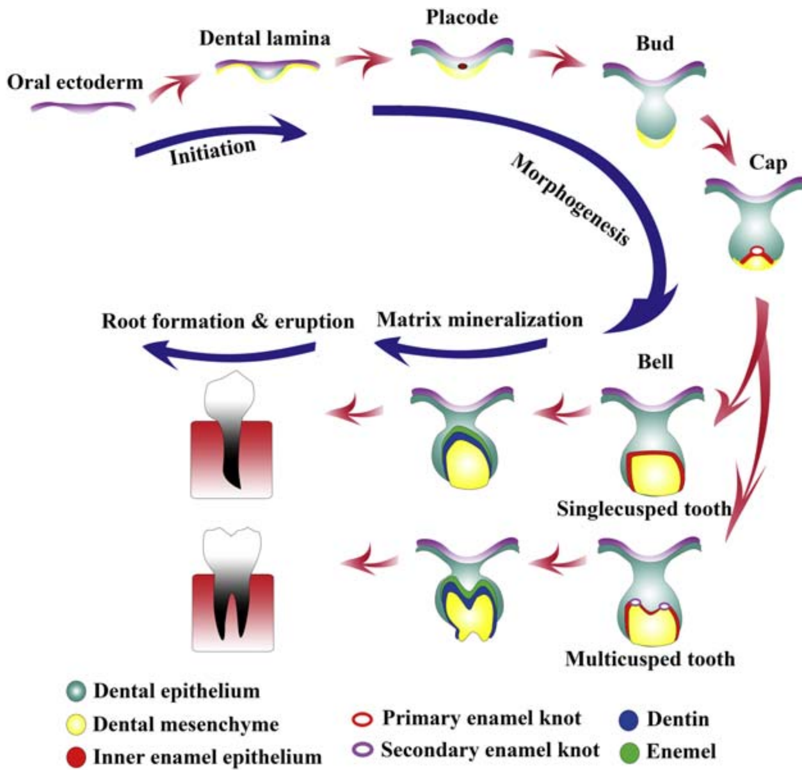


Figure 27.2 Schematic representation of the principal stages of tooth formation. Tooth development initiates by formation of dental lamina within the dental epithelium. Individual teeth subsequently develop within specific domains of the lamina, called placodes. The dental epithelium undergo invagination into the dental mesenchyme and form a bud. During the cap stage the epithelium extends further into the mesenchymal tissue and surrounds the condensing mesenchyme where a primary enamel knot appears. A species-specific-cusp pattern emerges in the bell stage as the primary enamel knot gives rise to the tip of the crown in a single-cusped tooth. In multicusped teeth, secondary and tertiary enamel knots form at the places for future cusps. In the final stage the epithelial and mesenchymal cells differentiate into ameloblasts and odontoblasts which produce enamel and dentin at the boundary surface between the epithelium and mesenchyme, respectively. Dental follicle cells give rise to periodontal tissues, including the cementum, periodontal ligament, and alveolar bone.

enamel knot forms the tooth crown, whereas in multicusped molar teeth, the secondary enamel knot appears at the site of future cusp tips. In a review by Thesleff et al. the authors reported numerous attempts that have been made to determine the molecular mechanisms of these signals during tooth development (Balic and Thesleff, 2015).

In differentiation stage, ameloblasts and odontoblasts produce an organic matrix of dentin and enamel which, in turn, mineralizes to fix the tooth's shape. The inner

and OEE form Hertwig epithelial root sheath (HERS) which is morphologically located between the dental papilla and follicle (Huang and Chai, 2012). Proliferation and migration of HERS downwards guides root formation and also induces the dental papilla cells (DPCs) adjacent to the inner epithelial layer of the HERS to differentiate into odontoblasts that form root dentin. HERS then disintegrates, creating the mesh-like structure called epithelial cell rests of Malassez (ERM). Some of the HERS cells located near the cementoenamel junction undergo apoptosis which is induced by dental follicle cells through the Fas/FasL pathway (Lee et al., 2012). This structure allows the dental follicle cells to generate contact with the root dentin and differentiate towards a cementoblast lineage. Simultaneously, dental follicle cells secrete collagen fibers into the cementum and secure the root in the jaw bone (Huang and Chai, 2012).

27.4 Triad of tooth tissue engineering

TE and regenerative medicine are multidisciplinary fields that combine the principles and knowledge of biological sciences, chemistry, physics, and engineering to develop a fully functioning engineered product that restores damaged or lost tissues (Griffith and Naughton, 2002). Various strategies under consideration for tooth TE can be categorized into three major approaches: conductive, inductive, and cell based (Nguyen et al., 2013). Conductive approaches utilize biomaterial components that passively promote the growth and regenerative capacity of a desired tissue. The induction strategy relies on activating cells in proximity to the defect site with specific biological signals. The cell-based TE approach is currently the most promising approach that involves the direct seeding of biodegradable scaffolds with cells and bioactive factors. Cells, scaffolds, and growth factors constitute three fundamental elements in this approach. Fine orchestration of these elements leads to efficient regeneration upon implantation in vivo. This section provides a detailed discussion of these elements in terms of tooth TE.

27.4.1 Cells: The first element

The cell, as a living component of the TE approach, is a potent mediator to improve the success rate of regenerative medicine. However, several challenges limit the use of this approach in the clinical setting (Almela et al., 2016). The appropriate choice of cell source, identification of efficient methodologies to induce cell proliferation and differentiation, as well as long-term cell survival present challenges (Horst et al., 2012). The priority of the TE approach is to use autologous cells derived from the patient as well as the same tissue to be regenerated in order to achieve the best outcome with minimal immune rejection. Nevertheless, invasive cell isolation or impaired cells in some circumstances require the inevitable use of alternative cell sources. As previously stated, the existence of two different types of cells, ectodermal and mesenchyme, is an extra challenge that increases the complexity of

tooth engineering. Regardless of these issues, various cell sources that have the potential for tooth formation have been explored. This section reviews the cell populations of dental embryonic and postnatal cells as well as stem cells derived from dental and nondental tissues, and their odontogenic capacity which is currently used for tooth TE.

27.4.1.1 Postnatal and embryonic dental cells

Immature cells from embryonic and postnatal tooth germ are presumed to be an ideal option for tooth TE applications. Existing dental progenitor cells that originate from the ectoderm and mesenchyme can be used to generate both teeth and supporting tissues. To date a variety of TE studies have attempted to direct tooth formation using immature dental cells in animal models (Honda et al., 2005; Sumita et al., 2006; Young et al., 2005a; Yelick and Vacanti, 2006). Initially, Yung et al. obtained the required cell populations from porcine postnatal third molar tooth bud. They generated a single-cell suspension by enzymatic dissociation of whole tooth bud tissue. The researchers observed generation of the tooth structure, including dentin and enamel, 30 weeks after implantation. Subsequently, a similar strategy with tooth bud cells from 4-day postnatal rats and stage E14 embryonic mice led to the formation of a tooth crown structure (Duailibi et al., 2004; Iwatsuki et al., 2006). The results of these studies have proven the potential of postnatal and embryonic dental cells to regenerate teeth. However, differences exist in crown morphogenesis and depend on the stage that the dental cells are harvested, as the normal crown shape is achieved from embryonic tooth bud cells. Researchers propose that the best outcome is caused by the presence of regulating factors that most probably originate from the enamel knot in the early stage of tooth development (Vaahtokari et al., 1996; Iwatsuki et al., 2006).

27.4.1.2 Stem cells from dental and nondental tissues

Amongst different cell sources, stem cells are of crucial importance due to their exclusive potential (van der Kooy and Weiss, 2000). They may either act as an internal source of unspecialized progenitors for healing after injuries or differentiate into specialized cells for normal tissue renewal. Stem cells possess the capability of self-renewal and generation of differentiated progenies (Blau et al., 2001). Their ability to proliferate and develop into many different cell types holds great promise for prospective therapeutic applications.

Somatic or adult stem cells (MSCs) as well as pluripotent stem cells, which include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), are the most commonly used in TE studies. The different types of stem cell sources for tooth TE applications are as follows.

27.4.1.2.1 Adult stem cells

Adult stem cells are known as MSCs or multipotent stromal stem cells. These cells comprise a group of clonogenic cells first isolated from the nonhematopoietic compartment of the bone marrow (Zomorodian and Baghaban Eslaminejad, 2012). Thus

far, in addition to bone marrow, MSCs have also been obtained from most adult tissues, such as skeletal muscle, adipose tissue, umbilical cord (UC), synovium, the liver, and lungs (Martin et al., 2008; Zuk et al., 2002; Hu et al., 2001). Although MSC populations obtained from most tissues exhibit functional heterogeneity, they test positive for several surface receptors—CD29, CD44, CD49a-f, CD51, CD73, CD105, CD106, CD166, and Stro1. In addition, they are negative for certain hematopoietic lineage markers—CD11b, CD14, and CD45 (Phinney and Prockop, 2007). MSCs characteristically contain a fibroblast-like morphology as well as plastic adherence properties (Dominici et al., 2006). Additionally, they exhibit the ability to self-renew and differentiate to generate mesodermal lineages such as osteoblasts, chondrocytes, and adipocytes (Aldahmash et al., 2012; Abdallah and Kassem, 2008). Hence, these features make MSCs ideal stem cell sources for regenerative applications.

MSCs can be derived from dental and nondental tissues in terms of tooth TE applications. To date, postnatal MSCs from various dental regions that include dental pulp, periodontal ligament, dental follicle, and dental apical papilla have been isolated. Postnatal dental pulp stem cells (DPSCs) were the first stem cells derived from dental tissues (permanent third molar) by Gronthos et al. (2000). Subsequently, Miura et al. (2003) isolated these cells from human exfoliated deciduous teeth (SHED). DPSCs have been extensively studied compared to other dental stem cells (Eslaminejad et al., 2010; Karamzadeh et al., 2012). A benefit of DPSCs compared to MSCs derived from other tissues is that they are derived from discarded tissues which are naturally lost or surgically removed. Although they can differentiate towards a variety of cell types, their cell fate is more directed towards an odontogenic lineage rather than an osteoblastic one (Tatullo et al., 2015). Diseased dental pulp (pulp polyps or chronic hyperplastic pulpitis) contains stem cells comparable to normal pulp stem cells in terms of colonigenic efficacy, population doubling time, differentiation ability, and cell surface antigen markers (Attar et al., 2014).

Numerous investigations reported the regenerative capacity of DPSCs both in vitro and in vivo (Eslaminejad et al., 2013; Khorsand et al., 2013). El-Backly et al. (2008) examined the potential for dental pulp cells to generate dentin/pulp tissue when subcutaneously transplanted in a rabbit model. They observed that a DPSC-seeded poly(lactic-co-glycolic acid) (PLGA) scaffold formed a tubular-like structure which resembled a natural dentin matrix. However, this structure was less organized compared to the results obtained using DPSC/HA/TCP. In contrast, DPSC/dentin transplants failed to generate an organized dentin structure and instead formed a structure that resembled reparative dentin (Batouli et al., 2003). Recently, the authors conducted a study which proved that the DPSCs scaffold used in treated dentin matrix (TDM) effectively enhanced the regenerative response of tissue (Bakhtiar et al., 2016). These findings highlighted the ability of DPSCs to form dentin and periodontium, yet this discrepancy in the generated structure was most probably attributed to different microenvironment and matrix construction.

Dental follicle stem cells (DFSC) and periodontal ligament stem cells (PDLSC) are other sources that have the capability for multilineage differentiation into

cementoblasts, osteocytes, chondrocytes, and adipocytes (Seo et al., 2004; Morsczeck et al., 2005). The odontogenic potential of both DSFCs and PDLSCs has been demonstrated in the inductive microenvironment of TDM following implantation into the dorsum of immune-deficient mice (Tian et al., 2015). Although both cells contributed to dentin formation, DSFCs showed greater dentinogenic potential compared to PDLSCs which resulted in complete dentin regeneration. Apart from odontogenic potential, DSFCs had a higher proliferation activity compared to PDLSCs. This feature has made DSFCs more convenient for TE applications due to efficient expansion and cryopreservation in large quantities (Tian et al., 2015). Although dental stem cells possess the ability to undergo odontoblastic differentiation, their clinical application remains limited due to several drawbacks. For instance, the use of DPSCs and DFSCs is problematic due to the decreased availability of freshly extracted third molars (wisdom teeth) from patients who lack their third molars, either congenitally or surgical removal.

In terms of dental epithelial stem cells located in the apical bud, there is no stunning report on epithelial stem cell extraction because ameloblasts commit to apoptosis coincide with tooth eruption. Shinmura et al. (2008) observed the differentiation potential of epithelial cell ERM into ameloblast-like cells. Transplantation of subcultured ERM in combination with primary dental pulp cells seeded onto scaffolds resulted in the generation of enamel-like tissues in mice.

Researchers attempted to address the ability of MSCs derived from nondental tissues to give rise to dental mesenchymal or epithelial-like cells. According to research, bone marrow-derived MSCs (BMMSCs) had the ability to differentiate toward ameloblast and odontoblast lineages due to their heterogeneous cell populations (i.e., hematopoietic and MSCs) (Hu et al., 2006). Hematopoietic stem cells have been shown to commit to several epithelial lines. A primary study demonstrated that the recombination of BMMSCs with mice embryonic oral epithelium provoked an odontogenic response in mesenchymal cells, which was similar to embryonic ectomesenchyme (Ohazama et al., 2004). The ability of BMMSCs to give rise to ameloblasts has been examined by mixing a more competent subpopulation of BMMSCs (c-Kit+ cells) with embryonic dental epithelial cells, followed by reassociation with dental mesenchyme (Hu et al., 2006). In this experiment, concurrent with ameloblast differentiation, the BMMSCs gave rise to odontoblasts at the epithelial–mesenchymal junction.

Hair follicles are another proposed source for MSCs that have been assessed for dental regeneration in vitro and in vivo (Wu et al., 2009). Stem cells in dermal papilla possess a high degree of plasticity and robust regenerative capacity. Morphogenesis of the hair follicle resembles the tooth as it is initiated by reciprocal epithelial–mesenchymal interactions during the embryo stage that persist to adulthood (Yang and Cotsarelis, 2010). Mice follicle dermal papilla-derived MSCs (FDPMC) in direct coculture with apical bud cells and DPCs (ABCs/DPCs) give rise to odontoblast-like cells (Wu et al., 2009). The morphological features, along with expression of tooth specific markers (dentin sialoprotein (DSP) and DSPP)) have confirmed the presence of odontoblasts in the ABC/DPC microenvironment. An explant of ABCs/DPCs/FDPMCs in renal capsules of adult mice developed into

tooth crown structure that contained enamel, dentin, pulp, and bone-like tissue. Apparently, MSCs from various sources can reprogram to differentiate into odontoblasts. However, the presence of an epithelial layer as an essential factor is a drawback for their use in clinical settings.

Ozeki et al. (2014) first examined the odontogenic potential of human skeletal muscle stem cells. They induced α7 integrin-positive human skeletal muscle stem cells to differentiate toward an odontoblastic lineage using an optimized differentiation protocol without the need for epithelial−mesenchymal interaction. The appearance of odontoblastic phenotypes and deposition of abundant mineralized ECM supported the generation of odontoblast-like cells in the presence of BMP-4 in gelatin scaffold.

Human (hUC) tissue is considered to be a promising MSC source for dental applications (Li et al., 2013). UC is a waste tissue and its isolation is noninvasive, therefore, there is no ethical or technical controversy related to their use. UC-derived MSCs are believed to have greater proliferative potential and capacity to differentiate into various cell types compared to BMMSCs (Baksh et al., 2007). Li et al. have demonstrated the possibility of UC-MSC differentiation into an odontoblast lineage in an odontogenic microenvironment. Immunocytochemical staining for DSP and dentin matrix protein 1 (DMP-1) showed a significant increase in hUCMSCs treated with tooth germ cell-conditioned medium (TGC-CM).

MSCs are used in numerous clinical trials to regenerate bone in oral cavity (Padial-Molina et al., 2015). However, tremendous effort is needed to pave before MSCs can be used for tooth regeneration. Aside from the common challenges with MSCs that include the lack of reliable markers to define true stem cells, genetic instability, and unknown cell fate, an extra limitation includes regulation of the complicated process of the epithelia−mesenchymal interaction. More importantly, the difference in embryological origin between dental mesenchymal and MSCs derived from other tissues that may lead to different functions is another important concern.

27.4.1.2.2 Embryonic stem cells

ESCs are pluripotent cell lines derived from blastocysts as the inner cell mass of an embryo during the early stages of development. They have potent differentiation capability into various cell types in response to internal and external stimuli. ESCs can also proliferate and renew themselves without differentiation for extended periods of time. In the proper environment, they can give rise to any specialized cell by acquiring epigenetic marks in their DNA to modulate gene expression. Osteoblasts (Bielby et al., 2004), cardiomyocytes (Laflamme et al., 2007; Kado et al., 2008), neurons (Kriks et al., 2011; Chambers et al., 2009), hematopoietic cells (Kaufman et al., 2001; Ledran et al., 2008), hepatic cells (Cai et al., 2007; Lavon et al., 2004), retinal cells (Klimanskaya et al., 2004; Lund et al., 2006), trophoblasts (Gerami-Naini et al., 2004; Chen et al., 2008), and endothelial cells (Wang et al., 2007) are various types of specialized cells derived from ESCs.

Several studies have explored the odontogenic differentiation ability of ESCs by formation of embryoid bodies (EBs) and direct culture of ESCs as monolayers

(Kawai et al., 2014; Kidwai et al., 2014). Kidway et al. demonstrated that ESCs could give rise to an odontogenic lineage under optimized conditions. In this study, ESCs were first differentiated into H9-MSCs through EBs which, in turn, induced toward odontoblast cells. Odontoblast-like cells expressed specific odontoblastic cell markers (DSSP) and underwent morphological changes from a fibroblast-like shape into rounded shape in the presence of FGF-8 and BMP-4. Another research group differentiated the cells from EBs towards an odontoblast lineage by coculture with pulp fibroblast (Jiang et al., 2006). The differentiation of ESCs in a coculture with adult somatic cells was an efficient approach initially developed for osteogenic differentiation.

Similarly, the differentiation potential of murine ESCs to dental epithelial cells, ameloblasts, has been evaluated in ameloblast serum-free CM (ASF-CM) (Ning et al., 2010). ASF-CM was introduced as an effective strategy that provided an appropriate microenvironment which contained signaling molecules (Notch 1), transforming growth factor (TGF)-βs, BMP, and FGF to induce mESCs differentiation into odontogenic epithelial cells. The higher expression of ameloblast-specific proteins that included cytokeratin (CK) 14, ameloblastin (AMBN), and amelogenin (AMGN) proved the enhanced odontogenesis of EB-derived mESCs under this condition. Implantation of ASF-CM-treated EBs into subcutaneous pockets of 6-week-old nude mice showed that the transplant had the capability to produce odontogenic epithelial-like tissues, which were similar to tissues of enamel organ explants (Ning et al., 2010).

Although ESCs hold great promise as potent tools for tooth regeneration by producing both mesenchymal and epithelial cells, there are a number of issues. Most importantly, ethical concerns exist in terms of ESCs isolated from human embryos. The necessity for embryo destruction and lack of accessibility to ESCs from patients has hampered their use in regenerative medicine. The tumorigenic potential of ESCs after transplantation and immune rejection urged scientists to seek alternative cell sources.

27.4.1.2.3 Induced pluripotent stem cells

Tremendous efforts to locate novel cell sources that contain beneficial properties of ESCs without their drawbacks have led to the discovery of iPSCs. iPSCs can be directly generated by reprogramming somatic cells using ectopic expressions of Oct3/4, Sox2, Klf4, and c-Myc3 transcription factors (Takahashi and Yamanaka, 2006). These cells resemble ESCs in pluripotency, yet lack the ethical consideration. iPSCs can be obtained from autologous and easily accessible cells such as fibroblasts, keratinocytes, and blood cells (Park et al., 2008; Li et al., 2009; Loh et al., 2009). They keep donor-specific immune characteristics to avoid immune rejection. This approach has paved the way to generate a specialized cell lineage from reprograming adult stem cells of different origins. As an example, functional neurons and endothelial CD341 progenitor cells have been derived from iPSCs of skin dermal fibroblasts and bone marrow, respectively (Oki et al., 2012; Xu et al., 2012).

In the dentistry field, researchers successfully programmed various cell types obtained from dental tissues into iPSCs. Stem cells from exfoliated deciduous teeth

(SHED), stem cells from apical papilla, and DPSCs were reprogrammed into iPSCs via viral delivery systems (Yan et al., 2010). The resultant iPSCs from all three cells exhibited an ESC-like morphology and expressed ESC markers at a relatively higher rate than human adult fibroblasts cells. Recently, root and crown cells obtained from primary teeth were induced into the pluripotent state (Toriumi et al., 2015). The reprogramming efficiency of root cells into iPSCs was four times higher than crown cells, which made them more a potent source for iPSC generation. MSCs from third molars, oral mucosa fibroblasts, and gingiva-derived stem cells have been used to generate iPSCs (Malhotra, 2016).

To date, numerous attempts have explored the differentiation potential of iPSCs toward either a dental epithelial or mesenchymal lineage for tooth regeneration (Ozeki et al., 2016; Arakaki et al., 2012). Researchers reported several approaches and factors involved in dental differentiation of iPSCs. Arakaki et al. (2012) established a coculture system that created ameloblasts from mouse iPSCs by direct contact with dental epithelium. The enamel-secreting ameloblast feeder cells stimulated differentiation of iPSCs into ameloblast-like cells, which has confirmed the necessity for bioactive factors such as AMBN which is secreted from differentiated dental epithelial cells. The importance of metabolic factors released from extracellular matrices for iPSCs differentiation was further confirmed by a feeder-free cell culture. Higher expressions of AMGN and AMBN were recorded in differentiated iPSCs under ERM-conditioned medium (Yoshida et al., 2015).

Otsu et al. demonstrated the capability of iPSCs to differentiate into an odontogenic mesenchymal lineage. The authors reported that coculture of dental epithelium and neural crest-like cells (NCLC) derived from mouse iPSCs gave rise to odontoblast-like cells without teratoma formation after subcutaneous injection in immunodeficient mice (Otsu et al., 2012). Several groups successfully differentiated iPS-derived cells into odontogenic cells by novel strategies without any epithelial−mesenchymal interaction. Seki et al. forced mouse iPSC-derived NCLCs (iNCLCs) to give rise to odontoblast-like cells by overexpression of Pax9 and BMP-4 in iNCLCs, as an essential factor in odontogenesis (Seki et al., 2015). Another group established a hanging drop culture method on a collagen/BMP-4 scaffold in order to differentiate mouse iPSCs towards odontoblasts (Ozeki et al., 2013). Despite the encouraging results obtained from in vitro and preclinical studies, an efficient directed differentiation has not been introduced. Additionally, the functionality of iPSCs in clinical applications are limited in terms of epigenetic memory of the former phenotype, use of viral transduction, tumorigenesis, and teratoma formation. These issues must be addressed before their applications in regenerative medicine.

27.4.2 Scaffolds in tissue engineering of teeth

Both natural and synthetic materials have been extensively used as replacements for missing teeth. However, TE approaches have used these biomaterials to regenerate dental tissues. Bottom-up and top-down approaches are two basic methods to create the proper constructs in TE applications. In both approaches, scaffolds are of great

importance for the success of TE (Nichol and Khademhosseini, 2009). A scaffold provides a three-dimensional (3D) environment to support cell adhesion, proliferation, and differentiation as well as allow delivery of growth/bioactive factors that generate the desired tissues (Dutta and Dutta, 2009). A scaffold is a temporary structure which degrades over time in a controlled manner and is eventually replaced by deposited ECM and newly formed tissue of interest. The types of materials and design of a scaffold are challenging. Several features should specifically be considered for each tissue in order to achieve the ultimate goal of TE (Chan and Leong, 2008). A tooth, like bone, is categorized as a hard tissue, though the main differences between the osteogenic and odontogenic processes affect their scaffold design. For instance, signals from epithelial cells are required to initiate differentiation of preodontoblasts into odontoblasts. Odontoblasts show polarity as their protoplasm exists in dentinal tubules such that the prerequisite to generate dentin is the alignment of odontoblasts on the surface of matrix or existing dentin, whereas osteoblasts do not show polarity.

Adequate mechanical properties, porosity (pore size, volume, and structure), biodegradation rate, biocompatibility, and low immunogenicity are important features for consideration in scaffold selection in order to ensure the desired outcome. Scaffolds are typically made from natural or synthetic biomaterials. Reported results highlight the importance of scaffold development for future clinical applications.

27.4.2.1 Synthetic materials

Synthetic biomaterials can be made from organic polymers such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), PLGA, and polycaprolactone (PCL) or from inorganic calcium phosphate materials such as hydroxyapatite (HA) or beta-tricalcium phosphate (β-TCP). Synthetic materials have numerous advantages. There is the ability to fabricate these materials under controlled conditions to produce the desired structure in terms of degradation time, porosity, stiffness-elasticity, texture, shape, and hydrophilicity. Synthetic materials can be tailored to obtain desired modifications suited for specific applications (O'Brien, 2011). However, they lack inherent bioactivity and biological recognition sites, which mandate additional manipulation to improve their biological activity.

The use of PGA alone or in combination with other synthetic or natural materials constitutes the most extensively studied scaffold for tooth regeneration, particularly dental pulp tissue (Mooney et al., 1996; Buurma et al., 1999). PGA is a biodegradable material that produces a natural metabolite, glycolic acid, which is eliminated from the body through naturally occurring metabolic pathways. This makes PGA more appropriate for clinical applications. It has been shown that the construct fabricated from PGA fibers and human dental pulp-derived cells could generate new pulp-like tissue over a 60-day period in vitro (Mooney et al., 1996). Bohl et al. (1998) observed that PGA constructs were more efficient in terms of cell density and collagen deposition compared to collagen and alginate scaffold when seeded

with dental pulp cells in vitro. However, vascularization and establishment of a neural network remained a primary obstacle in the newly formed tissues. In another study a comparison of subcutaneous implantation of PGA scaffold with fibrin and collagen gels in mice to generate a dental structure showed a higher capacity of PGA in dentin-like materials (Ohara et al., 2010).

Scaffolds fabricated from PLGA have been successfully used for bioengineering of a tooth crown (Young et al., 2002). PLGA is a copolymer that take advantages of both PGA and PLA as its monomers to generate an optimal scaffold with suitable mechanical and physicochemical properties, and an adjustable degradation rate. Generation of tooth structures occurred when researchers loaded the tooth bud cells onto the PLGA scaffolds and implanted them in the omentum of athymic rats (Young et al., 2002). Orientation of the PLGA fiber determines stem cell morphology, while it has no long-term impact on cell alignment (van Manen et al., 2014). The incorporation of inorganic materials like HA into PLGA scaffolds also provides a suitable environment to support efficient tooth tissue regeneration. HA-incorporated PLGA scaffolds promote the differentiation of porcine tooth bud stem cells towards an odontoblastic phenotype (van Manen et al., 2014). HA constitutes the main mineral component of dentin. Therefore the use of HA, particularly in a nanosized form mimics HA nanocrystals in natural tissues. Since nanosized HA supports favorable cell adhesion and growth, its combination with other polymers improves the biological activity of the constructs (Hosseini et al., 2014). Zheng et al. have compared a PLGA composite that contained different types of calcium phosphate that included HA, tricalcium phosphate (TCP), and calcium carbonate HA (CDHA). They found that PLGA/TCP had superior ability to promote proliferation and differentiation of DPSCs and dentin-like material formation compared to the other types (Zhang et al., 2011).

PCL is another synthetic polymer used for hard tissue regeneration like bone. PCL scaffolds are biocompatible, have low degradation rates, and support cell adhesion, proliferation, and odontogenic differentiation (Kim et al., 2014). The addition of nHA into an electrospun nanocomposite of PCL/gelatin significantly promoted odontogenic differentiation of the seeded rat DPSCs both in vitro and in vivo compared to the PCL/gelatin that lacked nHA (Yang et al., 2010).

Efficient substrates for dental application include a new synthetic silica-based nanocomposite scaffold that mimics the tubular porous morphology of mineralized tissues of natural dentin (Lluch et al., 2009). In this approach, poly(ethyl methacrylate-co-hydroxyethyl acrylate) (PEMA-co-HEA) and silica have been polymerized using a fiber-templating fabrication method. This organic−inorganic scaffold-induced deposition of apatite on their surfaces in vitro, and offered suitable substrates for the regeneration of dental mineralized tissue. Subcutaneous implantation of these scaffolds in immunodeficient mice showed immunopatterns and ultrastructural differentiations histologically similar to dentinal structure. There was improved cell colonization and viability when the scaffolds were premineralized in vitro and implanted with the HA layer coating (Valles-Lluch et al., 2010).

27.4.2.2 Natural materials

Natural materials that originate from either plants or animals mostly exhibit excellent properties for biomedical applications that include enhanced biocompatibility, bioactivity, and biodegradability. Numerous natural polymers like collagen, fibrin, alginate, hyaluronic acid (HyA), and silk are frequently used in tooth tissue alone as a hydrogel, or in combination with other synthetic materials (Sharma et al., 2014).

Collagen is a fibrous protein widely distributed in the body and found in the ECM of several dental tissues, particularly dentin. Given the robust biocompatibility, biodegradability, and nonimmunogenic properties, it has been extensively grown for biomedical applications. Collagen, as a 3D environment for various types of cells, was investigated in number of preclinical and clinical settings to promote cellular proliferation and dental tissue regeneration (d'Aquino et al., 2009). It provides superior substrate for initial cell attachment compared to synthetic polymers like PGA (Iwatsuki et al., 2006). A 3D matrix of collagen/chitosan has been proven to support both mesenchymal and epithelial cell migration, proliferation, and odontogenic differentiation through mimicking the in vivo microenvironment (Ravindran et al., 2010). Preclinical studies revealed that the implantation of a collagen sponge in the omentum of immunocompromised rats has resulted in the formation of complete tooth morphology (Sumita et al., 2006). Although collagen naturally promotes cellular adhesion and migration, the effective regeneration occurred in the presence of other components such as cells and growth factors. All three components, including DPSCs, dental matrix protein 1 as a growth factor and the collagen scaffold lead to the generation of dental pulp-like tissue following subcutaneous transplantation in mice (Prescott et al., 2008). Collagen naturally promotes cellular adhesion, migration and proliferation, yet its mechanical properties are not high. The composite of collagen with other materials is needed to improve its mechanical stiffness.

Alginate, a natural polysaccharide, forms a stable hydrogel which provides a favorable matrix for cell proliferation and differentiation in dental engineering. Alginate is an excellent material for encapsulation of cells and delivery of growth factors due to its ability to degrade under normal physiological conditions. The alginate hydrogel that contains TGF-β1 can induce odontoblastic differentiation of dental pulp cells and enhance dentin matrix secretion (Dobie et al., 2002). It has been shown that immobilized DPSCs in an alginate microsphere could preserve their viability and enhance mineralization activity over three weeks (Kanafi et al., 2014). Similarly, Moshavernia et al. encapsulated two dental stem cells, PDLSCs and gingival MSCs (GMSCs), in oxidized alginate microbeads and demonstrated that both cell populations retained their viability and differentiation properties (Moshaverinia et al., 2012).

Chitosan has been extensively used in various medical applications in different forms such as nanoparticle, microparticles, sponge, and hydrogel scaffolds due to their unique physiochemical and biological properties. A research group studied various forms of chitosan and developed a bilayer membrane that consisted of dense film on one side and a macroporous sponge which contained TGF-β/chitosan microparticles on the other side for dentinogenesis (Li et al., 2014). They observed

a drastic increase in proliferation of odontoblast-like cells and dentin formation in vitro and in vivo. The biomembrane (BM) fabricated from chitosan/collagen embedded with calcium-aluminate microparticles was studied for odontogenic differentiation of human pulp cells (Soares et al., 2016). Cells seeded on the BM underwent higher proliferation and odontoblastic differentiation, as confirmed by robust ALP activity and the level of DMP-1/DSPP gene expression. Implantation of the BMP-7 gene-activated chitosan/collagen scaffolds and showed efficient commitment of loaded DPSCs into odontoblast-like cells in mice (Yang et al., 2012b).

HyA is a natural polysaccharide highly conserved among mammals that constitutes the main component of the ECM in connective tissues. In addition to excellent biocompatibility and biodegradability, its viscoelastic properties make it an ideal material for teeth TE (Cowman et al., 2015). HyA is proposed to contribute to dentin formation upon the initial developmental process mediated via ligation of HyA and CD44 on the odontoblast cell surface (Inuyama et al., 2010; Chen et al., 2016). Odontoblasts have been reported to effectively proliferate on the HyA-treated surface. There were significantly greater viable cells compared to dentin adhesive (Excite) (Bogovic et al., 2011). Similarly, spongy 3D scaffolds of HyA provided appropriate structure to support cell growth in vitro and in vivo for dental pulp regeneration (Inuyama et al., 2010). The HyA scaffold showed less inflammatory responses as evidenced by expression of inflammatory cytokine tumor necrosis factor (TNF)-a and interleukin-6 as well as lower granulated leukocyte infiltration into the HyA sponge compared to the collagen sponge (Inuyama et al., 2010).

Fibrin is a fibrous, insoluble protein produced naturally by polymerization of fibrinogen, a soluble plasma protein, in response to vascular system damage and bleeding. It can serve as an autologous material from patients with simple preparation and has no immunogenic reaction, along with high biocompatibility. Despite these favorable features, fibrin suffers from several drawbacks, including its tendency to shrink, poor mechanical properties, and high degradation rate. Various strategies have been implemented to prevent shrinkage and improve mechanical properties such as alterations in the polymerization parameter, use of chemical fixing agents (e.g., poly-L-lysine) or a mixture with other natural or synthetic materials for TE applications (Lee and Kurisawa, 2013). Fibrin gel could support the growth of dental papilla-derived cells for early stage tooth bud regeneration for teeth TE (Ohara et al., 2010). It has been shown that the presence of fibrin gel leads to better cell infiltration and formation of new tissue similar to normal pulp with an abundant vascular network (Ruangsawasdi et al., 2014). Yang et al. (2012a) have seeded dental bud cells into fibrin glue enriched by platelet-rich fibrin and demonstrated the regeneration of tooth tissue structures in a porcine alveolar socket. Although they observed the presence of complete tooth tissues (e.g., crown, root, pulp, enamel, dentin, odontoblast, cementum, blood vessels, and periodontal ligaments), the irregular structure of the erupted tooth should be addressed by further experiments.

Recently, investigators have examined natural ECM as a scaffold for successful tissue regeneration. ECM contains essential signals that control cell behavior and is a major part of the cell niche. Hence, these types of scaffolds present new directions for the fabrication of tissues similar to the native tissue. Tissue decellularization is

an effective method to prepare an acellular scaffold by retaining the natural tissue's composition and organization (Tapias and Ott, 2014). It has been used for various tissues and organs (Ott et al., 2008; Uygun et al., 2010). Traphagen et al. have decellularized natural porcine tooth buds. Immunohistochemistry (IHC) comparison between natural ECM and acellular tissue confirmed the preservation of natural ECM protein gradients that included collagen I, fibronectin, collagen IV, and laminin in acellular tissues. More precise image analysis that used second harmonic generation, however, showed a lower density, yet higher organization of collagen fibers in processed tissue which directed matrix deposition in the recellularized scaffolds by tooth bud cells (Traphagen et al., 2012). These studies have shown the potential of natural decellularized tooth scaffolds for dental TE applications, however additional detailed studies are needed to evaluate the effects of these scaffold with various cell types in dental tissues and the formation of dentin in preclinical settings.

27.4.3 Growth factors

An appropriate construct for implantation and generation of new tissue in the host needs to be reinforced with exogenous bioactive molecules such as growth factors, morphogen, or ECM-derived molecules in order to generate proper tissues or organs. These biomolecules can promote the healing process and achieve effective tissue regeneration. The growth factors incorporated into the scaffolds recruit cells to the defect site for treatment of diseased tissues. They generally regulate cellular activity and induce progenitor or stem cells to proliferate and undergo differentiation towards a specific lineage by binding to specific cell membrane receptors (Zhao et al., 2004; Ripamonti et al., 2005). Several signaling molecules are versatile and have the same stimulatory effects in different cell types. In contrast, varying responses with a degree of specificity may be caused by the same signal in a variety of tissues or even over different periods of time. Various signaling pathways and molecules have been identified that are implicated in determining morphological features of teeth such as crown size, type of cusp patterning, and tooth length as well as repair of pulp and dentin. These signaling components—Shh, FGF, BMPs, and Wnt—regulate the cellular activity in dental tissue regeneration by mediating the reciprocal interactions between epithelial and mesenchymal cells.

Recently, several growth factors combined with various materials and cells have successfully been used to promote tooth regeneration in preclinical and clinical settings. TGFs, BMPs, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), and FGF-2 are the most studied signaling molecules in terms of tooth TE.

27.4.3.1 Platelet-derived growth factor

PDGF is the first growth factor whose recombinant form has been approved by the FDA (Wieman et al., 1998). Promising results of extensive preclinical studies, particularly in periodontal and peri-implant regeneration, show the potent ability of PDGF in clinical settings. PDGF is a well-characterized growth factor for dental

applications. It is naturally a glycoprotein with a high molecular weight ($\sim$30,000 MW), mostly released by platelets, inflammatory cells, and damaged bone (Alvarez et al., 2006). PDGF consists of two subunits that can form either homo- or heterodimers via a disulfide-bond. The PDGF family consists of PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD (Reigstad et al., 2005). PDGF has the capability to control cellular behavior such as chemotaxis and mitosis by binding to two tyrosine kinase receptors, PDGFRα and PDGFRβ (Heldin et al., 1998). PDGFRα predominantly binds to PDGF-A whereas PDGF-B has a high affinity for PDGFRβ. PDGF regulates the proliferation rate and affects odontoblastic differentiation, however the differentiation potential varies among PDGF dimers. PDGFRα and PDGF-A specifically regulate the prolonged epithelial−mesenchymal interaction during tooth and palate morphogenesis (Xu et al., 2005). PDGF has a stimulatory effect on the formation of mineralized dentin matrix as well as tremendous potential to promote regeneration in a variety of oral tissues including the cementum, gingival and alveolar bone (Lin et al., 2008; Lynch et al., 2006). Interestingly, although the vital role of PDGF in tissue mineralization is undeniable, continuous exogenous delivery of PDGF inhibits cementogenesis (Anusaksathien et al., 2004).

Considering the robust regenerative capacity of PDGF, many research groups have been used PDGF in combination with biomaterials in preclinical studies. Zhang et al. developed a chitosan-mediated expression of the *PDGF* gene in a collagen/coral construct to induce periodontal tissue regeneration. Subcutaneous implantation of gene-activated coral scaffolds into athymic mice resulted in improved vascularized tissue in-growth compared with pure coral scaffold (Zhang et al., 2007). Research showed that adenovirus which encoded the *PDGF-B* gene (Ad-PDGF-B) delivered in the collagen matrix had no remarkable histopathological symptoms for future clinical applications (Chang et al., 2009). Several studies evaluated the use of PDGF-BB in the clinical setting (Camelo et al., 2003; Nevins et al., 2003). A large-scale clinical trial of 180 patients (Nevins et al., 2005) evaluated PDGF-BB combined with β-TCP matrix to regenerate advanced periodontal bone defects. The results of this study verified the safety and effectiveness of PDGF-BB-TCP and the positive outcome of its long-term evaluation (24 months) led to FDA approval of 0.3 mg/ml rhPDGF and β-TCP for periodontal defects (Kaigler et al., 2011; McGuire et al., 2006).

27.4.3.2 Bone morphogenetic proteins

BMPs are a unique group of signaling proteins that belong to the TGF-β superfamily. A thorough literature research has revealed the importance of BMPs in cellular signaling for odontoblast differentiation and stimulation of reparative dentine formation; hence, their necessity for tooth regeneration (Nakashima, 2005). BMPs are considered to be osteoinductive factors that have the ability to regulate bone and dentin formation (Rutherford et al., 1993). Systemic analysis of BMP expression show different functions during tooth morphogenesis as well as concurrent temporal and spatial expression patterns (Aberg et al., 1997). They play an important role

throughout different morphological stages of tooth generation, from initiation of tooth development to matrix formation. BMP-2, BMP-4, BMP-6, BMP-7, and Gdf11 are detected during odontoblast differentiation, and BMP-4 and BMP-5 during ameloblast differentiation (Heikinheimo, 1994; Aberg et al., 1997). BMP-2 and -7 share a similar expression pattern. They express in dental epithelium and the enamel knot (Thesleff, 2003). However, BMP-2 (but not BMP-7) is needed to induce the differentiation of DPSCs into odontoblasts (Casagrande et al., 2010). The presence of BMP receptors (BMPR)-IA, BMPR-IB, and BMP-II on dental pulp cells enable these cells to respond potently to BMP-mediated signals. BMPR are two types of serine/threonine kinases that include type I (BMPR-IA, BMPR-IB) and type II (BMPR-II) receptors (Sebald et al., 2004).

Studies reported use of various types of BMPs, as an essential mediator, in tooth TE. In order to determine whether BMP-7 could be used in tooth TE, human DPSCs were genetically modified to express BMP-7 (Yang et al., 2012c). In vitro and in vivo evaluation of seeded BMP-7-transfected cells onto a ceramic scaffold has shown the commitment of transfected cells to the odontoblast phenotype and obvious calcified tissue formation. The differentiation of various stem cells into a dental lineage requires an epithelial—mesenchymal interaction. However, the use of proper growth factors and substrates can eliminate the need for this interaction. According to research, BMP-2, -4, and -7 are used for communication and signaling between epithelial and mesenchymal cells (Thesleff, 2003). Ozeki et al. (2013) successfully differentiated mouse iPSCs into odontoblast-like cells in a BMP-4/collagen construct. Amongst various BMPs, BMP-4 has higher expression of the odontoblast markers, DSPP and DMP-1. These findings provided the support for application of BMP members in dental TE.

27.4.3.3 Transforming growth factors

TGF-β signaling has been implicated in dentin formation and pulp protection through expression of TGF-β receptors I and II in both odontoblasts and pulp cells (Sloan et al., 1999). It mediates a variety of functions such as odontoblast differentiation, ECM synthesis, and matrix mineralization, however the exact molecular mechanism is not clearly elucidated. It has been suggested that TGF-β signaling stimulates dentin matrix formation with BMP and Wnt signaling (Ahn et al., 2015). The presence of various TGF-β isoforms detected within the tissues of healthy human molars and those with caries show that TGF-β3 has the highest level in the healthy tissues, whereas TGF-β1 showed the weakest intensity. This finding was reversed in diseased tissues (Sloan et al., 2000). The stimulatory effect of TGF-β associated with scaffold materials was explored on odontoblast proliferation and dentin formation of dental pulp cells both in vitro and in vivo (Li et al., 2011). Appearance of odontoblast-like cells and calcification nodules in TGF-β containing groups confirmed the positive role of TGF-β in the in vitro culture. Researchers observed deposition of tubular dentin matrix following the implantation of a construct under the renal capsule in rats after 3 months. Similarly, incorporated chitosan microporous TGF-β generated more repaired dentin compared to the controls in

a dog pulp model (Li et al., 2014). The beneficial effects of TGF in tooth TE must be addressed by additional preclinical and clinical studies.

27.4.3.4 Insulin-like growth factor

IGFs are present at different intensities during different stages of tooth development (Joseph et al., 1993). IGF-1 and -2 are members of the IGF family composed of a single polypeptide chain and recognized by IGF receptors. IGFs have the potential for proliferation and differentiation of dental pulp cells (Kim et al., 2012). IGF-1 promotes proliferation and odontogenic differentiation of human DPSCs (hDPSCs) by activation of MAPK pathways (Lv et al., 2016). Application of IGF-1 onto exposed pulp tissues of rabbit molars has shown the vitality of pulp tissues even 6 weeks after IGF-1 application (Haddad et al., 2003). Research explored the impact of IGFs on growth and differentiation of dental pulp cells in serum-free culture conditions (Onishi et al., 1999). The authors proposed that pulp cells underwent differentiation into odontoblast-like cells in serum-free culture. Wang et al. (2012) reported a different influence of IGFs on apical papilla stem cells (APSCs). The results of this study indicated that IGFs drove APSCs to osteogenic differentiation and reduced their odontogenic and dentinogenesis capabilities. Therefore inconsistent results under various circumstances must be taken into consideration prior to the administration of IGF.

27.5 Whole teeth regeneration: Recent strategies and techniques

Bioengineered teeth are considered a new generation of therapeutic approaches. The creation of natural-like teeth by means of TE requires the synchronized generation of the crown, root, and periodontal ligament. In order to create a functional bioengineered tooth the natural process of tooth germ formation through reciprocal epithelium−mesenchyme interaction needs to be imitated. Research has shown that each mesenchymal or epithelial cell cannot independently regenerate proper teeth (Yadav et al., 2016). Thus far, two major strategies are under development to fully reconstitute the following sequences of events in order to generate a bioengineered tooth: (1) transplantation of in vitro preprepared cell/scaffold construct in the host and (2) recreation of the embryonic development of natural teeth through implantation of competent stem cells (Fig. 27.3). Different investigators have studied a variety of cell populations and materials based on these two strategies (Table 27.1).

Young et al. (2002) pioneered production of bioengineered teeth by the former technique. The researchers cultured the enzymatically dissociated porcine third molar tooth bud cells on various polymeric scaffolds to allow for epithelial−mesenchymal cell interactions and subsequently implanted the constructs into the omenta of rats. Histological and molecular analyses of implanted constructs showed a recognizable tooth structure that consisted of the dentin, enamel organ,

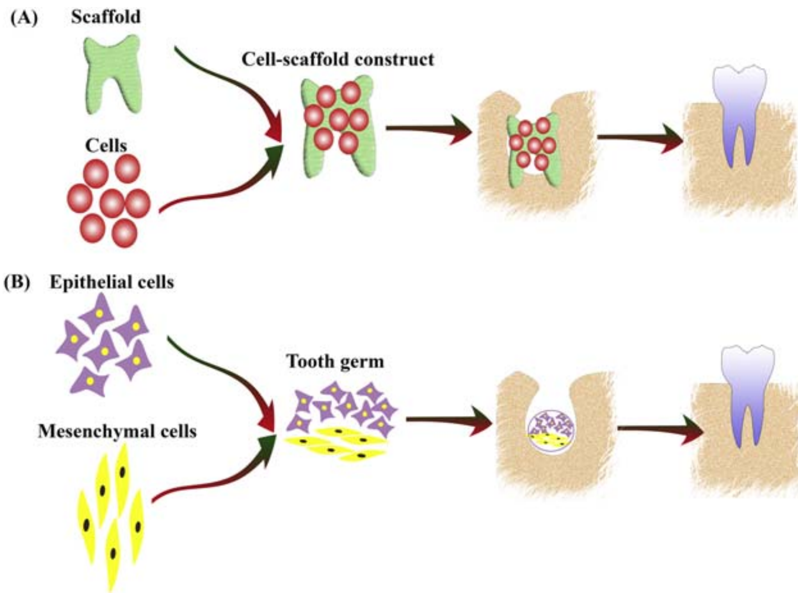


Figure 27.3 Two current approaches for whole tooth tissue engineering (TE).
(A) Implantation of a cell/scaffold construct and (B) the cell recombination approach.

and HERS at 35 weeks after implantation. However, the regenerated teeth were quite small and 85% of the newly formed tissues developed irregular tooth morphologies which was probably related to the disability of the dental epithelium in formation of an accurate cap-stage tooth bud (Honda et al., 2005; Young et al., 2005b). Improvements to this approach included optimizing the age of the tooth bud cells and exclusive use of single-cell suspensions of 6-day cultured rat tooth bud cells (Duailibi et al., 2004). The same tooth crown structure was achieved 12 weeks after implantation. Long-term development of bioengineered porcine teeth was most probably allocated to its prolonged natural growth patterns (Bivin and McClure, 1976). In addition, the regenerated teeth were smaller than normal teeth. Different parameters such as scaffold materials and mechanical stress were also examined in an attempt to enhance the success rate of the tooth regeneration (Honda et al., 2006b; Sumita et al., 2006).

Despite promising achievements in bioengineered teeth, irregular, and inaccurate structure of the bioengineered tooth were the driving forces for additional attempts. Nakao et al. (2007) developed bioengineered tooth germ in vitro from dissociated single cells of the epithelial and mesenchymal embryonic tooth germ (ETG) and transplanted them into subrenal capsules in mice. They confirmed that ETG at the cup-stage had the ability to accurately reconstitute the crown structure. Accordingly, the capability of dissociated ETG on 3D scaffold was explored with the aim to successfully generate a bioengineered tooth (Iwatsuki et al., 2006). The enzymatically dissociated first molar tooth germ derived from E14 mice at the cap

Table 27.1 **Studies that describe whole tooth regeneration**

Cell type	Scaffold	In vitro/ in vivo	Implantation site	Results	References
Porcine third molar tooth bud cells	PGA/PLLA and PLGA	In vivo	Rat omentum	Recognizable tooth structure consisted of the dentin, enamel organ, and HERS in 35 weeks	Young et al. (2002)
3−7 dpn rat tooth bud cells	PGA and PLGA	In vivo	Rat omentum	Mature tooth structures containing dentin, pulp enamel in 12 weeks	Duailibi et al. (2004)
Porcine third molar tooth bud cells	PGA	In vivo	Rat omentum	Regenerated tooth structure, including the enamel, dentin and cementum`	Honda et al. (2005)
Porcine third molar tooth bud cells	PGA/PLLA	In vivo	Rat omentum	Tooth structure including thick enamel, dentin, odontoblasts and pulp tissue	Young et al. (2005b)
Porcine third molar tooth bud cells	PGA	Both	Rat omentum	Enhanced expression level of amelogenin, bone sialoprotein and vimentin protein in vitro and formation of enamel and dentin tissues in vivo	Honda et al. (2006b)
Canine first molar tooth bud cells	PGA	In vivo	Canine tooth socket	Formation of dentin and bone without enamel and root	Honda et al. (2006a)
Mouse embryonic tooth bud cells	PGA	In vivo	Mouse kidney capsule	Small bioengineered teeth with normal crown shape	Iwatsuki et al. (2006)
Porcine third molar tooth bud cells	Collagen And PGA	Both	Rat omentum	Better performance of collagen sponge for tooth regeneration	Sumita et al. (2006)

(*Continued*)

Table 27.1 **(Continued)**

Cell type	Scaffold	In vitro/ in vivo	Implantation site	Results	References
Mouse incisor tooth germ	Collagen gel	Both	Mouse subrenal capsule	Reconstituted tooth germ generated complete tooth	Nakao et al. (2007)
Embryonic tooth bud cells	Collagen	In vivo	Mouse jaw	Functional tooth with sufficient hardness for mastication and a functional responsiveness to mechanical stress in the maxillofacial region	Ikeda et al. (2009)
Porcine dental mesenchymal cells, human DPSC, gingival epithelial cells	Collagen	In vivo	Rat subcutaneous	Formation of mineralized dental tissues and tooth crown structure	Zhang et al. (2014)
Human gingival epithelial cells. With mouse molar mesenchymal tissue	–	In vivo	Mouse kidney capsule	Bioengineered teeth containing dentin and enamel with ameloblast-like cells	Angelova Volponi et al. (2013)
Embryonic dental follicle stem cells	HA-coated implant	In vivo	Murine tooth loss model	Restoration of physiological function by periodontal ligament and cementum	Oshima et al. (2014)
BMSCs and embryonic oral epithelium	–	In vivo	Mouse renal capsules	Developed teeth (crowns) with associated bone and soft tissues	Ohazama et al. (2004)

stage were seeded on PGA fiber mesh and then implanted in the kidney capsule of adult mice. This led to the formation of a normal crown structure that contained dentin and enamel, however the small size of the tissue-engineered teeth and lack of root formation and periodontium remained challenging.

Complete reconstitution of a new tooth located at its proper position is another challenge to be addressed. A new study has intended to determine the ability of a seeded PGA scaffold with a dissociated tooth bud to generate a tooth when directly transplanted into a canine jaw (Honda et al., 2006a). All previous studies were performed in the omentum area of the abdomen. In contrast to results obtained from omentum graft models the odontogenic cells only generated dentin structure but not enamel and root regeneration. Ikeda et al. (2009) successfully developed a completely functional tooth following transplantation of bioengineered tooth germ in alveolar bone of a murine model.

It was assumed that the existence of both types of mesenchymal and epithelial dental cells in a random manner and their interactions, as well as the ability of a subpopulation of tooth bud cells for tooth development was the reason behind abnormal morphogenesis of the tooth crown. Honda et al. (2007) developed a new approach by manipulating the contact positions between epithelial and mesenchymal cells. They separately seeded the mesenchymal and epithelial components of a porcine tooth bud in a collagen sponge and placed the epithelial cells on the top mesenchyme in both groups of direct cell–cell contact (group I) or with the microporous membrane in the interface (group II). They observed the formation of only one tooth germ in each scaffold in group I. The outcome of this experiment clarified the necessity of direct cell–cell interaction for normal tooth generation.

Despite the vast majority of achievements in bioengineered teeth formation by the use of embryonic and postnatal dental tissues, the lack of availability of human autologous embryonic tissues remains one of the main challenges for clinical applications of this strategy. Therefore engineering functional teeth by cells from postnatal dental or nondental tissues is necessary for progression. Ohazama et al. (2004) for the first time have exploited adult nondental cells to generate a complete tooth. They replaced the mesenchymal compartment by bone marrow-derived cells (BMDCs) and then recombined them with an oral epithelium from an ED10 mouse embryo. The results showed stimulation of an odontogenic response in BMDCs and led to tooth formation in vivo. However, the random reassociation led correct site of mesenchyme and epithelial interactions to be unrecognizable. The exact nature of BMDCs that contribute to tooth formation should also be addressed.

27.6 Future trends and concluding remarks

Teeth can be lost due to several reasons including caries, periodontal disease, trauma, hypodontia, tooth wear, oral cancer, and iatrogenic damage to the teeth. TE technology for tooth engineering that closely approximate the same features of natural teeth has been recently developed in an attempt to replace traditional

therapeutic approaches. The encouraging results obtained from reconstituted teeth by tooth bud cells makes the dream of regeneration of a whole tooth a possibility in the future. There are several hurdles that must be addressed before successful tooth regeneration. Finding competent cells to imitate the complicated dental mesenchyme and epithelia interactions is the main and basic requirement for cell-based tooth TE. Currently, embryonic and adult stem cells constitute two major cell sources for preclinical investigations that have also been used in clinical trials (Feng et al., 2010; Gault et al., 2010). In terms of whole tooth TE, numerous attempts have been made to obtain epithelial cells from postnatal tissues. The differentiation ability of keratinocytes from human foreskin and gingival epithelial cells toward enamel-forming ameloblasts combined embryonic molar mesenchyme has been reported (Angelova Volponi et al., 2013; Wang et al., 2010). Consistent epithelial cell sources obtainable from adults are needed. Apart from determining suitable epithelial and mesenchymal cell sources the odontogenic potential of isolated cells is a tremendous challenge that must be overcome. In TE, scaffolds are paramount as they act as conducive substrates for cell migration, attachment, and differentiation as well as carriers for delivery of bioactive agents. Constructing scaffolds with the highest degree of similarity to the natural environment to provide optimum physiochemical and mechanical cues for cell growth and differentiation is the focus of current biomaterials research. The advancement of a decellularized scaffold and cell sheet engineering provide a promising approach for tooth engineering, although the investigations in this field are in their infancy. The use of bioactive molecules such as growth factors or mineralizing peptides associated with the scaffolds is a common strategy to direct cell function. This has resulted in human clinical trials that combined TCP and PDGF to treat periodontal bone defects (Nevins et al., 2005; McGuire et al., 2006). Nevertheless, further studies are underway to substitute this construct with more optimized combinations.

In summary, investigation in the field of tooth TE is rapidly advancing to overcome current obstacles. However, despite encouraging progress, it remains necessary to identify the appropriate epithelial and mesenchymal cells from postnatal tissues and related molecular mechanisms for initiation of odontogenesis for successful outcomes.

References

Abdallah, B.M., Kassem, M., 2008. Human mesenchymal stem cells: from basic biology to clinical applications. Gene Ther. 15, 109−116.

Aberg, T., Wozney, J., Thesleff, I., 1997. Expression patterns of bone morphogenetic proteins (Bmps) in the developing mouse tooth suggest roles in morphogenesis and cell differentiation. Dev. Dyn. 210, 383−396.

Ahn, Y.H., Kim, T.H., Choi, H., Bae, C.H., Yang, Y.M., Baek, J.A., Lee, J.C., Cho, E.S., 2015. Disruption of Tgfbr2 in odontoblasts leads to aberrant pulp calcification. J. Dent. Res. 94, 828−835.

Aldahmash, A., Zaher, W., Al-Nbaheen, M., Kassem, M., 2012. Human stromal (mesenchymal) stem cells: basic biology and current clinical use for tissue regeneration. Ann. Saudi Med. 32, 68–77.

Almela, T., Brook, I.M., Moharamzadeh, K., 2016. The significance of cell-related challenges in the clinical application of tissue engineering. J. Biomed. Mater. Res. A. 104, 3157–3163.

Alvarez, R.H., Kantarjian, H.M., Cortes, J.E., 2006. Biology of platelet-derived growth factor and its involvement in disease. Mayo Clin. Proc. 81, 1241–1257.

Angelova Volponi, A., Kawasaki, M., Sharpe, P.T., 2013. Adult human gingival epithelial cells as a source for whole-tooth bioengineering. J. Dent. Res. 92, 329–334.

Anusaksathien, O., Jin, Q., Zhao, M., Somerman, M.J., Giannobile, W.V., 2004. Effect of sustained gene delivery of platelet-derived growth factor or its antagonist (PDGF-1308) on tissue-engineered cementum. J. Periodontol. 75, 429–440.

Arakaki, M., Ishikawa, M., Nakamura, T., Iwamoto, T., Yamada, A., Fukumoto, E., Saito, M., Otsu, K., Harada, H., Yamada, Y., Fukumoto, S., 2012. Role of epithelial-stem cell interactions during dental cell differentiation. J. Biol. Chem. 287, 10590–10601.

Arana-Chavez, V.E., Massa, L.F., 2004. Odontoblasts: the cells forming and maintaining dentine. Int. J. Biochem. Cell Biol. 36, 1367–1373.

Attar, A., Eslaminejad, M.B., Tavangar, M.S., Karamzadeh, R., Dehghani-Nazhvani, A., Ghahramani, Y., Malekmohammadi, F., Hosseini, S.M., 2014. Dental pulp polyps contain stem cells comparable to the normal dental pulps. J. Clin. Exp. Dent. 6, e53–e59.

Bakhtiar, H., Mirzaei, H., Bagheri, M.R., Fani, N., Mashhadiabbas, F., Baghaban Eslaminejad, M., Sharifi, D., Nekoofar, M.H., Dummer, P., 2016. Histologic tissue response to furcation perforation repair using mineral trioxide aggregate or dental pulp stem cells loaded onto treated dentin matrix or tricalcium phosphate. Clin Oral Investig. 1–10.

Baksh, D., Yao, R., Tuan, R.S., 2007. Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. Stem Cells. 25, 1384–1392.

Balic, A., Thesleff, I., 2015. Tissue interactions regulating tooth development and renewal. Curr. Top. Dev. Biol. 115, 157–186.

Balintjoseph Orban, H.S., Bhaskar, S.N., 1972. Orban's Oral Histology and Embryology. *Mosby Company*, Missouri, United States, p. 393.

Batouli, S., Miura, M., Brahim, J., Tsutsui, T.W., Fisher, L.W., Gronthos, S., Robey, P.G., 2003. Comparison of stem-cell-mediated osteogenesis and dentinogenesis. J. Dent. Res. 82, 976–981.

Bielby, R.C., Boccaccini, A.R., Polak, J.M., Buttery, L.D., 2004. In vitro differentiation and in vivo mineralization of osteogenic cells derived from human embryonic stem cells. Tissue Eng. 10, 1518–1525.

Bivin, W.S., Mcclure, R.C., 1976. Deciduous tooth chronology in the mandible of the domestic pig. J. Dent. Res. 55, 591–597.

Blau, H.M., Brazelton, T.R., Weimann, J.M., 2001. The evolving concept of a stem cell: entity or function? Cell. 105, 829–841.

Bogovic, A., Nizetic, J., Galic, N., Zeljezic, D., Micek, V., Mladinic, M., 2011. The effects of hyaluronic acid, calcium hydroxide, and dentin adhesive on rat odontoblasts and fibroblasts. Arh. Hig Rada Toksikol. 62, 155–161.

Bohl, K.S., Shon, J., Rutherford, B., Mooney, D.J., 1998. Role of synthetic extracellular matrix in development of engineered dental pulp. J. Biomater. Sci. Polym. Ed. 9, 749–764.

Buurma, B., Gu, K., Rutherford, R.B., 1999. Transplantation of human pulpal and gingival fibroblasts attached to synthetic scaffolds. Eur. J. Oral Sci. 107, 282–289.

Cai, J., Zhao, Y., Liu, Y., Ye, F., Song, Z., Qin, H., Meng, S., 2007. Directed differentiation of human embryonic stem cells into functional hepatic cells. Hepatology. 45, 1229−1239.

Camelo, M., Nevins, M.L., Schenk, R.K., Lynch, S.E., Nevins, M., 2003. Periodontal regeneration in human Class II furcations using purified recombinant human platelet-derived growth factor-BB (rhPDGF-BB) with bone allograft. Int. J. Periodontics Restorative Dent. 23, 213−225.

Casagrande, L., Demarco, F.F., Zhang, Z., Araujo, F.B., Shi, S., Nor, J.E., 2010. Dentin-derived BMP-2 and odontoblast differentiation. J. Dent. Res. 89, 603−608.

Chambers, S.M., Fasano, C.A., Papapetrou, E.P., Tomishima, M., Sadelain, M., Studer, L., 2009. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat. Biotechnol. 27, 275−280.

Chan, B.P., Leong, K.W., 2008. Scaffolding in tissue engineering: general approaches and tissue-specific considerations. Eur. Spine J. 17 (Suppl. 4), 467−479.

Chang, P.C., Cirelli, J.A., Jin, Q., Seol, Y.J., Sugai, J.V., D'silva, N.J., Danciu, T.E., Chandler, L.A., Sosnowski, B.A., Giannobile, W.V., 2009. Adenovirus encoding human platelet-derived growth factor-B delivered to alveolar bone defects exhibits safety and biodistribution profiles favorable for clinical use. Hum. Gene Ther. 20, 486−496.

Chen, G., Ye, Z., Yu, X., Zou, J., Mali, P., Brodsky, R.A., Cheng, L., 2008. Trophoblast differentiation defect in human embryonic stem cells lacking PIG-A and GPI-anchored cell-surface proteins. Cell Stem Cell. 2, 345−355.

Chen, K.L., Yeh, Y.Y., Lung, J., Yang, Y.C., Yuan, K., 2016. Mineralization effect of hyaluronan on dental pulp cells via CD44. J. Endod. 42, 711−716.

Cowman, M.K., Schmidt, T.A., Raghavan, P., Stecco, A., 2015. Viscoelastic properties of hyaluronan in physiological conditions. F1000Res. 4, 622.

d'Aquino, R., de Rosa, A., Lanza, V., Tirino, V., Laino, L., Graziano, A., Desiderio, V., Laino, G., Papaccio, G., 2009. Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. Eur. Cell Mater. 18, 75−83.

Demarco, F.F., Conde, M.C., Cavalcanti, B.N., Casagrande, L., Sakai, V.T., Nor, J.E., 2011. Dental pulp tissue engineering. Braz. Dent. J. 22, 3−13.

Dobie, K., Smith, G., Sloan, A.J., Smith, A.J., 2002. Effects of alginate hydrogels and TGF-beta 1 on human dental pulp repair in vitro. Connect Tissue Res. 43, 387−390.

Dominici, M., le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., Deans, R., Keating, A., Prockop, D., Horwitz, E., 2006. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 8, 315−317.

Duailibi, M.T., Duailibi, S.E., Young, C.S., Bartlett, J.D., Vacanti, J.P., Yelick, P.C., 2004. Bioengineered teeth from cultured rat tooth bud cells. J. Dent. Res. 83, 523−528.

Dutta, R.C., Dutta, A.K., 2009. Cell-interactive 3D-scaffold; advances and applications. Biotechnol. Adv. 27, 334−339.

El-Backly, R.M., Massoud, A.G., El-Badry, A.M., Sherif, R.A., Marei, M.K., 2008. Regeneration of dentine/pulp-like tissue using a dental pulp stem cell/poly(lactic-co-glycolic) acid scaffold construct in New Zealand white rabbits. Aust. Endod. J. 34, 52−67.

Eslaminejad, M.B., Bordbar, S., Nazarian, H., 2013. Odontogenic differentiation of dental pulp-derived stem cells on tricalcium phosphate scaffolds. J. Dent. Sci. 8, 306−313.

Eslaminejad, M.B., Vahabi, S., Shariati, M., Nazarian, H., 2010. In vitro growth and characterization of stem cells from human dental pulp of deciduous versus permanent teeth. J. Dent. (Tehran). 7, 185−195.

Feng, F., Akiyama, K., Liu, Y., Yamaza, T., Wang, T.M., Chen, J.H., Wang, B.B., 2010. Utility of PDL progenitors for in vivo tissue regeneration: a report of 3 cases. Oral. Dis. 16, 20–28.
Galler, K.M., Cavender, A.C., Koeklue, U., Suggs, L.J., Schmalz, G., D'souza, R.N., 2011. Bioengineering of dental stem cells in a PEGylated fibrin gel. Regen. Med. 6, 191–200.
Gault, P., Black, A., Romette, J.L., Fuente, F., Schroeder, K., Thillou, F., Brune, T., Berdal, A., Wurtz, T., 2010. Tissue-engineered ligament: implant constructs for tooth replacement. J. Clin. Periodontol. 37, 750–758.
Gerami-Naini, B., Dovzhenko, O.V., Durning, M., Wegner, F.H., Thomson, J.A., Golos, T.G., 2004. Trophoblast differentiation in embryoid bodies derived from human embryonic stem cells. Endocrinology. 145, 1517–1524.
Goldberg, M., Kulkarni, A.B., Young, M., Boskey, A., 2011. Dentin: structure, composition and mineralization. Front Biosci. (Elite Ed.). 3, 711–735.
Griffith, L.G., Naughton, G., 2002. Tissue engineering—current challenges and expanding opportunities. Science. 295, 1009–1014.
Gronthos, S., Mankani, M., Brahim, J., Robey, P.G., Shi, S., 2000. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc. Natl. Acad. Sci. USA. 97, 13625–13630.
Gupte, M.J., Ma, P.X., 2012. Nanofibrous scaffolds for dental and craniofacial applications. J. Dent. Res. 91, 227–234.
Haddad, M., Lefranc, G., Aftimos, G., 2003. Local application of IGF1 on dental pulp mechanically exposed; in vivo study on rabbit. Bull. Group Int. Rech Sci. Stomatol. Odontol. 45, 12–17.
Heikinheimo, K., 1994. Stage-specific expression of decapentaplegic-Vg-related genes 2, 4, and 6 (bone morphogenetic proteins 2, 4, and 6) during human tooth morphogenesis. J. Dent. Res. 73, 590–597.
Heldin, C.H., Ostman, A., Ronnstrand, L., 1998. Signal transduction via platelet-derived growth factor receptors. Biochim. Biophys. Acta. 1378, F79–113.
Honda, M.J., Ohara, T., Sumita, Y., Ogaeri, T., Kagami, H., Ueda, M., 2006a. Preliminary study of tissue-engineered odontogenesis in the canine jaw. J. Oral Maxillofac. Surg. 64, 283–289.
Honda, M.J., Shinohara, Y., Sumita, Y., Tonomura, A., Kagami, H., Ueda, M., 2006b. Shear stress facilitates tissue-engineered odontogenesis. Bone. 39, 125–133.
Honda, M.J., Sumita, Y., Kagami, H., Ueda, M., 2005. Histological and immunohistochemical studies of tissue engineered odontogenesis. Arch. Histol. Cytol. 68, 89–101.
Honda, M.J., Toriumi, T., Oka, K., Saito, Y., Isokawa, K., 2015. Chapter 27—dental stem cells for tooth tissue engineering A2— Vishwakarma, Ajaykumar. In: Sharpe, P., Shi, S., Ramalingam, M. (Eds.), Stem Cell Biology and Tissue Engineering in Dental Sciences. Academic Press, Boston.
Honda, M.J., Tsuchiya, S., Sumita, Y., Sagara, H., Ueda, M., 2007. The sequential seeding of epithelial and mesenchymal cells for tissue-engineered tooth regeneration. Biomaterials. 28, 680–689.
Horst, O.V., Chavez, M.G., Jheon, A.H., Desai, T., Klein, O.D., 2012. Stem cell and biomaterials research in dental tissue engineering and regeneration. Dent. Clin. North Am. 56, 495–520.
Hosseini, S., Naderi-Manesh, H., Vali, H., Faghihi, S., 2014. Improved surface bioactivity of stainless steel substrates using osteocalcin mimetic peptide. Mater. Chem. Phys. 143, 1364–1371.
Hu, B., Unda, F., Bopp-Kuchler, S., Jimenez, L., Wang, X.J., Haikel, Y., Wang, S.L., Lesot, H., 2006. Bone marrow cells can give rise to ameloblast-like cells. J. Dent. Res. 85, 416–421.

Hu, Y., Zhang, L.Y., Ma, G.J., Jiang, X.Y., Zhao, C.H., 2001. Phenotypical and biological characteristics of human fetal marrow and liver mesenchymal stem cells. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 9, 289–293.

Huang, X.F., Chai, Y., 2012. Molecular regulatory mechanism of tooth root development. Int. J. Oral Sci. 4, 177–181.

Ikeda, E., Morita, R., Nakao, K., Ishida, K., Nakamura, T., Takano-Yamamoto, T., Ogawa, M., Mizuno, M., Kasugai, S., Tsuji, T., 2009. Fully functional bioengineered tooth replacement as an organ replacement therapy. Proc. Natl. Acad. Sci. USA. 106, 13475–13480.

Inuyama, Y., Kitamura, C., Nishihara, T., Morotomi, T., Nagayoshi, M., Tabata, Y., Matsuo, K., Chen, K.K., Terashita, M., 2010. Effects of hyaluronic acid sponge as a scaffold on odontoblastic cell line and amputated dental pulp. J. Biomed. Mater. Res. B: Appl. Biomater. 92, 120–128.

Iwatsuki, S., Honda, M.J., Harada, H., Ueda, M., 2006. Cell proliferation in teeth reconstructed from dispersed cells of embryonic tooth germs in a three-dimensional scaffold. Eur. J. Oral. Sci. 114, 310–317.

Jain, N., Gupta, A., N, M., 2013. An insight into neurophysiology of pulpal pain: facts and hypotheses. Korean J. Pain. 26, 347–355.

Jheon, A.H., Seidel, K., Biehs, B., Klein, O.D., 2013. From molecules to mastication: the development and evolution of teeth. Wiley Interdiscip. Rev. Dev. Biol. 2, 165–182.

Jiang, Z.M., Ji, P.H., Liu, J., Tang, Y.J., Li, S.J., Li, W.L., 2006. Induction of mouse embryonic stem cells forming odontoblast-like cells by co-culture with pulp fibroblast. Shanghai Kou Qiang Yi Xue. 15, 653–656.

Joseph, B.K., Savage, N.W., Young, W.G., Gupta, G.S., Breier, B.H., Waters, M.J., 1993. Expression and regulation of insulin-like growth factor-I in the rat incisor. Growth Factors. 8, 267–275.

Jung, R.E., Zembic, A., Pjetursson, B.E., Zwahlen, M., Thoma, D.S., 2012. Systematic review of the survival rate and the incidence of biological, technical, and aesthetic complications of single crowns on implants reported in longitudinal studies with a mean follow-up of 5 years. Clin. Oral Implants Res. 23 (Suppl 6), 2–21.

Jussila, M., Thesleff, I., 2012. Signaling networks regulating tooth organogenesis and regeneration, and the specification of dental mesenchymal and epithelial cell lineages. Cold Spring Harb Perspect. Biol. 4, a008425.

Kado, M., Lee, J.K., Hidaka, K., Miwa, K., Murohara, T., Kasai, K., Saga, S., 2008. Paracrine factors of vascular endothelial cells facilitate cardiomyocyte differentiation of mouse embryonic stem cells. Biochem. Biophys. Res. Commun. 377, 413–418.

Kaigler, D., Avila, G., Wisner-Lynch, L., Nevins, M.L., Nevins, M., Rasperini, G., Lynch, S. E., Giannobile, W.V., 2011. Platelet-derived growth factor applications in periodontal and peri-implant bone regeneration. Expert Opin. Biol. Ther. 11, 375–385.

Kanafi, M.M., Ramesh, A., Gupta, P.K., Bhonde, R.R., 2014. Dental pulp stem cells immobilized in alginate microspheres for applications in bone tissue engineering. Int. Endod. J. 47, 687–697.

Karamzadeh, R., Eslaminejad, M.B., Aflatoonian, R., 2012. Isolation, characterization and comparative differentiation of human dental pulp stem cells derived from permanent teeth by using two different methods. J. Vis. Exp. 69, pii: 4372.

Kaufman, D.S., Hanson, E.T., Lewis, R.L., Auerbach, R., Thomson, J.A., 2001. Hematopoietic colony-forming cells derived from human embryonic stem cells. Proc. Natl. Acad. Sci. USA. 98, 10716–10721.

Kawai, R., Ozeki, N., Yamaguchi, H., Tanaka, T., Nakata, K., Mogi, M., Nakamura, H., 2014. Mouse ES cells have a potential to differentiate into odontoblast-like cells using hanging drop method. Oral Dis. 20, 395−403.

Khorsand, A., Eslaminejad, M.B., Arabsolghar, M., Paknejad, M., Ghaedi, B., Rokn, A.R., Moslemi, N., Nazarian, H., Jahangir, S., 2013. Autologous dental pulp stem cells in regeneration of defect created in canine periodontal tissue. J. Oral Implantol. 39, 433−443.

Kidwai, F.K., Movahednia, M.M., Iqbal, K., Jokhun, D.S., Cao, T., Fawzy, A.S., 2014. Human embryonic stem cell differentiation into odontoblastic lineage: an in vitro study. Int. Endod. J. 47, 346−355.

Kim, J.J., Bae, W.J., Kim, J.M., Kim, J.J., Lee, E.J., Kim, H.W., Kim, E.C., 2014. Mineralized polycaprolactone nanofibrous matrix for odontogenesis of human dental pulp cells. J. Biomater. Appl. 28, 1069−1078.

Kim, S.G., Zhou, J., Solomon, C., Zheng, Y., Suzuki, T., Chen, M., Song, S., Jiang, N., Cho, S., Mao, J.J., 2012. Effects of growth factors on dental stem/progenitor cells. Dent. Clin. North Am. 56, 563−575.

Klimanskaya, I., Hipp, J., Rezai, K.A., West, M., Atala, A., Lanza, R., 2004. Derivation and comparative assessment of retinal pigment epithelium from human embryonic stem cells using transcriptomics. Cloning Stem Cells. 6, 217−245.

Kriks, S., Shim, J.W., Piao, J., Ganat, Y.M., Wakeman, D.R., Xie, Z., Carrillo-Reid, L., 2011. Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. Nature. 480, 547−551.

L.Mescher, A., 2013. Junqueira's Basic Histology: Text and Atlas. thirteenth ed. McGraw-Hill Education, USA, p. 559.

Laflamme, M.A., Chen, K.Y., Naumova, A.V., Muskheli, V., Fugate, J.A., Dupras, S.K., Reinecke, H., 2007. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nat. Biotechnol. 25, 1015−1024.

Lavon, N., Yanuka, O., Benvenisty, N., 2004. Differentiation and isolation of hepatic-like cells from human embryonic stem cells. Differentiation. 72, 230−238.

Ledran, M.H., Krassowska, A., Armstrong, L., Dimmick, I., Renstrom, J., Lang, R., Yung, S., 2008. Efficient hematopoietic differentiation of human embryonic stem cells on stromal cells derived from hematopoietic niches. Cell Stem Cell. 3, 85−98.

Lee, F., Kurisawa, M., 2013. Formation and stability of interpenetrating polymer network hydrogels consisting of fibrin and hyaluronic acid for tissue engineering. Acta Biomater. 9, 5143−5152.

Lee, J.H., Lee, D.S., Nam, H., Lee, G., Seo, B.M., Cho, Y.S., Bae, H.S., Park, J.C., 2012. Dental follicle cells and cementoblasts induce apoptosis of ameloblast-lineage and Hertwig's epithelial root sheath/epithelial rests of Malassez cells through the Fas-Fas ligand pathway. Eur. J. Oral Sci. 120, 29−37.

Li, F., Liu, X., Zhao, S., Wu, H., Xu, H.H., 2014. Porous chitosan bilayer membrane containing TGF-beta1 loaded microspheres for pulp capping and reparative dentin formation in a dog model. Dent. Mater. 30, 172−181.

Li, T.X., Yuan, J., Chen, Y., Pan, L.J., Song, C., Bi, L.J., Jiao, X.H., 2013. Differentiation of mesenchymal stem cells from human umbilical cord tissue into odontoblast-like cells using the conditioned medium of tooth germ cells in vitro. Biomed. Res. Int. 2013, 218543.

Li, W., Zhou, H., Abujarour, R., Zhu, S., Young Joo, J., Lin, T., Hao, E., 2009. Generation of human-induced pluripotent stem cells in the absence of exogenous Sox2. Stem Cells. 27, 2992−3000.

Li, Y., Lu, X., Sun, X., Bai, S., Li, S., Shi, J., 2011. Odontoblast-like cell differentiation and dentin formation induced with TGF-beta1. Arch. Oral Biol. 56, 1221−1229.

Lin, Z., Sugai, J.V., Jin, Q., Chandler, L.A., Giannobile, W.V., 2008. Platelet-derived growth factor-B gene delivery sustains gingival fibroblast signal transduction. J. Periodontal. Res. 43, 440−449.

Lluch, A.V., Fernandez, A.C., Ferrer, G.G., Pradas, M.M., 2009. Bioactive scaffolds mimicking natural dentin structure. J. Biomed. Mater. Res. B: Appl. Biomater. 90, 182−194.

Loh, Y.H., Agarwal, S., Park, I.H., Urbach, A., Huo, H., Heffner, G.C., Kim, K., Miller, J.D., Ng, K., Daley, G.Q., 2009. Generation of induced pluripotent stem cells from human blood. Blood. 113, 5476−5479.

Lumsden, A.G., 1988. Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. Development. 103 (Suppl), 155−169.

Lund, R.D., Wang, S., Klimanskaya, I., Holmes, T., Ramos-Kelsey, R., Lu, B., Girman, S., 2006. Human embryonic stem cell-derived cells rescue visual function in dystrophic RCS rats. Cloning Stem Cells. 8, 189−199.

Lv, T., Wu, Y., Mu, C., Liu, G., Yan, M., Xu, X., Wu, H., Du, J., Yu, J., Mu, J., 2016. Insulin-like growth factor 1 promotes the proliferation and committed differentiation of human dental pulp stem cells through MAPK pathways. Arch. Oral Biol. 72, 116−123.

Lynch, S.E., Wisner-Lynch, L., Nevins, M., Nevins, M.L., 2006. A new era in periodontal and periimplant regeneration: use of growth-factor enhanced matrices incorporating rhPDGF. Compend. Contin. Educ. Dent. 27, 672−678, quiz 679-80.

Malhotra, N., 2016. Induced pluripotent stem (iPS) cells in dentistry: a review. Int. J. Stem Cells. 9 (2), 176−185.

Martin, J., Helm, K., Ruegg, P., Varella-Garcia, M., Burnham, E., Majka, S., 2008. Adult lung side population cells have mesenchymal stem cell potential. Cytotherapy. 10, 140−151.

McGuire, M.K., Kao, R.T., Nevins, M., Lynch, S.E., 2006. rhPDGF-BB promotes healing of periodontal defects: 24-month clinical and radiographic observations. Int. J. Periodontics Restorative Dent. 26, 223−231.

Miura, M., Gronthos, S., Zhao, M., Lu, B., Fisher, L.W., Robey, P.G., Shi, S., 2003. SHED: stem cells from human exfoliated deciduous teeth. Proc. Natl. Acad. Sci. USA. 100, 5807−5812.

Mooney, D.J., Powell, C., Piana, J., Rutherford, B., 1996. Engineering dental pulp-like tissue in vitro. Biotechnol. Prog. 12, 865−868.

Morsczeck, C., Gotz, W., Schierholz, J., Zeilhofer, F., Kuhn, U., Mohl, C., Sippel, C., Hoffmann, K.H., 2005. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol. 24, 155−165.

Moshaverinia, A., Chen, C., Akiyama, K., Ansari, S., Xu, X., Chee, W.W., Schricker, S.R., 2012. Alginate hydrogel as a promising scaffold for dental-derived stem cells: an in vitro study. J. Mater. Sci. Mater. Med. 23, 3041−3051.

Nakao, K., Morita, R., Saji, Y., Ishida, K., Tomita, Y., Ogawa, M., Saitoh, M., Tomooka, Y., Tsuji, T., 2007. The development of a bioengineered organ germ method. Nat. Methods. 4, 227−230.

Nakashima, M., 2005. Bone morphogenetic proteins in dentin regeneration for potential use in endodontic therapy. Cytokine Growth Factor Rev. 16, 369−376.

Nanci, A., 2013. Ten Cate's Oral Histology, Development, Structure, and Function. Elsevier, Amsterdam, The Netherlands.

Nevins, M., Camelo, M., Nevins, M.L., Schenk, R.K., Lynch, S.E., 2003. Periodontal regeneration in humans using recombinant human platelet-derived growth factor-BB (rhPDGF-BB) and allogenic bone. J. Periodontol. 74, 1282−1292.

Nevins, M., Giannobile, W.V., Mcguire, M.K., Kao, R.T., Mellonig, J.T., Hinrichs, J.E., Mcallister, B.S., 2005. Platelet-derived growth factor stimulates bone fill and rate of attachment level gain: results of a large multicenter randomized controlled trial. J. Periodontol. 76, 2205–2215.

Nguyen, T.T., Mui, B., Mehrabzadeh, M., Chea, Y., Chaudhry, Z., Chaudhry, K., Tran, S.D., 2013. Regeneration of tissues of the oral complex: current clinical trends and research advances. J. Can. Dent. Assoc. 79, d1.

Nichol, J.W., Khademhosseini, A., 2009. Modular tissue engineering: engineering biological tissues from the bottom up. Soft Matter. 5, 1312–1319.

Ning, F., Guo, Y., Tang, J., Zhou, J., Zhang, H., Lu, W., Gao, Y., Wang, L., Pei, D., Duan, Y., Jin, Y., 2010. Differentiation of mouse embryonic stem cells into dental epithelial-like cells induced by ameloblasts serum-free conditioned medium. Biochem. Biophys. Res. Commun. 394, 342–347.

O'Brien, F.J., 2011. Biomaterials & scaffolds for tissue engineering. Mater. Today. 14, 88–95.

Ohara, T., Itaya, T., Usami, K., Ando, Y., Sakurai, H., Honda, M.J., Ueda, M., Kagami, H., 2010. Evaluation of scaffold materials for tooth tissue engineering. J. Biomed. Mater Res. A. 94, 800–805.

Ohazama, A., Modino, S.A., Miletich, I., Sharpe, P.T., 2004. Stem-cell-based tissue engineering of murine teeth. J. Dent. Res. 83, 518–522.

Oki, K., Tatarishvili, J., Wood, J., Koch, P., Wattananit, S., Mine, Y., Monni, E., Tornero, D., 2012. Human-induced pluripotent stem cells form functional neurons and improve recovery after grafting in stroke-damaged brain. Stem Cells. 30, 1120–1133.

Onishi, T., Kinoshita, S., Shintani, S., Sobue, S., Ooshima, T., 1999. Stimulation of proliferation and differentiation of dog dental pulp cells in serum-free culture medium by insulin-like growth factor. Arch. Oral Biol. 44, 361–371.

Oshima, M., Inoue, K., Nakajima, K., Tachikawa, T., Yamazaki, H., Isobe, T., Sugawara, A., Ogawa, M., Tanaka, C., Saito, M., Kasugai, S., Takano-Yamamoto, T., Inoue, T., Tezuka, K., Kuboki, T., Yamaguchi, A., Tsuji, T., 2014. Functional tooth restoration by next-generation bio-hybrid implant as a bio-hybrid artificial organ replacement therapy. Sci. Rep. 4, 6044.

Otsu, K., Kishigami, R., Oikawa-Sasaki, A., Fukumoto, S., Yamada, A., Fujiwara, N., Ishizeki, K., Harada, H., 2012. Differentiation of induced pluripotent stem cells into dental mesenchymal cells. Stem Cells Dev. 21, 1156–1164.

Ott, H.C., Matthiesen, T.S., Goh, S.K., Black, L.D., Kren, S.M., Netoff, T.I., Taylor, D.A., 2008. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat. Med. 14, 213–221.

Ozeki, N., Hase, N., Higuchi, N., Hiyama, T., Yamaguchi, H., Kawai, R., Matsumoto, T., Nakata, K., Mogi, M., 2016. Gelatin scaffold combined with bone morphogenetic protein-4 induces odontoblast-like cell differentiation involving integrin profile changes, autophagy-related gene 10, and Wnt5 sequentially in human induced pluripotent stem cells. Differentiation. 93, 1–14.

Ozeki, N., Mogi, M., Kawai, R., Yamaguchi, H., Hiyama, T., Nakata, K., Nakamura, H., 2013. Mouse-induced pluripotent stem cells differentiate into odontoblast-like cells with induction of altered adhesive and migratory phenotype of integrin. PLoS One. 8, e80026.

Ozeki, N., Mogi, M., Yamaguchi, H., Hiyama, T., Kawai, R., Hase, N., Nakata, K., Nakamura, H., Kramer, R.H., 2014. Differentiation of human skeletal muscle stem cells into odontoblasts is dependent on induction of alpha1 integrin expression. J. Biol. Chem. 289, 14380–14391.

Padial-Molina, M., O'valle, F., Lanis, A., Mesa, F., Dohan Ehrenfest, D.M., Wang, H.L., Galindo-Moreno, P., 2015. Clinical application of mesenchymal stem cells and novel supportive therapies for oral bone regeneration. Biomed. Res. Int. 2015, 341327.

Park, I.H., Zhao, R., West, J.A., Yabuuchi, A., Huo, H., Ince, T.A., Lerou, P.H., 2008. Reprogramming of human somatic cells to pluripotency with defined factors. Nature. 451, 141−146.

Phinney, D.G., Prockop, D.J., 2007. Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair—current views. Stem Cells. 25, 2896−2902.

Pihlstrom, B.L., Michalowicz, B.S., Johnson, N.W., 2005. Periodontal diseases. Lancet. 366, 1809−1820.

Prescott, R.S., Alsanea, R., Fayad, M.I., Johnson, B.R., Wenckus, C.S., Hao, J., John, A.S., 2008. In vivo generation of dental pulp-like tissue by using dental pulp stem cells, a collagen scaffold, and dentin matrix protein 1 after subcutaneous transplantation in mice. J. Endod. 34, 421−426.

Ravindran, S., Song, Y., George, A., 2010. Development of three-dimensional biomimetic scaffold to study epithelial-mesenchymal interactions. Tissue Eng. A. 16, 327−342.

Reigstad, L.J., Varhaug, J.E., Lillehaug, J.R., 2005. Structural and functional specificities of PDGF-C and PDGF-D, the novel members of the platelet-derived growth factors family. FEBS J. 272, 5723−5741.

Ripamonti, U., Herbst, N.N., Ramoshebi, L.N., 2005. Bone morphogenetic proteins in craniofacial and periodontal tissue engineering: experimental studies in the non-human primate Papio ursinus. Cytokine Growth Factor Rev. 16, 357−368.

Rosa, V., Zhang, Z., Grande, R.H., Nor, J.E., 2013. Dental pulp tissue engineering in full-length human root canals. J. Dent. Res. 92, 970−975.

Ruangsawasdi, N., Zehnder, M., Weber, F.E., 2014. Fibrin gel improves tissue ingrowth and cell differentiation in human immature premolars implanted in rats. J. Endod. 40, 246−250.

Rutherford, R.B., Wahle, J., Tucker, M., Rueger, D., Charette, M., 1993. Induction of reparative dentine formation in monkeys by recombinant human osteogenic protein-1. Arch. Oral Biol. 38, 571−576.

Schilke, R., Lisson, J.A., Bauß, O., Geurtsen, W., 2000. Comparison of the number and diameter of dentinal tubules in human and bovine dentine by scanning electron microscopic investigation. Arch. Oral Biol. 45, 355−361.

Sebald, W., Nickel, J., Zhang, J.L., Mueller, T.D., 2004. Molecular recognition in bone morphogenetic protein (BMP)/receptor interaction. Biol. Chem. 385, 697−710.

Seki, D., Takeshita, N., Oyanagi, T., Sasaki, S., Takano, I., Hasegawa, M., Takano-Yamamoto, T., 2015. Differentiation of odontoblast-like cells from mouse induced pluripotent stem cells by Pax9 and Bmp4 transfection. Stem Cells Transl. Med. 4, 993−997.

Seo, B.M., Miura, M., Gronthos, S., Bartold, P.M., Batouli, S., Brahim, J., Young, M., 2004. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet. 364, 149−155.

Sharma, S., Srivastava, D., Grover, S., Sharma, V., 2014. Biomaterials in tooth tissue engineering: a review. J. Clin. Diagn. Res. 8, 309−315.

Shinmura, Y., Tsuchiya, S., Hata, K., Honda, M.J., 2008. Quiescent epithelial cell rests of Malassez can differentiate into ameloblast-like cells. J. Cell Physiol. 217, 728−738.

Simmer, J.P., Papagerakis, P., Smith, C.E., Fisher, D.C., Rountrey, A.N., Zheng, L., Hu, J.C., 2010. Regulation of dental enamel shape and hardness. J. Dent. Res. 89, 1024−1038.

Sloan, A.J., Matthews, J.B., Smith, A.J., 1999. TGF-beta receptor expression in human odontoblasts and pulpal cells. Histochem. J. 31, 565−569.

Sloan, A.J., Perry, H., Matthews, J.B., Smith, A.J., 2000. Transforming growth factor-beta isoform expression in mature human healthy and carious molar teeth. Histochem. J. 32, 247−252.

Soares, D.G., Rosseto, H.L., Basso, F.G., Scheffel, D.S., Hebling, J., Costa, C.A., 2016. Chitosan-collagen biomembrane embedded with calcium-aluminate enhances dentinogenic potential of pulp cells. Braz. Oral Res. 30, e54.

Sumita, Y., Honda, M.J., Ohara, T., Tsuchiya, S., Sagara, H., Kagami, H., Ueda, M., 2006. Performance of collagen sponge as a 3-D scaffold for tooth-tissue engineering. Biomaterials. 27, 3238−3248.

Takahashi, K., Yamanaka, S., 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 126, 663−676.

Tapias, L.F., Ott, H.C., 2014. Decellularized scaffolds as a platform for bioengineered organs. Curr. Opin. Organ Transplant. 19, 145−152.

Tatullo, M., Falisi, G., Amantea, M., Rastelli, C., Paduano, F., Marrelli, M., 2015. Dental pulp stem cells and human periapical cyst mesenchymal stem cells in bone tissue regeneration: comparison of basal and osteogenic differentiated gene expression of a newly discovered mesenchymal stem cell lineage. J. Biol. Regul. Homeost. Agents. 29, 713−718.

Thesleff, I., 2003. Epithelial-mesenchymal signalling regulating tooth morphogenesis. J. Cell Sci. 116, 1647−1648.

Tian, Y., Bai, D., Guo, W., Li, J., Zeng, J., Yang, L., Jiang, Z., Feng, L., Yu, M., Tian, W., 2015. Comparison of human dental follicle cells and human periodontal ligament cells for dentin tissue regeneration. Regen. Med. 10, 461−479.

Toriumi, T., Takayama, N., Murakami, M., Sato, M., Yuguchi, M., Yamazaki, Y., Eto, K., 2015. Characterization of mesenchymal progenitor cells in the crown and root pulp of primary teeth. Biomed. Res. 36, 31−45.

Traphagen, S.B., Fourligas, N., Xylas, J.F., Sengupta, S., Kaplan, D.L., Georgakoudi, I., Yelick, P.C., 2012. Characterization of natural, decellularized and reseeded porcine tooth bud matrices. Biomaterials. 33, 5287−5296.

Uygun, B.E., Soto-Gutierrez, A., Yagi, H., Izamis, M.L., Guzzardi, M.A., Shulman, C., Milwid, J., Kobayashi, N., Tilles, A., Berthiaume, F., Hertl, M., Nahmias, Y., Yarmush, M.L., Uygun, K., 2010. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. Nat. Med. 16, 814−820.

Vaahtokari, A., Aberg, T., Jernvall, J., Keranen, S., Thesleff, I., 1996. The enamel knot as a signaling center in the developing mouse tooth. Mech. Dev. 54, 39−43.

Valles-Lluch, A., Novella-Maestre, E., Sancho-Tello, M., Pradas, M.M., Ferrer, G.G., Batalla, C.C., 2010. Mimicking natural dentin using bioactive nanohybrid scaffolds for dentinal tissue engineering. Tissue Eng. A. 16, 2783−2793.

van der Kooy, D., Weiss, S., 2000. Why stem cells? Science. 287, 1439−1441.

van Manen, E.H., Zhang, W., Walboomers, X.F., Vazquez, B., Yang, F., Ji, W., Yu, N., Spear, D.J., 2014. The influence of electrospun fibre scaffold orientation and nano-hydroxyapatite content on the development of tooth bud stem cells in vitro. Odontology. 102, 14−21.

Wang, B., Li, L., Du, S., Liu, C., Lin, X., Chen, Y., Zhang, Y., 2010. Induction of human keratinocytes into enamel-secreting ameloblasts. Dev. Biol. 344, 795−799.

Wang, S., Mu, J., Fan, Z., Yu, Y., Yan, M., Lei, G., Tang, C., Wang, Z., 2012. Insulin-like growth factor 1 can promote the osteogenic differentiation and osteogenesis of stem cells from apical papilla, Stem Cell Res., 8. pp. 346−356.

Wang, Z.Z., Au, P., Chen, T., Shao, Y., Daheron, L.M., BaI, H., Arzigian, M., 2007. Endothelial cells derived from human embryonic stem cells form durable blood vessels in vivo. Nat. Biotechnol. 25, 317–318.

Wieman, T.J., Smiell, J.M., Su, Y., 1998. Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers. A phase III randomized placebo-controlled double-blind study. Diabetes Care. 21, 822–827.

Wu, G., Deng, Z.H., Fan, X.J., Ma, Z.F., Sun, Y.J., Ma, D.D., Wu, J.J., Shi, J.N., Jin, Y., 2009. Odontogenic potential of mesenchymal cells from hair follicle dermal papilla. Stem Cells Dev. 18, 583–589.

Xu, X., Bringas JR., P., Soriano, P., Chai, Y., 2005. PDGFR-alpha signaling is critical for tooth cusp and palate morphogenesis. Dev. Dyn. 232, 75–84.

Xu, Y., Liu, L., Zhang, L., Fu, S., Hu, Y., Wang, Y., Fu, H., Wu, K., 2012. Efficient commitment to functional CD34+ progenitor cells from human bone marrow mesenchymal stem-cell-derived induced pluripotent stem cells. PLoS One. 7, e34321.

Yadav, P., Tahir, M., Yadav, H., Sureka, R., Garg, A., 2016. Test tube tooth: the next big thing. J. Clin. Diagn Res. 10, ZE01–ZE03.

Yan, X., Qin, H., Qu, C., Tuan, R.S., Shi, S., Huang, G.T., 2010. iPS cells reprogrammed from human mesenchymal-like stem/progenitor cells of dental tissue origin. Stem Cells Dev. 19, 469–480.

Yang, C.C., Cotsarelis, G., 2010. Review of hair follicle dermal cells. J. Dermatol. Sci. 57, 2–11.

Yang, K.C., Wang, C.H., Chang, H.H., Chan, W.P., Chi, C.H., Kuo, T.F., 2012a. Fibrin glue mixed with platelet-rich fibrin as a scaffold seeded with dental bud cells for tooth regeneration. J. Tissue Eng. Regen. Med. 6, 777–785.

Yang, X., Han, G., Pang, X., Fan, M., 2012b. Chitosan/collagen scaffold containing bone morphogenetic protein-7 DNA supports dental pulp stem cell differentiation in vitro and in vivo. J. Biomed. Mater. Res. A.

Yang, X., Yang, F., Walboomers, X.F., Bian, Z., Fan, M., Jansen, J.A., 2010. The performance of dental pulp stem cells on nanofibrous PCL/gelatin/nHA scaffolds, J. Biomed. Mater. Res. A, 93. pp. 247–257.

Yang, X., Zhang, S., Pang, X., Fan, M., 2012c. Mineralized tissue formation by bone morphogenetic protein-7-transfected pulp stem cells, J. Endod., 38. pp. 170–176.

Yelick, P.C., Vacanti, J.P., 2006. Bioengineered teeth from tooth bud cells. Dent. Clin. North Am. 50, 191–203, viii.

Yoshida, K., Sato, J., Takai, R., Uehara, O., Kurashige, Y., Nishimura, M., Chiba, I., Saitoh, M., Abiko, Y., 2015. Differentiation of mouse iPS cells into ameloblast-like cells in cultures using medium conditioned by epithelial cell rests of Malassez and gelatin-coated dishes. Med. Mol. Morphol. 48, 138–145.

Young, C.S., Abukawa, H., Asrican, R., Ravens, M., Troulis, M.J., Kaban, L.B., Vacanti, J. P., Yelick, P.C., 2005a. Tissue-engineered hybrid tooth and bone. Tissue Eng. 11, 1599–1610.

Young, C.S., Kim, S.W., Qin, C., Baba, O., Butler, W.T., Taylor, R.R., Bartlett, J.D., 2005b. Developmental analysis and computer modelling of bioengineered teeth. Arch. Oral Biol. 50, 259–265.

Young, C.S., Terada, S., Vacanti, J.P., Honda, M., Bartlett, J.D., Yelick, P.C., 2002. Tissue engineering of complex tooth structures on biodegradable polymer scaffolds. J. Dent. Res. 81, 695–700.

Zhang, W., Ahluwalia, I.P., Literman, R., Kaplan, D.L., Yelick, P.C., 2011. Human dental pulp progenitor cell behavior on aqueous and hexafluoroisopropanol based silk scaffolds. J. Biomed. Mater. Res. A. 97, 414–422.

Zhang, W., Vazquez, B., Yelick, P.C., 2014. Bioengineered post-natal recombinant tooth bud models. J. Tissue Eng. Regen. Med. Available from: http://dx.doi.org/10.1002/term.1962.

Zhang, Y., Wang, Y., Shi, B., Cheng, X., 2007. A platelet-derived growth factor releasing chitosan/coral composite scaffold for periodontal tissue engineering. Biomaterials. 28, 1515–1522.

Zhang, Y.D., Chen, Z., Song, Y.Q., Liu, C., Chen, Y.P., 2005. Making a tooth: growth factors, transcription factors, and stem cells. Cell Res. 15, 301–316.

Zhao, M., Jin, Q., Berry, J.E., Nociti, F.H., Giannobile, W.V., Somerman, M.J., 2004. Cementoblast delivery for periodontal tissue engineering. J. Periodontol. 75, 154–161.

Zomorodian, E., Baghaban Eslaminejad, M., 2012. Mesenchymal stem cells as a potent cell source for bone regeneration. Stem Cells Int. 2012, 980353.

Zuk, P.A., Zhu, M., Ashjian, P., De Ugarte, D.A., Huang, J.I., Mizuno, H., Alfonso, Z.C., 2002. Human adipose tissue is a source of multipotent stem cells. Mol. Biol. Cell. 13, 4279–4295.

This page intentionally left blank

Pharmacological agents for bone remodeling: An experimental approach

28

Massoud Seifi[1,2], *Parisa Amdjadi*[1,2] *and Lobat Tayebi*[1]
[1]Marquette University School of Dentistry, Milwaukee, WI, United States, [2]Shahid Beheshti University of Medical Sciences, Tehran, Iran

28.1 Introduction

Bone formation or osteogenesis involves two modes of ossifications, i.e., intramembranous, that is direct bone formation from mesenchymal tissue and endochondral, which starts by conversion of mesenchymal tissue to cartilage template and continues until replacement by bone cells. Intramembranous bone formation in which osteoprogenitor cells intermediate into osteoblasts has an important role in growth and development of craniofacial skeleton and healing of the fractured bones. The results of both modes of osteogenesis are biochemically and histologically identical. Bone tissue is supplied with blood vessels and has the ability to turnover its constituents, i.e., remodeling process.

Bone remodeling is a crucial process during life from infancy to adolescence, and from adulthood to senility. It is also important from different perspectives, e.g., athletic activities, astronauts, or cosmonauts stay in space, postmenopausal osteoporosis and even eating during the day or nocturnal fasting. In all of the abovementioned periods or situations, bone remodeling takes place and any interruption in its normal condition will lead to a skewness toward inequality between bone formation and bone resorption. Many pharmacological agents can change the balance or skewed curve of apposition/resorption toward targeted intention of the drug formulation. However, the whole process of bone remodeling governed by the osteoblastic (bone apposition) and osteoclastic (bone resorption) activities during life should not be stopped.

The dynamic tissue of bone as a reservoir for calcium and a metabolic organ is remodeled constantly to maintain its vitality and health. The aforementioned phenomena are under the influence of growth factors, hormones, and in general; pharmacological agents affecting bone turnover. In this chapter the effects of different drugs that have been administered to living subjects and can influence the bone development and remodeling are discussed. These include calcium, vitamin D, prostaglandin (PG) E, thyroid hormone, pamidronate, zoledronate, aspirin, acetaminophen, ibuprofen, and fibroblast growth factor as well gonadal hormones with an experimental perspective towards bone remodeling by dentoalveolar changes that are adapted from author's own experiences.

Biomaterials for Oral and Dental Tissue Engineering. DOI: http://dx.doi.org/10.1016/B978-0-08-100961-1.00028-1
© 2017 Elsevier Ltd. All rights reserved.

28.2 Calcium

28.2.1 Overview

Calcium has a pivotal role in dentoalveolar turnover and remodeling. Alveolar bone changes are under the influence of calcium ion levels in extracellular matrix or bone environment (Seifi et al., 2003). It is an essential body electrolyte and has important roles in many vital functions in the human body. A dynamic equilibration exists between the calcium in the blood and the bones, which is called "Homeostasis." Concentration of calcium in the extracellular fluid is regulated by homeostasis phenomenon and calcium ion has an stabilizing effect on the voltage-gated ion channels, i.e., transmembrane proteins that are activated by changes in electrical membrane potential near the channels. The radius of Ca^{2+} ion is just right to fit into the folds of many proteins, peptide chains and their side groups. It stabilizes critical tertiary structure of both catalytic and structural proteins (Carafoli and Penniston, 1985).

28.2.2 Calcium homeostasis

Parathyroid hormone (PTH) and 1,25$(OH)_2$ cholecalciferol (the active form of vitamin D) are the most important hormones in controlling calcium homeostasis. PTH is part of negative feedback loop with calcium level. In hypocalcemia condition, PTH stimulates bone resorption, calcium ion reabsorption in kidneys, and synthesis of active vitamin D in kidneys. By stimulating the production of 1,25 $(OH)_2$ vitamin D in the kidney, PTH (indirectly) increases calcium ion absorption by the effect of active vitamin D on the small intestine.

Calcium intake has an important effect on the bone mass as demonstrated by manipulating calcium intake in experimental animals. When cats, dogs, and rats were put on low-calcium diet, they showed severe bone loss and after nutrient being restored, recovery was noted (Bodansky and Duff, 1939). These studies emphasize the important role of bones as reservoir of calcium for maintaining the homeostasis. The prerequisite for the above experiment's condition is normal function of parathyroid gland. If the function of the PTH secretion is impaired, the bone loss will be prevented at the expense of severe "Hypocalcemia."

28.2.3 Calcium absorption and bone density

Chronic dietary calcium scarcity is among diseases of civilization in human societies. Randomized controlled clinical trials have shown that calcium intake enhances bone acquisition during growth, diminishes bone loss in postmenopausal women, and reduces fracture rates at spine, hip, and other extremity sites (Heaney, 2000). The extremities and hip fractures have increased exponentially by continual increase in life expectancy. Vitamin D insufficiency and deficit in calcium intake are very common in elderly people (Meunier, 1996). The cumulative response to the deficit in calcium intake and low vitamin D status is a negative calcium balance

which stimulates PTH secretion. The senile secondary hyperparathyroidism is one of the determinants of femoral bone loss and can be reversed by calcium and vitamin D supplements (Meunier et al., 1994). Hypovitaminosis D and a low-calcium intake contribute to increased parathyroid function in elderly persons. Calcium and vitamin D supplements reduce secondary hyperparathyroidism. The effect of tricalcium phosphate, containing 1.2 g of elemental calcium; and vitamin D3 supplements have been studied on the risk of hip and other nonvertebral fractures among elderly women. The number of hip fractures was 43% lower and the total number of nonvertebral fractures was 32% lower than the control group. The mean serum PTH concentration had decreased by 44% from the baseline value; the bone density of the proximal femur increased 2.7% in the vitamin D3—calcium group and decreased 4.6% in the placebo group ($P < 0.001$) (Chapuy et al., 1992). The vitamin D individual patient data analysis indicated that vitamin D given alone in doses of 10−20 μg was not effective in preventing fractures. By contrast, calcium and vitamin D given together reduce hip fractures and total fractures, and probably vertebral fractures, irrespective of age, sex, or previous fractures (Abrahamsen et al., 2010).

In a study of a population of elderly Japanese men with relatively low dietary calcium intake several bone-related parameters were evaluated. Areal bone mineral density (aBMD) at the lumbar spine (LS), total hip, and femoral neck were measured by dual-energy X-ray absorptiometry (DXA). Trabecular bone score (TBS) was assessed using DXA images at LS, and biochemical markers of bone turnover in serum were evaluated. Greater milk intake was associated with lower bone turnover, higher bone density, and higher bone microarchitecture index, i.e., TBS (Sato et al., 2015).

The elderly is more sensitive to calcium deprivation and more responsive to its supplementation. A gradient trend exists in age-specific fracture risk (increasing steepness with age) for bone mineral density of midshaft radius bone or forearm. For a determined amount of BMD, risk is raised with incremental stages of the age. A relatively small difference in BMD, produces a higher fracture rate in the elderly than in younger individuals. In other words, the age effect is larger than the bone density. A determined amount of increase in BMD has greater reduction in fracture risk in older patients (considering the steepness of the curve). Bone mass is a useful predictor of fractures but other age-related factors associated with fractures need to be identified (Hui et al., 1988).

The Food and Nutrition Board of the National Research Council (United States) produce guidelines for calcium, mineral, and vitamin daily requirements for a normal person engaged in average activities. Recommended daily (dietary) allowance (RDA) was established by the US Food and Nutrition Board in 1941 when sufficient nutritional diet was not common. RDAs are increased for increased activity, body growth and size, pregnancy, lactation, and adverse environmental conditions. Malnutrition is now rare in the United States, as many foods are supplemented with the necessary vitamins and minerals; many nutritionists now prefer the daily value standard.

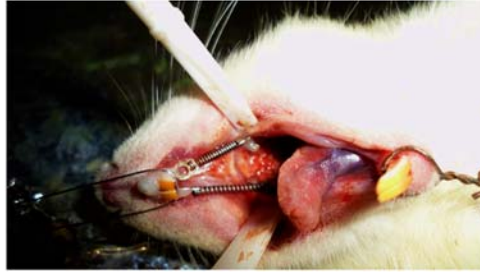


Figure 28.1 Illustrates the installation of NiTi coil spring for molar protraction.

28.2.4 Calcium in the experimental bone remodeling

The authors evaluated the role of a calcium compound, i.e., calcium gluconate on alveolar bone remodeling through orthodontic tooth movement (OTM) (Seifi et al., 2003). It was initially speculated that by maintenance of calcium balance, body itself acts against the injection of calcium gluconate and any effort to unbalance the calcium ions levels in the body in general and in the bone environment in specific; is ineffective. On the contrary the authors were convinced on the basis of their theoretical knowledge and clinical experiences that importing the administered amount of the calcium can change the balance level for some time. According to the aforementioned issues a change in the level of calcium ions can be reflected on their role in the process of the bone remodeling (Feng and McDonald, 2011). Metabolic bone disturbances like calcium deficient diets may influence the quality of bone, in the experimental bone remodeling, and in response to the mechanical demand of orthodontic forces (Kiliaridis et al., 1996).

Authors conducted an experimental tooth movement on rats and studied the effect of calcium on the OTM and root resorption. OTM was decreased by intraperitoneal injection of calcium gluconate (0.40 ± 0.1 mm) relative to the group that received only PG (0.47 ± 0.3 mm) as an arachidonic acid metabolite and primary messenger of OTM (Seifi et al., 2003) (Fig. 28.1).

Another important finding of the above-mentioned research was the stabilizing role of the calcium administration on the root resorption (Fig. 28.2). According to the data and histological findings, root resorption was diminished to a level that was not significant relative to the control group after injection of calcium gluconate. However, the amount of the OTM (0.40 ± 0.1 mm) remained significant relative to the control group (0.22 ± 0.1 mm) (Seifi et al., 2003).

28.3 Vitamin D

28.3.1 Overview

Cholecalciferol of skin is hydroxylated in the 25 position in the liver and in the 1 position in the kidney. Biologically active form of vitamin D is 1,25-dihydroxy

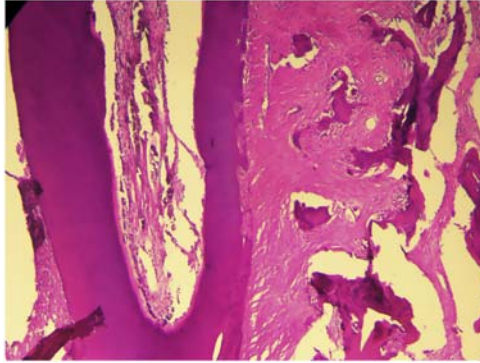


Figure 28.2 Illustrates the existence of orthodontic-induced inflammatory root resorption.

cholecalciferol, a steroid hormone that has a role in calcium homeostasis. It is a potent stimulator of bone resorptive activity by inducing differentiation of osteoclasts from their precursors. Vitamin D as a secosteroid hormone increases calcitonin receptors on multinucleated giant cells in bone marrow cultures but not in mature osteoclasts in vivo which shows the potential for activation of precursor cells and not the mature osteoclasts. Vitamin D increases the absorption of calcium ions from intestine that increases plasma calcium ions, and consequently will help osteogenesis. Vitamin D receptors exist on osteoclasts, osteoblast-like cells, osteoprogenitor cells, and bone lining cells showing their important role in bone production (Reichel et al., 1989).

28.3.2 Pharmacological properties

Vitamin D facilitates the absorption of calcium ions and phosphate from small intestine to maintain their normal concentrations in plasma. It also interacts with PTH to enhance calcium ions and phosphate mobilization from bone and to decrease their excretion from the kidney. Normal bone formation occurs when calcium ions and phosphate concentrations in the plasma are adequate. It is now clear that vitamin D has both direct and indirect effects on the cells that are involved in bone remodeling (Marcus, 1996).

The osteoblasts contain calcitriol receptors, and calcitriol stimulates the release of several proteins including osteocalcin that promotes bone resorption (Spear et al., 1988). Calcitriol promotes the recruitment of osteoclast precursor cells to resorption sites as well as the development of differentiated functions that characterize mature osteoclasts. Osteoclasts are polykaryons and the principal resorptive cells of bone. They are members of the monocyte/macrophage family whose precursors differentiate under the influence of 1,25-dihydroxyvitamin D3 (Mimura et al., 1994).

28.3.3 Clinical applications of vitamin D compounds

Compounds of the vitamin D have clinical usefulness in several conditions such as diabetes, rickets, osteoporosis, psoriasis, and immunosuppression.

Vitamin D may contribute to maintaining insulin secretion because calcium ions are necessary for insulin secretion. Over the past decades, data linking vitamin D deficiency to Type 2 Diabetes Mellitus (T2DM) and metabolic disorders has accumulated (Boucher, 2011).

Rickets and oseomalacia have nutritional basis and in 1940s, vitamin D_2 became the treatment of choice because of its cheaper value. Vitamin D supplementation and food fortification (milk, margarin, and bread) is necessary by law, a reason that rickets is uncommon in developed countries. Vitamin D promotes calcium absorption and utilization, preventing and curing rickets and osteomalacia (Boucher, 2011).

Etiology of the osteoporosis is complex and multifactorial presumably. There have been consistent claims that levels of 1α,25-$(OH)_2D_3$ are low in osteoporosis (Riggs and Melton, 1992). Vitamin D (1α,25-$(OH)_2D_3$) treatment can be expected to exacerbate the excessive bone resorption component of osteoporosis but this hormone also raises the calcium levels of the plasma and stimulates synthesis of bone matrix formation in osteoblasts (Hock et al., 1986).

Of great interest is that 1,25(OH)2D3 is a potent antiproliferative and prodifferentiation mediator and it can offer some relief in psoriasis and cancers of the breast, prostate, and colon (Holick, 1995). Most immunosuppressants have a narrow margin between efficacy and side effects. The active form of vitamin D, 1,25(OH)2D3, is an immunomodulator that interacts with T cells but mainly targets antigen-presenting cells and synergism is observed between vitamin D and cyclosporine, rapamycin, and FK506. This synergism could be observed with other immunosuppressants (mycophenolate mofetil, leflunomide, and the methylxanthine). Vitamin D and its analogs are potent dose-reducing drugs for other immunomodulators, making them potentially interesting for clinical use in autoimmunity and transplantation (van Etten et al., 2000; Bouillon et al., 1995).

28.3.4 Specific characteristics

The physiologically active form of vitamin D, 1,25-(OH)2D3,4 can initiate differentiation of myeloid cells into monocyte-macrophage-like cells and can increase monocyte characteristics in the human monocytic cell line. In addition, induction of monocyte-like characteristics in HL-60 cells with pure and physiologically relevant substances may provide an ideal system for identifying biochemical events underlying this maturational process (Spear et al., 1988).

Vitamin D has an antiinflammatory role in various bacterial infections. It can enhance the bactericidal activity of macrophages against *Mycobacterium tuberculosis*, causative microorganism of tuberculosis that shows its role in the immune response to bacterial pathogens. 25-Hydroxyvitamin D (25OHD) in circulation is bound to vitamin D binding protein and is released to the monocyte/macrophage and is then

converted to 1,25(OH)2D3 in the mitochondria. A link exists between vitamin D-triggered antimicrobial activity in monocytes/macrophages and cathelicidins (antimicrobial peptides), a family of polypeptides found in lysosomes of macrophages, polymorphonuclear leukocytes, and keratinocytes (Di Rosa et al., 2011).

28.3.5 Vitamin D in the experimental bone remodeling

Vitamin D is actually a hormone rather than a vitamin because it is produced in the body, transported in the blood to the distant sites, and like other hormones affects multiple tissues. The effect of vitamin D as a hormone and bone metabolite on bone remodeling have been studied by assessing tooth movement through the maxillary bone and root resorption (Seifi et al., 2013). The orthodontic appliance was comprised of a 5 mm long NiTi closed-coil spring connected posteriorly to the right first molar and anteriorly to the upper right incisor by a ligature wire and a force of 60 g was applied. Composite bonding material was used to fix the ligature wires to the teeth. OTM was measured with a feeler gauge with an accuracy of 0.01 mm (Fig. 28.3).

Results showed that experimental group (vitamin D) had significant increase in OTM 0.4 ± 0.13 (mm) relative to the control group 0.2 ± 0.06 (mm) ($P < 0.05$) (Seifi et al., 2013).

It has been shown that by increasing tooth movement, root resorption is also increased (Gonzales et al., 2008). In the aforementioned study, vitamin D increased the OTM significantly. Root resorption was almost stabilized and even diminished slightly but not significantly. Root resorption in the control group was $2.4 \times 10^{-3} \pm 1.3 \times 10^{-3}$ (mm^2) and in vitamin D group was $2 \times 10^{-3} \pm 1.3 \times 10^{-3}$ (mm^2) and despite of the increased tooth movement, inflammatory process of root resorption was controlled (Seifi et al., 2013).

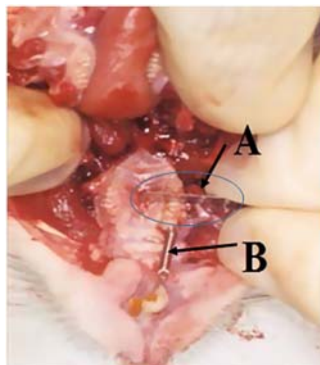


Figure 28.3 (A) Feeler gauge shows the amount of tooth movement. (B) First molar has been protracted mesially by NiTi closed coil spring.

Authors believe that control of the inflammatory phenomenon of the root resorption can be a consequence of the administration of the vitamin D and its role in reducing the inflammatory response (Di Rosa et al., 2011).

28.4 Prostaglandins

28.4.1 Overview

Eicosanoids are oxygenated 20-carbon fatty acids from either omega-3 (ω-3) or omega-6 (ω-6) group. There are multiple subfamilies of eicosanoids, including the PGs, thromboxanes, and leukotrienes. PGs are derived from arachidonic acid (cleaved from phospholipids in the lipid bilayer of cell membranes) and have important physiologic and pathologic roles in skeletal metabolism. Most cells including osteoblasts in the bone secrete PGs and PGE_2 is the prominent PG in the bone. PGs are associated with stimulation of osteoprogenitor cells to proliferate and differentiate and increased number of osteoclasts. PGs initially inhibit the activity of osteoclasts in tissue culture (Yonaga and Morimoto, 1979; Ibbotson et al., 1984).

28.4.2 Pharmacological properties

PGs have been shown to play an important role in the bone-healing process and, consequently, the decrease in PG levels caused by nonsteroidal antiinflammatory drug (NSAIDs) may impair the healing process. Under experimental conditions, many NSAIDs including the cyclooxygenase-2 (COX-2) inhibitors have been shown to reduce healing. However, clinical evidence of such an effect is rare (James et al., 1993).

The diversity of receptors means that PGs exhibit versatile biological activities. They cause constriction or dilation in vascular smooth muscle cells, bronchoconstriction (receptor EP1), and bronchodilation (receptor EP2). They also cause aggregation or disaggregation of platelets, sensitize spinal neurons to pain, induce uterus contraction, have roles in immune responses, blood pressure, gastrointestinal integrity, and fertility. They decrease intraocular pressure, regulate inflammation, calcium movement, and hormones, control cell growth, and acts on thermoregulatory center of hypothalamus to produce fever. They decrease gastric acid secretion, increase gastric mucus secretion and inhibit lipolysis (Fig. 28.4) (Ricciotti and FitzGerald, 2011; Nelson, 2005; Rang et al., 2014).

28.4.3 Prostaglandins antagonists

NSAIDs inhibit cyclooxygenase and reduce PG synthesis. Corticosteroids inhibit phospholipase A2 production by boosting production of lipocortin, an inhibitor protein. Drugs that affect PG activity fall into two categories; first, corticosteroids

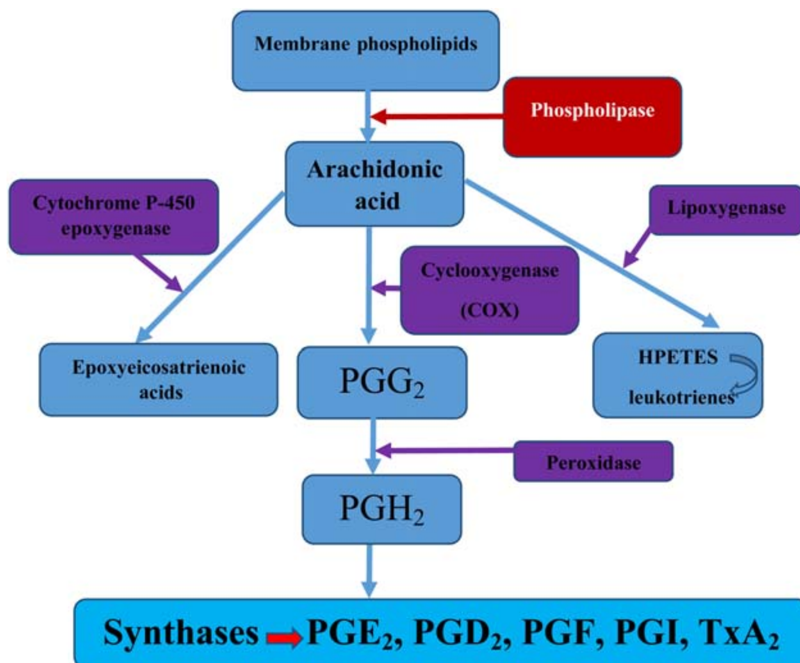


Figure 28.4 Diagram illustrates the three major families of eicosanoids derived from arachidonic acid, i.e. prostanoids (PGs and thromboxane), leukotrienes, and products of the cytochrome P-450-dependent epoxygenase pathway.

and NSAIDs that interfere with PGs synthesis and second; other agents that have mixed agonistic and antagonistic effects on various PGs. In the body, PGs are formed from arachidonic acid, which in turn is derived from phospholipids. Corticosteroids reduce PG synthesis by inhibiting the formation of arachidonic acid, and NSAIDs inhibit the conversion of arachidonic acid to PGs (Ricciotti and FitzGerald, 2011).

Relatively new drugs, known as COX-2 selective inhibitors or coxibs, are used as specific inhibitors of COX-2. The development of these drugs allowed the circumvention of the negative gastrointestinal effects while effectively reducing inflammation (Funk et al., 2013). Prescriptions of a specific COX-2 inhibitor has no effect on PGE2 synthesis because the drug selectively blocks the COX-2 enzyme and impedes the production of PGs that cause pain and swelling. Since it selectively blocks COX-2 enzyme and not cyclooxygenase-1 (COX-1) enzyme, it was suggested that the drug can be safely employed during orthodontic mechanotherapy, without causing negative effects on tooth movement (Diravidamani et al., 2012).

28.4.4 Prostaglandin E_2 in the experimental bone remodeling

As an important local regulator of bone, PGE2 have an essential role in bone remodeling. Osteoblasts and osteoclasts are stimulated by PGE2 that may have intrinsic (physiologic/pathologic) or extrinsic (injections or dinoprostone) origin, directly or indirectly.

Four groups of male Wistar rats (each group consisted of eight rats) are compared in this section, i.e., control, PGE2, PGE2 plus calcium, and PGE2 plus vitamin D. Eight week-old rats, weighing 230−300 g, were randomly divided between the aforementioned groups. Method of force application and installation of the pull coil springs were similar to the above-mentioned methodology. PGE2 (0.1 ml of 1 mg/ml) dissolved in 1% lidocaine was injected submucosally at the mesiobuccal mucosa of the first molars. In PGE2 + calcium group, PGE2 was injected submucosally and calcium gluconate 10% (200 mg/kg) was injected intraperitoneally (Marcus, 1996) and the injections were administered on days 0 and 7.

Orthodontic tooth movements were increased from the control group (0.23 ± 0.07 mm) to PGE2 (0.47 ± 0.28 mm) and PGE2 + calcium group (0.46 ± 0.1 mm) significantly ($P < 0.05$) (Seifi et al., 2003). A synergistic effect was observed with the combination of PGE2 and vitamin D in OTM (Seifi et al., 2013). Tooth movement was significantly increased in PGE2 + vitamin D group (0.7 ± 0.13 mm) relative to the other three groups, i.e., control, PGE2, and PGE2 + calcium. No information was available regarding the combined injection of vitamin D and PGE2 during OTM when the research was conducted.

Root resorption was the least in the utmost OTM group interestingly. Root resorption in PGE2 + vitamin D group which had the highest amount of tooth movement was 0.0021 ± 0.0001 mm^2 followed by the control group (0.0081 ± 0.0043 mm^2) which had the least amount of OTM. PGE2 + calcium (0.0113 ± 0.0111 mm^2) and PGE2 (0.0192 ± 0.0198 mm^2) groups had more root resorption than the previous groups respectively (Seifi et al., 2003, 2013).

It has been reported that there was no significant difference in OTM between PGE2 and 1,25-DHCC groups (Kale et al., 2004). This statement is in agreement with the findings mentioned by the authors that in both groups (PGE2 about 0.47 mm and vitamin D about 0.4 mm), an increased OTM took place but it does not mean that the synergistic effect would equal to mathematical addition of both values (PGE2 + vitamin D about 0.7 mm) (Seifi et al., 2003, 2013). Researches have reported that the numbers of Howship's lacunae and capillaries on the pressure side are significantly greater in the PGE2 group than in the 1,25-DHCC group. On the other hand the number of osteoblasts on the external surface of the alveolar bone on the pressure side are significantly greater in the 1,25-DHCC group than in the PGE2 group indicating that bone formation and bone resorption are well balanced (Kale et al., 2004). The balance between resorption and formation of hard tissue of bone leads to less resistance to tooth movement and better stabilization of tooth root structure. Root resorption is very complicated phenomenon but it seems that synergistic effects of PGE2 and vitamin D can play an essential role in maintaining the root resorption at its lowest level, i.e., about 0.002 (mm^2) (Seifi et al., 2013).

28.5 Thyroid hormone

28.5.1 Pharmacological properties

Thyroid hormones are produced by parafollicular cells and increase cellular metabolism in general and in particular increase bone growth, development and remodeling. Thyroxin (the inactive form of hormone) is the main hormone secreted into the bloodstream by thyroid gland and is converted to its active form (T3) by organs such as the kidneys and liver. Tri-iodothyronine (T3) and its prohormone, thyroxine (T4), are tyrosine-based hormones that are primarily responsible for regulation of metabolism. Osteoprogenitor cells proliferate and differentiate into osteoblasts by the stimulation of thyroid hormone receptors on their plasma membrane (Krieger et al., 1988).

28.5.2 Thyroid hormone in the experimental bone remodeling

Administration of thyroid hormone (T_3 and T_4) leads to increased bone remodeling and bone resorptive activity, reduced bone density and stimulation of bone formation. This phenomenon can be observed following administration of levothyroxine causing incremental increase of the amount of OTM compared to the control group (0.23 ± 0.06 mm) to thyroid hormone group (0.45 ± 0.13 mm) (Seifi et al., 2015). The present study has utilized the same model that has been described in the previous sections. 20 μg/kg thyroxine was injected intraperitoneally in the groups that received thyroid hormone.

As previously stated, calcium may play a stabilizing role in bone resorption and this pattern is observed when it is combined with levothyroxine. The amount of OTM was diminished from 0.45 ± 0.13 mm in thyroid group to 0.37 ± 0.05 mm in thyroid plus calcium group (T + Ca). According to the aforementioned reasons, by administration of PG E_2, OTM is increased significantly ($P < 0.05$). When levothyroxine is injected in conjunction with PGE_2, an increase in the amount of OTM can be postulated. The OTM of thyroid plus PGE_2 group (T + PGE) (0.74 ± 0.14 mm) was significantly greater than those of the control (about 0.23 mm) and thyroid hormone groups (approximately 0.45 mm) ($P < 0.05$). This synergistic phenomenon of the increased OTM following combined administration of levothyroxine and PGE_2 resembles the earlier mentioned synergistic effect of PGE2 and $1,25(OH)_2D_3$ (Seifi et al., 2013, 2015). After incorporating calcium gluconate in the combination of pharmacologic agents administered for the group of thyroid hormone plus PGE plus calcium (T + PGE + Ca), a decline in OTM can be anticipated. The highest amount of tooth movement was in T + PGE group (about 0.74 mm) which decreased to 0.65 ± 0.06 mm in the T + PGE + Ca group.

The present findings are in agreement with the other researchers who have studied the effect of exogenous thyroxine on OTM in a rat model in which an orthodontic force of 25 cN was applied on the first molar for 21 days. After an induction period of 4 weeks, in which 0.003% thyroxine was added to the drinking water, a significant increase in the rate of OTM was found (Verna et al., 2000). The highest

amount of root resorption was observed in T + PGE group ($0.0042 \pm 0.0036\ mm^2$) which had also the highest amount of OTM (about 0.74 mm) among the thyroid groups. This indicates that the amount of tooth movement is increased and PGE_2 is a potent pharmacologic agent to induce root resorption (Diravidamani et al., 2012). Authors have shown that the incorporation of calcium in the above-mentioned group, i.e., (T + PGE) to establish a group of (T + PGE + Ca) can reduce the amount of root resorption ($0.0017 \pm 0.0019\ mm^2$) significantly ($P < 0.05$). Administration of levothyroxine can have a reducing effect on root resorption from the control group ($0.0024 \pm 0.0013\ mm^2$) to thyroid hormone group ($0.0020 \pm 0.0013\ mm^2$). This can be correlated to the increased bone remodeling following the administration of levothyroxine and less mechanical resistance against tooth movement. However, its dynamic metabolic action should be taken into the account as well.

28.6 Gonadal hormones

28.6.1 Pharmacological properties

Androgens that stimulate or control the development and maintenance of male characteristics in vertebrates by binding to androgen receptors, estrogens, as primary female sex hormone, and progestins all have osteo-stimulatory actions.

Osteogenic activity of vitamin D and calcitonin is increased by the action of estrogen and estrogen increases circulating levels of biologically active free calcitriol in postmenopausal women (Cheema et al., 1989). Therefore estrogen therapy tends to decrease the osteoporosis and selective estrogen receptor modulator increases bone mineral density and reduces the risk of vertebral fracture (Ettinger et al., 1999). The protective effects of estrogen against postmenopausal osteoporosis are mediated in part by the direct induction of apoptosis of the bone-resorbing osteoclasts via an estrogen receptor-mediated mechanism (Kameda et al., 1997).

28.6.2 Gonadal hormones in the experimental bone remodeling

The effect of the lack of gonadal hormones on the calcium level of tibia bone in rats has been studied by the author. Gonadal hormones affect the growth through increased activity of the osteoblastic cells. They have an influence on the calcium metabolism and therefore, are influential on the calcium level of serum and the strength of the bones. The purpose of our study was to examine the effects of gonadal hormones deficiency on the percentage of bone calcium of the tibia in rats (Seifi et al., 2011).

Twenty-five male rats were randomly divided into two groups; experimental group, i.e., orchiectomy ORX ($n = 15$) and the control group ($n = 10$). Twenty-five female rats were divided in the same way into two groups; experimental group, i.e., ovariectomy OVX ($n = 15$) and control group ($n = 10$). Animals were sacrificed 6 months after the surgery and the percentage of bone calcium was determined by atomic absorption spectrophotometry. Serum testosterone, progesterone, and estradiol

levels were measured by ELISA. Serum testosterone level significantly decreased in the ORX group as compared with the male control group. In this study estradiol level was not changed in OVX group, but despite the significant decrease in progesterone level ($P < 0.001$), no significant differences in all variables were found between the OVX group and female control group. No significant difference in bone calcium percentage was found (Student's *t*-test) between the control and experimental groups. In conclusion, it seems that the suppression of gonadal hormones in the growth phase cannot decrease calcium percentage of tibia bone in rats (Seifi et al., 2011).

In another study by the authors, changes of craniofacial dimensions and growth were studied following gonadal elimination in rats. Sex steroids increase osteoblasts activities and affect growth, remodeling and bone homeostasis. Body length and weight were registered in the control, OVX, and ORX groups monthly. The rats were sacrificed 6 months after the surgery. Direct millimetric measurements of the skeletodental variables and the tibial length were obtained using an electronic caliper. Serum testosterone, progesterone, and estradiol levels were measured by ELISA. One-way ANOVA, Tukey, and student *t*-tests were used to analyze the data. Serum testosterone level significantly decreased in the ORX group as compared with the male control group. In the ORX group, body length and weight, coronoid height, mandibular length, mandibular arch length, midfacial width, midfacial length, midfacial height, calvarial width, maxillary arch width, premaxillary length, nasal bone height, facial width, basisphenoid bone length, and tibia bone length were significantly smaller than those in the male control group (Fig. 28.5). Structures showing cartilaginous growth were influenced more than structures showing sutural growth. Estradiol level did not change in OVX group, but despite the significant decrease in progesterone level, no significant differences except for the weight were found between the OVX group and female control group. It is strongly suggested that the suppression of sex hormones secretion in the growth phase might inhibit craniofacial growth and development and may result in developing malocclusions (Seifi et al., 2008).

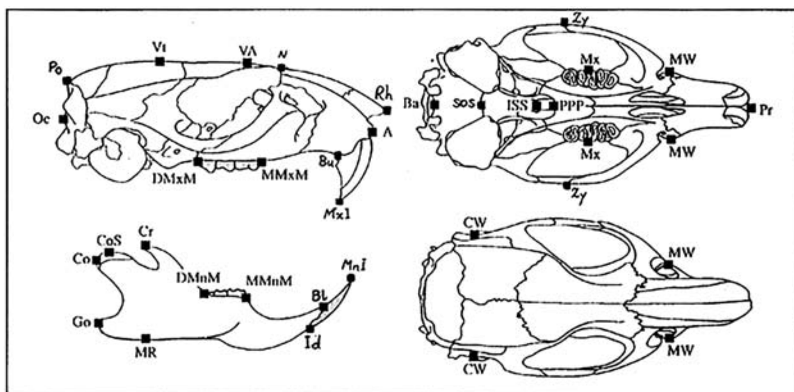


Figure 28.5 Schematic view of craniofacial and dental structures of rat.

To investigate the role of gonadal steroids on bone remodeling the authors conducted another study on the effect of ovariectomy and orchiectomy on OTM and root resorption in wistar rats. Hormonal deficiencies and their effect on bone turnover were reported to have influences on the rate of tooth movement and root resorption (multifactorial complication). Orthodontic appliances were placed on the right maxillary first molars of 10 ovariectomized female and 10 orchiectomized male wistar rats as experimental groups and 10 female and 10 male healthy Wistar rats as control groups. NiTi closed-coil springs (9 mm, medium, 011″ × .030″, Ortho Technology; Tampa, FL) were placed between the right incisors and the first right maxillary molars to induce tipping movement in the first molars with the application of a 60 g force. After 21 days the rats were sacrificed and the tooth movement was measured using a digital caliper (Guanglu, China). Orthodontic-induced root resorption (OIRR) was assessed by histomorphometric analysis of hematoxylin and eosin stained sections of the mesial root. The rate of tooth movement was significantly higher in all female rats, with the root resorption being lower in the experimental group. The rate of tooth movement in experimental male rats was significantly higher than that of the control group ($P = 0.001$) and the rate of root resorption was significantly lower in the experimental group ($P = 0.001$). It seems that alterations in plasma levels of estrogen, progesterone, and testosterone hormones can influence the rate of OTM and RR. The acceleration in tooth movement increased OTM and decreased RR (Seifi et al., 2015).

28.7 Fibroblast growth factor

28.7.1 Pharmacological properties

Fibroblast growth factor (FGF), transforming growth factor (alpha and beta), platelet-derived growth factor, and insulin-like growth factor are heterogeneous group of polypeptides. FGF is a single-chain polypeptide growth factor that plays a significant role in the process of wound healing and is a potent inducer of angiogenesis and a crucial factor in osteogenesis. In normal tissue, basic fibroblast growth factor (bFGF) is present in basement membranes and in the subendothelial extracellular matrix of blood vessels. Perivascular cells have mesenchymal cell qualities with a possible role as progenitors for osteoblast differentiation. Endothelial cells and osteoblasts can produce FGF and it is found in bone matrix that acts as a mitogen to osteoprogenitor cells, but also reduces the function of the osteoblasts (Globus et al., 1989).

28.7.2 Fibroblast growth factor in the experimental bone remodeling

The authors have studied the role of an angiogenic factor, i.e., bFGF on experimental tooth movement and bone remodeling. bFGF is a cytokine involved in angiogenesis, tissue remodeling and stimulation of osteoblasts and osteoclasts. Fifty rats

were randomly divided into five groups of 10 rats each. Rats received 0.02 cc injections of the following doses of bFGF: group A (10 ng), group B (100 ng), and group C (1000 ng). Group D (positive control) received an orthodontic force and injection of 0.02 cc phosphate-buffered saline whereas group E (negative control) only received the anesthetic drug. A nickel titanium spring was bonded to the right maxillary first molar and incisor. After 21 days the rats were sacrificed and the distance between the first and second right molars was measured using a leaf gauge with 0.05 mm accuracy. ANOVA and Tukey's HSD statistical tests were used for data analysis. The greatest mean value of OTM was 0.77 mm observed in the group C, followed by 0.66 mm in the group B, 0.53 mm in group A, 0.26 mm in group D and 0.02 mm in group E. There was a significantly higher rate of tooth movement in the test groups compared to the control groups ($P < 0.05$). Among the test groups the rate of tooth movement in the group C was significantly higher than the group A ($P < 0.05$). Weight changes after the intervention were not significant when compared to the baseline values, with the exception for the group E ($P > 0.05$). The effect of bFGF on the rate of tooth movement was dose-dependent. Injection of 1000 ng bFGF in rats showed the most efficacy (Seifi et al., 2013).

The effect of bFGF as angiogenic cytokine on orthodontically induced inflammatory root resorption (OIIRR) was studied. OIIRR is an undesirable sequel of tooth movement after sterile necrosis that takes place in periodontal ligament due to the blockage of blood vessels following exertion of orthodontic force. Number of resorption lacunae and the area of resorption lacunae in the experimental group (1000 ng) were 0.97 ± 0.80 and $1.\ 27 \pm 0.01 \times 10^{-3}$, respectively which were significantly lower than those in the control positive group (4.17 ± 0.90 and $2.77 \pm 0.01 \times 10^{-3}$, respectively, $P = 0.000$). Number of blood vessels, osteoclasts, and Howship's lacunae were significantly higher in the E1000 group compared to positive control group ($P < 0.05$). Tooth movement as the outcome of bone remodeling is concomitant with the formation of sterile necrosis in the periodontal ligament following blocked blood supply. Thus bFGF can significantly decrease the risk of root resorption by providing more oxygen and angiogenesis (Seifi et al., 2016).

28.8 Nonsteroidal antiinflammatory drugs (ibuprofen, aspirin) and acetaminophen

28.8.1 Pharmacological properties

Nonsteroidal antiinflammatory drugs, also called nonsteroidal antiinflammatory agents/analgesics or nonsteroidal antiinflammatory medicines, are a drug category that includes drugs that provide analgesic (pain-killing) and antipyretic (fever-reducing) effects, and, in higher doses, antiinflammatory effects. The term nonsteroidal distinguishes these drugs from steroids, which, among a broad range of other effects, have a similar eicosanoid-depressing, antiinflammatory action (Buer, 2014).

Most NSAIDs inhibit the activity of COX-1 and COX-2, and thereby, the synthesis of PGs and thromboxanes. As analgesics, NSAIDs are unusual in that they are non-narcotic and thus are used as a nonaddictive alternative to narcotics. The most prominent members of this group of drugs, aspirin, ibuprofen, and naproxen, are all available over the counter in most countries (Warden, 2010). Paracetamol (acetaminophen) is generally not considered an NSAID because it has only little antiinflammatory activity. It treats pain mainly by blocking COX-2 mostly in the central nervous system, but not much in the rest of the body (Hinz et al., 2008).

28.8.2 Nonsteroidal antiinflammatory drugs (ibuprofen and aspirin) and acetaminophen in the experimental bone remodeling

Effects of two commonly prescribed NSAIDs, i.e., aspirin and ibuprofen and acetaminophen have been studied on the rate of OTM and root resorption in rabbits. Twenty-four male New Zealand white rabbits ranging in weight from 1.35 to 2.5 kg were randomly assigned into one of the three test groups or a control group, each consisting of six animals. Under anesthesia NiTi closed coil springs were ligated between the left side mandibular incisor and the 1st molar with closed coil spring delivering 6 oz force. Aspirin group received 100 mg/kg of drug (tablet) in one dose per day. Ibuprofen group received 10 mg/kg of drug (suspension) in one dose per day. Acetaminophen group received 200 mg/kg of drug (tablet) in one dose per day. Control group received no drug during this study. The animals were sacrificed after 1 month and OTM of the 1st molar was measured with special gauges with 0.05 mm accuracy. After histological processing, sections of each group (mesio-distal) were prepared and midroot sections were chosen to calculate root resorption on mesial surface of the 1st molar with computerized histomorphometric method. Analysis of variance (ANOVA) was performed to assess tooth movement and root resorption and multiple comparisons analysis was used to compare different groups. Control and acetaminophen groups exhibited similar degree of tooth movement which had significant difference with ibuprofen and aspirin groups ($P < 0.001$). Aspirin group exhibited least root resorption which was significantly different from the other groups ($P < 0.05$). Acetaminophen had no effect on the rate of tooth movement but aspirin and ibuprofen decreased rate of tooth movement significantly (Seifi et al., 2004).

28.9 Bisphosphonates (pamidronic acid, zoledronic acid)

28.9.1 Pharmacological properties

Pamidronic acid (international nonproprietary name: INN) or pamidronate disodium (USAN), or pamidronate disodium pentahydrate is a nitrogen-containing bisphosphonate, used to prevent osteoporosis. It is used to prevent bone loss, treat

osteoporosis, to strengthen the bone in Paget's disease, to prevent bone loss due to steroid use, and in certain cancers with high propensity to bone.

Zoledronic acid (INN) or zoledronate is a bisphosphonate drug given intravenously to treat some bone diseases. It was sold under many trade names worldwide. Third-generation bisphosphonates are known to inhibit bone resorption and also appear to exhibit direct antitumor activity (Koto et al., 2010). Zoledronic acid slows down bone resorption, allowing the bone-forming cells time to rebuild normal bone and allowing bone remodeling.

The use of bisphosphonates for osteoporosis is effective in reducing the risk of fractures. However, oral formulations are sometimes not well tolerated or are contraindicated. The intravenous pamidronate in a group of postmenopausal women with predominant high risk of fracture, promoted an isolated gain in the spine bone mineral density or BMD (Zanatta et al., 2016).

28.9.2 Bisphosphonates (pamidronate, zoledronate) in the experimental bone remodeling

The authors have examined the effect of administration of bisphosphonate; an anti-osteoclastic agent, i.e., pamidronate, on the OTM and root resorption in rats. Experimental study was conducted on 30 male 8-week-old Wistar rats. The orthodontic appliance was first installed and then the control group received normal saline and the experimental groups received pamidronate 1 mg/kg, by intraperitoneal injection. After 21 days, rats were sacrificed and the upper jaws were dissected and the distance between upper 1st and 2nd molars were measured. Under light microscope, root resorptive lacunae were calculated using a computer software. Student *t*-test was used for the purpose of comparison between the groups. There were significant differences between the control (0.562 ± 0.168 mm) and the experimental group (0.346 ± 0.197 mm) for OTMs ($P < 0.01$ by paired *t*-test). Regarding the root resorption, no significant differences were observed between the control ($0.25 \pm 0.175 \times 10^{-2}$ mm^2) and the experimental group ($0.26 \pm 0.125 \times 10^{-2}$ mm^2). According to the present study, pamidronate can reduce tooth movement and at the same time no increase of the root resorption is observed (Seifi, 2009).

Another research conducted by the authors was evaluating the comparative effectiveness of two bisphosphonates, i.e., pamidronate and zoledronate on OTM and OIIRR in rats. Thirty-six, 8-week-old male, albino wistar rats weighing 200–250 g were randomly divided into three groups of twelve. On day zero the orthodontic appliances were installed. Control group received normal saline and for the experimental groups, pamidronate (1 mg/kg) and zoledronate (0.1 mg/kg) were injected intraperitoneally, respectively. After 21 days, rats of the control group and experimental group were sacrificed and the upper jaws were dissected and the distance between upper 1st and 2nd molars were measured. Under light microscope, root resorptive lacunae were calculated using a computer software. Kruskal–Wallis statistical test was used for purpose of the comparison. The highest mean of OTM was observed in the control group (0.56 ± 0.16 mm) that was significantly higher relative to the experimental groups

($P < 0.05$). The amount of OTM in pamidronate and zoledronate groups were 0.35 ± 0.19 (mm) and 0.31 ± 0.18 (mm), respectively. No significant difference in root resorption was found between control ($0.26 \pm 0.18 \times 10E^{-2}$ mm^2) and experimental groups (pamidronate ($0.24 \pm 0.12 \times 10E^{-2}$) and zoledronate ($0.23 \pm 0.13 \times 10E^{-2}$)). According to the present study, bisphosphonates (pamidronate and zoledronate) can reduce OTM but root resorption cannot be diminished despite OTM reduction.

28.10 Conclusion

1. Bones undergo dynamic remodeling that is physiologic but imbalances can influence their structure, quantitatively and qualitatively.
2. Vitamin D insufficiency can also influence the bone remodeling and regeneration. This means a higher dose of vitamin D may be necessary for some individuals with known risk factors for vitamin D insufficiency, such as those with dark skin, the elderly, photosensitive individuals, people with limited sun exposure, obese individuals or those with fat malabsorption.
3. Calcium balance and homeostasis and their relationship to dietary calcium intake and calcium supplementation are very important in healthy subjects and patients with chronic kidney disease and mineral bone disorders (chronic kidney disease-mineral and bone disorder: CKD-MBD) (Peacock, 2010).
4. There is some controversy regarding the effects of medications on bone health and bone remodeling. The administration of bisphosphonate drugs for delivering local effects without considering the systemic effects or long-term side-effects such as atypical bone fracture can be an area of concern. Adverse effects of the medications affecting the bone remodeling should be taken into account. A cost-benefit analysis will determine the net outcome of the intervention that can be difficult to carry out.

Acknowledgment

We would like to thank our colleagues who have helped us for preparing this chapter directly or indirectly; helped us in conducting different experimental research for evaluating the OTM and root resorption during the previous years including, Dr. Ziaee-Matin, Dr. Eslami, Dr. Lotfi, Dr. Naziri, Dr. Khavandgar, Dr. Ashiri, Dr. Hedayati, Dr. Ezzati, Dr. Aghaeepour, Dr. Asefi, Dr. Ravadgar, and Dr. Badiee.

References

Abrahamsen, B.M.T., Avenell, A., Anderson, F., Meyer, H.E., Cooper, C., Smith, H., et al., 2010. Patient level pooled analysis of 68 500 patients from seven major vitamin D fracture trials in US and Europe. BMJ. 340, b5463.

Bodansky, M., Duff, V.B., 1939. Regulation of the level of calcium in the serum during pregnancy. J. Am. Med. Assoc. 112 (3), 223–229.

Boucher, B.J., 2011. Vitamin D insufficiency and diabetes risks. Curr. Drug Targets. 12 (1), 61–87.

Bouillon, R., Okamura, W.H., Norman, A.W., 1995. Structure-function relationships in the vitamin D endocrine system. Endocr. Rev. 16 (2), 200–257.

Buer, J.K., 2014. Origins and impact of the term 'NSAID'. Inflammopharmacology. 22 (5), 263–267.

Carafoli, E., Penniston, J.T., 1985. The calcium signal. Sci. Am. 253 (5), 70–78.

Chapuy, M.C., Arlot, M.E., Duboeuf, F., Brun, J., Crouzet, B., Arnaud, S., et al., 1992. Vitamin D3 and calcium to prevent hip fractures in elderly women. New Engl. J. Med. 327 (23), 1637–1642.

Cheema, C., Grant, B.F., Marcus, R., 1989. Effects of estrogen on circulating "free" and total 1, 25-dihydroxyvitamin D and on the parathyroid-vitamin D axis in postmenopausal women. J. Clin. Invest. 83 (2), 537.

Di Rosa, M., Malaguarnera, M., Nicoletti, F., Malaguarnera, L., 2011. Vitamin D3: a helpful immuno-modulator. Immunology. 134 (2), 123–139.

Diravidamani, K., Sivalingam, S.K., Agarwal, V., 2012. Drugs influencing orthodontic tooth movement: an overall review. J. Pharm. Bioallied Sci. 4 (Suppl. 2), S299–S303.

Diravidamani, K., Sivalingam, S.K., Agarwal, V., 2012. Drugs influencing orthodontic tooth movement: an overall review. J. Pharm. Bioallied Sci. 4 (6), 299.

Ettinger, B., Black, D.M., Mitlak, B.H., Knickerbocker, R.K., Nickelsen, T., Genant, H.K., et al., 1999. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. JAMA. 282 (7), 637–645.

Feng, X., McDonald, J.M., 2011. Disorders of bone remodeling. Annu. Rev. Pathol. 6, 121.

Funk, C.D., Song, W.-C., FitzGerald, G.A., 2013. Biosynthesis of Eicosanoids. Yen & Jaffe's Reproduct. Endocrinol. Physiol. Pathophysiol. Clin. Manag. 108.

Globus, R.K., Plouet, J., Gospodarowicz, D., 1989. Cultured bovine bone cells synthesize basic fibroblast growth factor and store it in their extracellular matrix. Endocrinology. 124 (3), 1539–1547.

Gonzales, C., Hotokezaka, H., Yoshimatsu, M., Yozgatian, J.H., Darendeliler, M.A., Yoshida, N., 2008. Force magnitude and duration effects on amount of tooth movement and root resorption in the rat molar. Angle Orthodontist. 78 (3), 502–509.

Heaney, R.P., 2000. Calcium, dairy products and osteoporosis. J. Am. College Nutr. 19 (Suppl. 2), 83S–99S.

Hinz, B., Cheremina, O., Brune, K., 2008. Acetaminophen (paracetamol) is a selective cyclooxygenase-2 inhibitor in man. FASEB J. 22 (2), 383–390.

Hock, J.M., Gunness-Hey, M., Poser, J., Olson, H., Bell, N.H., Raisz, L.G., 1986. Stimulation of undermineralized matrix formation by 1,25 dihydroxyvitamin D3 in long bones of rats. Calcified Tissue Int. 38 (2), 79–86.

Holick, M.F., 1995. Noncalcemic actions of 1,25-dihydroxyvitamin D3 and clinical applications. Bone. 17 (2 Suppl.), 107s–111ss.

Hui, S.L., Slemenda, C.W., Johnston Jr, C.C., 1988. Age and bone mass as predictors of fracture in a prospective study. J. Clin. Investig. 81 (6), 1804.

Ibbotson, K.J., Roodman, G.D., McManus, L.M., Mundy, G.R., 1984. Identification and characterization of osteoclast-like cells and their progenitors in cultures of feline marrow mononuclear cells. J. Cell Biol. 99 (2), 471–480.

James, E., Reynolds, F., Martindale, W., 1993. The Extra Pharmacopoeia. Council of The Royal Pharmaceutical Society of Great Britain, London, p. 754.

Kale, S., Kocadereli, I., Atilla, P., Asan, E., 2004. Comparison of the effects of 1,25 dihydroxycholecalciferol and prostaglandin E2 on orthodontic tooth movement. Am. J. Orthodont. Dentofac. Orthoped. 125 (5), 607−614.

Kameda, T., Mano, H., Yuasa, T., Mori, Y., Miyazawa, K., Shiokawa, M., et al., 1997. Estrogen inhibits bone resorption by directly inducing apoptosis of the bone-resorbing osteoclasts. J. Exp. Med. 186 (4), 489−495.

Kiliaridis, S., Engstrom, C., Lindskog-Stokland, B., Katsaros, C., 1996. Craniofacial bone remodeling in growing rats fed a low-calcium and vitamin-D-deficient diet and the influence of masticatory muscle function. Acta Odontol. Scand. 54 (5), 320−326.

Koto, K., Murata, H., Kimura, S., Horie, N., Matsui, T., Nishigaki, Y., et al., 2010. Zoledronic acid inhibits proliferation of human fibrosarcoma cells with induction of apoptosis, and shows combined effects with other anticancer agents. Oncol. Rep. 24 (1), 233−239.

Krieger, N.S., Stappenbeck, T.S., Stern, P.H., 1988. Characterization of specific thyroid hormone receptors in bone. J. Bone and Min. Res. 3 (4), 473−478.

Marcus, R., 1996. Agents affecting calcification and bone turnover, Goodman & Gilman's The Pharmacological Basis of Therapeutics. 9th ed. MacGraw Hill, New York, pp. 1519−1546.

Meunier, P., 1996. Prevention of hip fractures by correcting calcium and vitamin D insufficiencies in elderly people. Scand. J. Rheumatol. Suppl. 103, 75−78, discussion 9-80.

Meunier, P., Chapuy, M., Arlot, M., Delmas, P., Duboeuf, F., 1994. Can we stop bone loss and prevent hip fractures in the elderly? Osteoporosis Int. 4 (1), S71−S76.

Mimura, H., Cao, X., Ross, F.P., Chiba, M., Teitelbaum, S.L., 1994. 1,25-Dihydroxyvitamin D3 transcriptionally activates the beta 3-integrin subunit gene in avian osteoclast precursors. Endocrinology. 134 (3), 1061−1066.

Nelson, R.J., 2005. An Introduction to Behavioral Endocrinology. Sinauer Associates, Sunderland, MA.

Peacock, M., 2010. Calcium metabolism in health and disease. Clin. J. Am. Soc. Nephrol. 5 (Suppl. 1), S23−S30.

Rang, H.P., Ritter, J.M., Flower, R.J., Henderson, G., 2014. Rang & Dale's Pharmacology: With Student Consult Online Access. Elsevier Health Sciences, Amsterdam, The Netherlands.

Reichel, H., Koeffler, H.P., Norman, A.W., 1989. The role of the vitamin D endocrine system in health and disease. New Engl. J. Med. 320 (15), 980−991.

Ricciotti, E., FitzGerald, G.A., 2011. Prostaglandins and inflammation. Arterioscler. Thromb. Vasc. Biol. 31 (5), 986−1000.

Riggs, B.L., Melton 3rd, L.J., 1992. The prevention and treatment of osteoporosis. New Engl. J. Med. 327 (9), 620−627.

Sato, Y., Iki, M., Fujita, Y., Tamaki, J., Kouda, K., Yura, A., et al., 2015. Greater milk intake is associated with lower bone turnover, higher bone density, and higher bone microarchitecture index in a population of elderly Japanese men with relatively low dietary calcium intake: Fujiwara-kyo Osteoporosis Risk in Men (FORMEN) Study. Osteoporosis Int. 26 (5), 1585−1594.

Seifi, M., 2009. Effect of pamidronate on tooth movement and root resorption in rat. J. Dental School. 27 (2), 4.

Seifi, M., Ashiri, M., Hedayati, M., 2008. Effect of sexual hormone elimination on the changes of craniofacial dimensions in rats. J. Dent. School Shahid Beheshti Univ. Med. Sci. 25 (4), 365−372.

Seifi, M., Ashiri, M., Hedayati, M., 2011. Effect of the lack of gonadal hormones on the calcium level of tibia bone in rats. J. Dent. Sch. 28 (4), 219−224.
Seifi, M., Badiee, M.R., Abdolazimi, Z., Amdjadi, P., 2013. Effect of basic fibroblast growth factor on orthodontic tooth movement in rats. Cell J. (Yakhteh). 15 (3), 230.
Seifi, M., Eslami, B., Saffar, A.S., 2003. The effect of prostaglandin E2 and calcium gluconate on orthodontic tooth movement and root resorption in rats. Eur. J. Orthodont. 25 (2), 199−204.
Seifi, M., Ezzati, B., Saedi, S., Hedayati, M., 2015. The effect of ovariectomy and orchiectomy on orthodontic tooth movement and root resorption in wistar rats. J. Dent. 16 (4), 302.
Seifi, M., Hamedi, R., Khavandegar, Z., 2015. The effect of thyroid hormone, prostaglandin E2, and calcium gluconate on orthodontic tooth movement and root resorption in rats. J. Dent. 16 (1 Suppl.), 35.
Seifi, M., Hamedi, R., Naziri, M., 2013. The synergistic effect of vitamine d and prostaglandin E2 on orthodontic tooth movement in rats. Iran. J. Orthodont. 8 (1), 1−5.
Seifi, M., Lotfi, A., Badiee, M.R., Abdolazimi, Z., Amdjadi, P., Bargrizan, M., 2016. The effect of an angiogenic cytokine on orthodontically induced inflammatory root resorption. Cell J. (Yakhteh). 18 (2), 271−280.
Seifi, M., Ravadgar, M., Eslami, B., 2004. The effect of Acetaminophen, Aspirin and Ibuprofen on the rate of orthodontic tooth movement and root resorption in rabbits. J. Dent. School Shahid Beheshti Univ. Med. Sci. 21 (4), 689−700.
Spear, G.T., Paulnock, D.M., Helgeson, D.O., Borden, E.C., 1988. Requirement of differentiative signals of both interferon-gamma and 1,25-dihydroxyvitamin D3 for induction and secretion of interleukin-1 by HL-60 cells. Cancer Res. 48 (7), 1740−1744.
van Etten, E., Branisteanu, D.D., Verstuyf, A., Waer, M., Bouillon, R., Mathieu, C., 2000. Analogs of 1,25-dihydroxyvitamin D3 as dose-reducing agents for classical immunosuppressants. Transplantation. 69 (9), 1932−1942.
Verna, C., Dalstra, M., Melsen, B., 2000. The rate and the type of orthodontic tooth movement is influenced by bone turnover in a rat model. Eur. J. Orthodont. 22 (4), 343−352.
Warden, S.J., 2010. Prophylactic use of NSAIDs by athletes: a risk/benefit assessment. Phys. Sportsmed. 38 (1), 132−138.
Yonaga, T., Morimoto, S., 1979. A calcitonin-like action of prostaglandin E 1. Prostaglandins. 17 (6), 801−819.
Zanatta, L.B., Marcatto, C., Ramos, C.S., Manas, N., Moreira, C., Borba, V., 2016. Use of pamidronate for osteoporosis treatment in public health care in Brazil. Revista Bras. Reumatol.. Available from: http://dx.doi.org/10.1016/j.rbr.2016.05.005.

Further reading

Marx, R.E., Cillo, J.E., Ulloa, J.J., 2007. Oral bisphosphonate-induced osteonecrosis: risk factors, prediction of risk using serum CTX testing, prevention, and treatment. J. Oral Maxillofac. Surg. 65 (12), 2397−2410.
Rosen, H., Moses, A., Garber, J., Iloputaife, I., Ross, D., Lee, S., et al., 2000. Serum CTX: a new marker of bone resorption that shows treatment effect more often than other markers because of low coefficient of variability and large changes with bisphosphonate therapy. Calcified Tissue Int. 66 (2), 100−103.

This page intentionally left blank

Index

Note: Page numbers followed by "*f*" and "*t*" refer to figures and tables, respectively.

A

Acellular afibrillar cementum, 280–281
Acellular biomaterials, 147
Acellular cadaveric dermis, 227
Acellular dermal matrix allograft (ADMA), 260–262
Acellular extrinsic fiber cementum (AEFC), 280–281
Acellular intrinsic fiber cementum (AIFC), 280–281
Acellularized human dermal matrix, 142
Acetaminophen, 517–518
Acetobacter xylinum, 128–129
Acid etching, 87
Acrylonitrile butadiene styrene (ABS), 38–39
Active Implantable Medical Devices Directive (AIMDD), 148–149
Additive manufacturing (AM), 163–165, 390–391
Additive surface modification methods, 88–90
  biochemical and organic compound coating, 90
  ceramic coating methods, 89
    electrolytic process, 89
    plasma spraying, 89
    sol–gel and dip-coating, 89
    vacuum deposition, 89
  fluoride-modified surface, 89
Adenovirus encoding human aquaporin-1 (AdhAQP1), 343
Adipose tissue-derived (ADSC) MSCs (ADSC-MSCs), 343, 434
Adipose-derived stem cells (ADSCs), 321, 327, 415–416
Adult stem cells, 47, 343, 446–447, 472–475
Agar overlay test, 110, 118–119
Agarose, 26*t*, 53–54
Alginate, 26*t*, 30, 53–55, 128, 480
Alginate gels, 54–55
Alginate hydrogel, 30, 54–55
AlloDerm, 227, 247, 260–261, 261*f*
Allogeneic bone grafts, 76
  types of, 76*t*
Allogenic materials, 260–262
Allografts, 76, 367, 408–409
Alloplastic grafts, 77
  types of, 77*f*
Alloplastic materials, 271–272
Alumina, 11
Alveolar bone, 282–283
Alveolar bone cells (ABC), 170–173
Alveolar bone defects and repairing techniques, 448–451
Alveolar bone loss, 279
Ame's test, 120
Ameloblast serum-free CM (ASF-CM), 476
Ameloblastin (AMBN), 476–477
Ameloblasts, 469–471, 474
Amelogenin (AMGN), 476–477
American Association of Endodntists (AAE), 310
American Association of Tissue Banks, 260–261
American Society for Testing Materials (ASTM), 109, 109*t*
Amniotic membrane, 227–228
Amorphous calcium phosphate (ACPs), 15, 412–413
Amylase, 264
Analysis of variance (ANOVA), 518
Anatomic repositioning, 406–408
  distraction osteoogenesis (DO), 406–407
Androgens, 514
Angiogenesis, 367
Angiogenic factors (AGFs), 368–369
Angiopoietin (ANG-1), 368–369
Animal and human-derived biomaterials, 142–144
  hard tissue biomaterials, 143–144
  soft tissue biomaterials, 142–143

Animal dentin, 143
Animal tests, 121–123
  implantation tests, 122–123
  mucous membrane irritation tests, 122
  skin sensitization tests, 121–122
Animal-derived collagen-based membranes, 143
Anodized surface implants, 88
Antisclerostin immunoglobulin coating, 92
Apical bud cells and DPCs (ABCs/DPCs), 474–475
Apical papilla stem cells (APSCs), 485
Aquaporins (AQPs), 343
Areal bone mineral density (aBMD), 505
Articular cartilage, 436–438
Aspirin, 517–518
Atomic force microscopy (AFM), 103–104, 104*f*
Auricular cartilages, 437–438
Autogenous bone grafts, 74–75
Autografts, 74, 367, 408–409
  advantages and disadvantages of using, 75*t*
Autologous grafts, 257–259, 430
Avulsed teeth, 307–308

B

Bacterial infection, 297
  oral mucosal models of, 251
Bacterial reverse mutation test (Ame's test), 120
Bacterial sialadenitis, 339
Bartholin's duct, 338
Basic fibroblast growth factor (bFGF), 516–517
Beer–Lambert law, 100–101
Beta tricalcium phosphate (β-TCP), 28–29, 199–200, 412–413, 478
Bilayer collagen matrix, 265–267
Bilayered scaffolds, synthesis of
  materials and techniques used for, 285*t*
Bioactive glasses (BG), 8–13, 162–163, 430–431
  applications of, in oral and maxillofacial region, 12–13
  composition of, 8–10
    borate bioactive glass, 9–10
    phosphate bioactive glass, 10
    silicate bioactive glass, 8–9
Bioactive glasses methods, preparation of, 10–12
  melt-derived methods, 10–11
  sol–gel methods, 11–12
Bioceramics, 7–8, 173–177
Biochemical and organic compound coating, 90
Biocompatibility of dental biomaterials, 117, 308
  animal tests, 121–123
    implantation tests, 122–123
    mucous membrane irritation tests, 122
    skin sensitization tests, 121–122
  biocomposite, 134–135
  ceramic biomaterials, 131–134
    bioglass, 133–134
    calcium phosphate (Ca-PO_4), 132–133
    hydroxyapatite, 131–132
  combining in vitro, animal, and usage tests together, 124–126
  concepts of biocompatibility testing, 117–118
  in vitro tests, 118–121
    cytotoxicity tests, 118–120
    hemocompatibilty testing, 121
    mutagenicity and genotoxicity testing, 120
  polymeric biomaterials, 126–131
    natural origin polymers, 127–129
    synthetic polymers, 129–131
  usage tests, 123–124
    dental pulp irritation tests, 123
    endodontic usage tests, 124
    mucosa and gingiva usage tests, 124
Biocomposites, 65, 134–135
  alloplastic grafts, 77
  applications of, 65, 66*f*, 72–73
  bone cements, 73–75
    allogeneic bone grafts, 76
    autogenous bone grafts, 74–75
  classification of, 65–67, 67*f*
  natural, 67–68
  restorative applications, 73
  shortcomings of, and recent developments, 77–79
  synthetic, 68–69
  unique properties and adaptability of, 69–72
  xenografts, 76

Biodegradable electrospun polymer scaffolds, 231
Biodegradable hydrogels, 49
Biodegradable polymers, 158, 167
Biodegradable Polyphosphazene/Nano-Hydroxyapatite, 164*t*
Biodegradation test, 112
Bioglass, 8−9, 133−134
Biological characterization technique, 108−112
  in vitro characterization, 110−111
    cytotoxicity testing, 110
    genotoxicity and carcinogenicity testing, 110−111
    hemocompatibility testing, 110
    reverse transcription-polymerase chain reaction (RT-PCR), 111
  in vivo characterization, 111−112
    biodegradation test, 112
    implantation testing, 111−112
    sensitization, irritation, and toxicity tests, 111
Biological structure of teeth, 468−469
Biomimetic apatite coatings, 33
Biomimetic scaffolds, 32−34
  with improved bioactivity, 33−34
  with improved hydrophilicity, 32−33
Bio-Oss, 408−409
Bioreactor design, 193, 196*f*
  application of bioreactors for tissue engineering of oral tissue, 197−201
  fundamentals of, 194−196
  oxygen transport in, 195
  types of, 196−197
Bioreactors, function of, 194−195
Bioseed, 230
Biphasic calcium phosphate (BCP), 16
Biphasic scaffold
  assembling onto a dentin block, 288*f*
  fabrication, using electrospinning, 171*f*
  fabrication of, 287*f*
Bisphenol A-glycidyl methacrylate (Bis-GMA), 68−69
Bisphosphonate-related osteonecrosis, 247−248
Bisphosphonates, 518−520
  in experimental bone remodeling, 519−520
  pharmacological properties, 518−519
Blasting and acid etching, 87
Bone, tissue-engineered, 391−398
  bioactive factors, 393−397
  cells, 392
  environmental factors, 397−398
  scaffolds, 392−393, 393*t*, 394*t*
Bone and cartilage tissue engineering
  cell sources for, 434−435
  scaffolds for, 432−434
Bone augmentation techniques, 408−412
Bone cells, 389−390
Bone cements, 71, 73−75
  allogeneic bone grafts, 76
  autogenous bone grafts, 74−75
Bone grafting, 74
Bone marrow mesenchymal stem cells, 343
Bone marrow-derived cells (BMDCs), 489
Bone marrow-derived mesenchymal stem cells (BMMSCs/BMSCs), 321, 327, 354−355, 413−416, 474
Bone matrix, 388−389
Bone morphogenetic proteins (BMPs), 28−29, 397, 449−450, 453, 457, 476, 483−484
  BMP-2, 416
Bone morphogenic proteins (BMPs), 205, 209−211, 430
  functions of, 212*t*
Bone repair, materials for, 73−74, 74*f*
Bone replacement graft tissues and materials, 75*t*
Bone scaffolds, 369−370
Bone tissue engineering, 31, 371, 375−376
  in maxillofacial region. *See* Maxillofacial region, bone tissue engineering in
Borate bioactive glass, 9−10
Bovine bone, 408−409
Bovine dentin-based bone material, 143
Bovine spongiform encephalopathy (BSE) prion transmission, 146
British Association of Oral and Maxillofacial Surgeons (BAOMS), 387
Buccal fat pad (BFP) grafts, 173−177
Buehler occluded patch tests, 122

C

Calcitonin, 389
Calcium, 504–506
  absorption and bone density, 504–505
  in experimental bone remodeling, 506
  homeostasis, 504
Calcium hydroxide, 71, 298, 311
Calcium phosphates, 13–17, 33, 132–133, 430–431
  chemical properties of, 14–16
    amorphous calcium phosphate (ACPs), 15
    biphasic calcium phosphate (BCP), 16
    hydroxyapatite (HAP), 14–15
    tricalcium phosphate, 15
  clinical application of, in oral and maxillofacial tissue regeneration, 16–17
  osteoinductivity of, 14
Canal disinfection, 311
Candida albicans, 71, 249–250
Carbon nanotubes (CNTs), 327
CD341 progenitor cells, 476
Cell culture, 193–194
Cell interactivity of biomaterial, 144
Cell sheet engineering, 169
Cell-based therapy, 368–369
Cell-containing products, 146–147
Cells, 471–477
  postnatal and embryonic dental cells, 472
  stem cells from dental and nondental tissues, 472–477
    adult stem cells, 472–475
    embryonic stem cells, 475–476
    induced pluripotent stem cells (iPSCs), 476–477
Cell-seeded scaffold, 368–369
Cellular intrinsic fiber cementum (CIFC), 280–281
Cellular mixed stratified fiber cementum (CMSC), 280–281
Cellulose, 67, 128–129
Cementoclasts, 452
Cementum, 68, 280–281, 469
Ceramic biomaterials, 131–134
  bioglass, 133–134
  calcium phosphate (Ca-PO_4), 132–133
  hydroxyapatite, 131–132
Ceramic coating methods, 89
  electrolytic process, 89
  plasma spraying, 89
  sol–gel and dip-coating, 89
  vacuum deposition, 89
Characterization of biomaterials, 97
  biological characterization technique, 108–112
    in vitro characterization, 110–111
    in vivo characterization, 111–112
  chemical characterization techniques, 97–102
    infrared spectroscopy, 97–99
    mass spectrometry, 102
    nuclear magnetic resonance (NMR) spectroscopy, 101–102, 101*f*
    Raman spectroscopy, 99
    ultraviolet–visible spectroscopy, 100–101
    X-ray photoelectron spectroscopy (XPS), 99–100
  physical characterizations techniques, 102–108
    atomic force microscopy (AFM), 103–104, 104*f*
    contact angle measurement, 105–106, 105*f*
    gas adsorption measurements, 108
    mercury intrusion porosimetry, 106–108
    scanning electron microscope (SEM), 102–103
    transmission electron microscopy (TEM), 103
    X-ray diffraction (XRD), 104–105
Chemical characterization techniques, 97–102
  infrared spectroscopy, 97–99
  mass spectrometry, 102
  nuclear magnetic resonance (NMR) spectroscopy, 101–102, 101*f*
  Raman spectroscopy, 99
  ultraviolet–visible spectroscopy, 100–101
  X-ray photoelectron spectroscopy (XPS), 99–100
Chitin, 51, 344
Chitin/chitosan, 127

Chitosan, 26*t*, 51–52, 127, 162–163, 229, 328*t*, 480–481
Chitosan scaffolds, 325
Chitosan/poly(lactic acid glycolic acid), 164*t*
Chitosan-based scaffolds, 344
Chlorhexidine (CHX), 310
Cholecalciferol, 506–507
Chondroitin sulfate, 128
Ciprofloxacin (CIPX), 300
Closed apices, 307
Collagen, 26*t*, 30, 52, 67, 127, 143, 328*t*, 343, 480
Collagen/HA composites, 134–135
Collagen-based scaffolds, 228–229, 324
  compound collagen scaffolds, 229
  fibrin-based scaffolds, 230
  gelatin-based scaffolds, 229–230
  pure collagen scaffolds, 228–229
Collagen-chitosan scaffold, 229
Collagen-elastin membrane, 229
Collagen-GAG-chitosan (CGC) scaffolds, 229
Collagen-glucosaminoglycan (C-GAG) matrix, 229
Composite biomaterials, 134–135
Composite scaffolds, 31–32
Composites, 65
Compound collagen scaffolds, 229
Computer-aided design (CAD), 34–37, 163, 284, 390–391, 458
Computer-aided manufacturing (CAM), 34–37, 284, 458
Computerized tomography (CT), 37
Conductive polymers, 327
Cone beam computerized tomography (CBCT), 458
Connective tissue, 52, 224
Connective tissue flap, 258*f*
Connective tissue graft (CTG), 257
Contact angle measurement, 105–106, 105*f*
Contracted bovine skin collagen gels (CCG), 228–229
COX-2 selective inhibitors/coxibs, 511
Craniofacial and dental tissue-engineering approaches, 368–369
Craniofacial defect, 3D virtual imaging of, 459*f*
Craniofacial disfigurement, 429
Craniofacial scaffolds, 34–37
Craniofacial surgery, orthodontics, and tissue engineering, 445
  alveolar bone defects and repairing techniques, 448–451
  future developments, 457–460
  low-intensity pulsed ultrasound, 455–457
    clinical use of low-intensity pulsed ultrasound in orthodontics, 456–457
    low-intensity pulsed ultrasound conclusions, 457
    mechanical action of low-intensity pulsed ultrasound, 455–456
  periodontal ligament (PDL) tissue engineering, 451–455
  scaffolds, 446
  stem cells, 446–447
Craniofacial tissue engineering, 435–438, 446
  hydrogels in. *See* Hydrogels, in craniofacial tissue engineering
  tissue-engineered bone grafts, 435–437
  tissue-engineered cartilage grafts for cartilage craniofacial features, 437–438
Cryopreservation, 146
Cryoprotectants, 146
CXCL8, 247
Cyclic olefin copolymer (COC), 35*t*
Cytokeratins (CK), 224
  CK 14, 224–225, 476
Cytotoxicity testing, 110, 118–120

D

Decellularization techniques, 142–143
Decellularized matrix, 375
Deciduous teeth, 468, 468*f*
Deepidermalized dermis (DED), 227
Demineralized freeze-dried bone allograft (DFDBA), 409–410
Dental epithelium, 469
Dental follicle progenitor cells (DFPCs), 187
Dental follicle stem cells (DFSC), 473–474
Dental implants, 12–13, 405, 407, 415, 467
  surface modification of. *See* Surface modification methods
Dental papilla cells (DPCs), 470–471
Dental prosthesis and preprosthetic surgery, 406

Dental pulp irritation tests, 123
Dental pulp stem cells (DPSCs), 173–177, 186, 327, 412–413, 434–435, 473, 476–477
attachment to the gelatin-based scaffolds, 188*f*
morphological changes of, through neuronal differentiation, 329*f*
Dentin, 68, 143–144, 468–469
Dentin matrix protein 1 (DMP-1), 30, 475
Dentin sialophosphoprotein (DSPP), 30
Dentin sialoprotein (DSP), 475
Dentine, 68
Dermagraft, 228
Dextran, 51
1,25-Dihydroxy cholecalciferol, 506–507
1,25-Dihydroxyvitamin D, 389
4-(*N*,*N*-Dimethylamino) phenethyl alcohol, 79
N,*N*-dimethylaminobenzyl alcohol, 79
Discrete crystalline deposition (DCD), 90
Distraction osteoogenesis (DO), 406–407
DOX, 173–177
Drug delivery systems, 251–252
Dual acid etching (DAE), 87
Dual-energy X-ray absorptiometry (DXA), 505
DynaMatrix, 263–265
Dysplasia and oral cancer, in vitro models of, 245–247
Dysplasia-scoring system, 245

E
Ectoderm, 193–194
Eicosanoids, 510, 511*f*
Electrolytic process, 89
Electromicroscopy, 88
Electrospinning, 35*t*, 159–162, 285, 321–322
effect of parameters, 161–162
materials properties, 161
principles and methods, 160
Embryoid bodies (EBs), 475–476
Embryonic stem cells (ESCs), 446–447, 472, 475–476
Embryonic tooth germ (ETG), 486–489
Enamel, 68
Enamel matrix derivatives (EMDs), 12
Endoderm, 193–194
Endodontic usage tests, 124
Endoneurium, 320
Engineered blood vessels, clinical transplantation of, 369
Engineered neural tissue (EngNT) constructs, fabrication of, 325*f*
Enzyme-linked immunosorbent assay (ELISA) kits, 198
Epidermal growth factor (EGF), 226
EpiGingival, 225
Epineurium, 320
EpiOral, 225
Epithelial cell rests of Malassez (ERM), 470–471
Epithelium dental stem cells, 453–454
Esophageal stenosis, 235
Estrogen, 389, 514
Ethyl-4-dimethylaminobenzoate, 79
Ethylenediamine tetraacetic acid (EDTA), 310
European regulators, 148–149
Expanded polytetrafluoroethylene (ePTFE), 409–410
Extracellular matrix (ECM), 30, 47–50, 52, 54–55, 127, 225–226, 246–247, 354, 371–372, 388–390, 405, 432–433, 436–437
Extracellular matrix membrane, 262–265
Extracellular matrix protein coating, 91
Extracted human tooth dentin, 144
Extraoral grafting, 234–235
Extrusion and rapid prototyping, 321

F
Facial muscles, 353
anatomy and structure of, 353–354
cells, 354–355
future trends, 360
laboratory and clinical challenges, 355–360
scaffolds, 355
tissue engineering approaches, 354
Feeler gauge, 509, 509*f*
Fiber formation, 159–160
Fibrin, 481
Fibrin and fibrinogen, 26*t*
Fibrin scaffolds, 326
Fibrin-based adhesive biomaterials, 143
Fibrin-based scaffolds, 230

Fibroblast growth factor (FGF), 393–397, 476, 516–517
in experimental bone remodeling, 516–517
pharmacological properties, 516
Fibroblast-populated skin substitutes, 228
Fibroblasts, 225–226, 229, 231–232, 452
Fibronectin, 26*t*
Fluoride ion, 71
Fluoride-modified surface, 89
Foam replica technique, 284
Follicle dermal papilla-derived MSCs (FDPMC), 474–475
Food and Drug Administration (FDA), 148
45S5 glass, 8–9
biocompatibility of, 9
Fourier transform infrared (FTIR) spectrometer, 98–99, 98*f*
Free gingival flap, 258*f*
Free gingival graft (FGG), 257
Freeze drying, 35*t*, 147, 159, 323–324, 408–409
Freeze-dried bone allograft (FDBA), 409–410
Full-thickness oral mucosa engineering, 225–232
scaffolds, 226–231
Fused deposition modeling (FDM), 38–39, 163, 284, 390–391
Fused filament fabrication (FFF), 163
Fusobacterium nucleatum, 250

G

Gas adsorption measurements, 108
Gas foaming, 34, 35*t*, 159
Geistlich Mucograft 3D Collagen Matrix, 266*f*
Gelatin, 53, 58, 127, 324–325
-based scaffolds, 229–230
Gelation process, 12
GelMA, 57–58
Gels, 11
Gene therapy, 343, 368–369
"Genotoxic" material/"genotoxin", 120
Genotoxicity and carcinogenicity testing, 110–111
Gingiva, 282
keratinized, 265–266
Gingival epithelium, 282
Gingival margin-derived cells (GMC), 170–173
Gingival recession, 260–262, 268
Glass ionomer, 71–72
Glycosaminoglycans (GAGs), 52, 128, 229
Gonadal hormones, 514–516
in experimental bone remodeling, 514–516
pharmacological properties, 514
G-rich alginate, 55
Grit blasting, 87
Growth and differentiation factor (GDF), 449–450
Growth factors (GFs), 205, 212*t*, 264, 448–450, 482–485
bone morphogenetic proteins (BMPs), 483–484
insulin-like growth factor, 485
in oral and maxillofacial nerve repair, 329–330
for oral and maxillofacial regeneration applications, 205
bone morphogenic proteins (BMPs), 209–211
future trends, 213
insulin-like growth factors (IGFs), 207–208
platelet-derived growth factor (PDGF), 205–207
platelet rich plasma (PRP), 211
transforming growth factor beta (TGF-β), 208–209
vascular endothelial growth factor (VEGF), 212
platelet-derived growth factor, 482–483
REP therapies, 310
transforming growth factors, 484–485
Guided bone regeneration (GBR), 406–407, 410–412, 414–415, 448–449
Guided tissue regeneration (GTR), 173, 284
Guinea pig maximization tests, 122

H

Hair follicles, 474–475
Hard tissue biomaterials, 143–144
Health Research Authority (HRA), 149
Hemifacial microsomia and ankyloglossia, 234

Hemocompatibility testing, 110, 120
Hemolysis, 121
Hertwig epithelial root sheath (HERS), 470–471
High-density polytetrafluoroethylene (dPTFE), 409–410
Human amniotic epithelial cells, 343
Human dental pulp cells (hDPSCs), 28–29
Human embryonic stem cells (hESCs), 392
Human exfoliated deciduous teeth, stem cells from, 186
Human MSCs (hMSCs), 368–369
Human Tissue Authority (HTA), 149
Human-derived hydrogels, 344
Hyaluronan (HA), 128
Hyaluronic acid (HA), 128, 325–326, 328*t*, 481
Hyaluronic acid-based hydrogels, 344
Hybrid scaffolds, 231, 345
Hydrogel polymers, injectable, 309
Hydrogel scaffolds, 50
Hydrogels, in craniofacial tissue engineering, 47–58, 49*t*, 50*t*
  current status and future prospects, 58
  natural hydrogels, 51–55
    agarose, 53
    alginate, 53–55
    chitosan, 51–52
    collagen, 52
    dextran, 51
    gelatin, 53
    glycosaminoglycans, 52
    silks, 52
  synthetic hydrogels, 56–58
    GelMA, 57–58
    polyacrylamide (PAM)-based hydrogel, 57
    polyethylene glycol (PEG), 57
    polyglycolic acid, 56
    polylactic acid, 56
    polypropylene fumarate (PPF), 56–57
Hydrogels, injectable, 324
Hydrophilicity, scaffolds with improved, 32–33
Hydroxyapatite (HA/HAP), 14–15, 31, 131–132, 162–163, 407, 412–413, 416, 478
Hydroxylapatite (HAp), 67
25-Hydroxyvitamin D (25OHD), 508–509
Hypermineralization, 389
Hypertrophic cartilage, tissue-engineered, 435–436
Hysteresis, 106

I

Ibuprofen, 517–518
ImageJ, 161
Imaging and spectroscopic diagnostic techniques, 248
Implantation testing, 111–112, 122–123
Impregnation, 86
In vitro characterization, 110–111
  cytotoxicity testing, 110
  genotoxicity and carcinogenicity testing, 110–111
  hemocompatibility testing, 110
  reverse transcription-polymerase chain reaction (RT-PCR), 111
In vitro tests, 108–109, 118–121
  cytotoxicity tests, 118–120
  hemocompatibilty testing, 121
  mutagenicity and genotoxicity testing, 120
In vivo bioreactor approach, 199–200, 199*f*
In vivo characterization, 111–112
  biodegradation test, 112
  implantation testing, 111–112
  sensitization, irritation, and toxicity tests, 111
Increment vascularization, current strategies to, 369–375
  decellularized matrix, 375
  growth factor and cytokines, 370–371
  mature and primary cell delivery, 371
  vascular inductive scaffold, 371–374
Induced pluripotent stem cells (iPSCs), 392, 476–477
Inferior alveolar nerve (IAN)
  encroachment, 408
  lateralization, 408, 408*f*
Inflamed periapical progenitor cells, 307
Infrared spectroscopy, 97–99
Initial drying, 159
Injectable hydrogels, 324
Inkjet 3-DP technique, 37–38
Inlay bone grafting, 407, 407*f*
Inner enamel epithelium (IEE), 469
Inosculation, 369–370

Institutional Review Board (IRB), 148
Insulin-like growth factors (IGFs), 205, 207–208, 416, 485
functions of, 212*t*
IGF-I, 393–397
Integrated Research Application System (IRAS), 149
Intensity-modulated radiation therapy (IMRT), 341–342
Interleukin (IL)-6, 247
Internal bubbling process (IBP), 51
International Organization for Standardization (ISO), 109, 109*t*
International regulations, 147–148
International Standards Organization (ISO), 147–148
Intracanal barrier, 311–312
Intracanal medication, 311
Intraoral grafting, 233–234
Invisalign, 458
IPSC-derived NCLCs (iNCLCs), 477
Irrigation, 310

K
Keratinization, 223–224
Keratinocytes, 223–225, 231–232, 282

L
Lamina dura, 282–283
Lamina propria, 225–226, 282
Laminin, 343–344
Laser ablation, 90–91
Laser etching and microarc oxidation, 88
Living cellular construct (LCC), 268–271
Local lymph node assay, 122
Low-intensity pulsed ultrasound (LIPUS), 455–457
clinical use of, in orthodontics, 456–457
mechanical action of, 455–456
Lyophilization, 147

M
Mass spectrometry, 102
Matrigel coating, 26*t*, 343–345
MatTek Corp., 251–252
Mature and primary cell delivery, 371
Maxillofacial defects, 197–198
Maxillofacial reconstruction, 429
Maxillofacial region, bone tissue engineering in, 385
challenges, 398
future trends, 399
natural alveolar bone structure, 388–390
bone cells, 389–390
bone components, 388
bone matrix, 388–389
strategies for, 390–391
tissue-engineered bone, 391–398
bioactive factors, 393–397
cells, 392
environmental factors, 397–398
scaffolds, 392–393, 393*t*, 394*t*
Maxillofacial tissues dental pulp stem cells, 186–187
Medical Devices Directive (MDD), 148–149
Medication-induced salivary gland dysfunction (MISGD), 341
Medicines and Healthcare products Regulatory Agency (MHRA), 149
Melt molding, 35*t*
Melt-derived methods, 10–11
Membrane integrity tests, 119–120
Mercury intrusion porosimetry (MIP), 106–108
Mesenchymal cells, 469
Mesenchymal stem, coapplication of cells with growth factors, 416–417
Mesenchymal stem cells (MSCs), 14, 16–17, 29–30, 32, 49, 132, 185, 307, 354–355, 368–369, 389, 392, 412, 433–434, 446–447, 453–454
markers, 308
in peri-implant hard tissue engineering, 414–416
in preprosthetic hard tissue engineering, 413–414
Mesoderm, 193–194
Methyl thiazolyl tetrazolium assay (MTT), 119–120, 198
Metronidazole (MTZ), 300
Microencapsulation techniques, 146–147
Microfabrication approach, 165–168
micromolding in microcapillaries (MIMIC), 167–168
replica molding (RM), 167
soft lithography, 166–167

Micromolding in microcapillaries (MIMIC), 167–168
Microsphere sintering, 162–163, 162*f*, 164*t*
Mineral trioxide aggregate (MTA), 307, 311–312
Minocycline (MIN), 300
Modified coronally advanced flap (MCAF), 268
Molecular self-assembly, 35*t*
Mucograft, 265
MucomatrixX, 262–263, 262*f*
Mucosa and gingiva usage tests, 124
Mucosa-/gingiva-derived mesenchymal stem cell (MSC), 368–369
Mucosal models, 246–247, 249–250
  of bacterial infection, 250–251
Mucous membrane irritation tests, 122
Multipotent stromal stem cells, 472–475
Multi-tissue scaffolds for oral and dental regeneration, 169–173
Mutagenicity and genotoxicity testing, 120
Myoblasts, 353–355
Myogenic potency, 354–355

N

Nanohydroxyapatite (n-HA), 132, 173–177, 391
Nanotechnology coatings, 92–93
Nasal cartilage, tissue-engineered, 437–438
National Aeronautics and Space Administration (NASA), 197
National Research Ethics Service (NRES), 149
Natural alveolar bone structure, 388–390
  bone cells, 389–390
  bone components, 388
  bone matrix, 388–389
Natural biocomposites, 65, 67–68
Natural biodegradable three-dimensional scaffolds, 355
Natural bone, 412–413
Natural ECM, 127, 173–177, 481–482
Natural hydrogels, 51–55
  agarose, 53
  alginate, 53–55
  chitosan, 51–52
  collagen, 52
  dextran, 51
  gelatin, 53
  glycosaminoglycans, 52
  silks, 52
Natural materials, 50, 57, 67–68, 230, 480–482
Natural origin polymers, 127–129
  alginate, 128
  cellulose, 128–129
  chitin/chitosan, 127
  collagen/gelatin, 127
  glycosaminoglycan (GAGs), 128
Natural polymers, 30, 72–73, 126–127, 129, 323–326, 432–433, 480
Natural scaffolds, 30, 173–177, 309, 415–416
Naturally derived polymers, 25–28, 26*t*, 29*t*, 30–31
Naturally derived scaffolds, 227–228, 343–344
  acellular cadaveric dermis, 227
  amniotic membrane, 227–228
Neovascularization, 367, 369–371
Nerve tissue engineering, 319–327
  materials, 324–327, 328*t*
    conductive polymers, 327
    natural polymers, 324–326
    synthetic polymers, 326–327
  techniques, 321–324
    electrospinning, 321–322
    extrusion and rapid prototyping, 321
    freeze drying, 323–324
    injectable hydrogels, 324
    phase separation, 324
    porogen leaching, 322–323
Neural crest-like cells (NCLC), 477
Neural stem/progenitor cells (NSCs), 323*f*, 326–327
Neuramidase, 264
Neutral red uptake, 119–120
Nonsteroidal antiinflammatory drugs (NSAIDs), 510–511, 517–518
  in experimental bone remodeling, 518
  pharmacological properties, 517–518
Nonvital pulp therapy (NPT), 298, 299*f*
Nuclear magnetic resonance (NMR) spectroscopy, 101–102, 101*f*

O

Odontoblast-like cells, 30, 310, 474–477, 480–481, 484–485
Odontoblasts, 123, 299–300, 308, 470–471, 474–478, 481
Onlay bone grafting, 411–412, 411*f*
Open apices, 143–144, 306–307
Optical coherence tomography (OCT), 248
Oral and maxillofacial nerve repair
  applicable stem cells in, 327–328
  growth factors in, 329–330
Oral and maxillofacial region, applications of bioactive glasses in, 12–13
Oral and maxillofacial tissues, stem cells from. *See* Stem cells: from oral and maxillofacial tissues
Oral candidiasis disease modeling, 249–250
Oral epithelium, 223–224, 236, 249, 282
Oral mucosa, 4, 143, 159, 282
Oral mucosa tissue engineering, 221
  applications of tissue-engineered oral mucosa, 232–237
    in vitro applications, 235–237
    in vivo clinical applications, 233–235
  collagen-based scaffolds, 228–229
    compound collagen scaffolds, 229
    fibrin-based scaffolds, 230
    gelatin-based scaffolds, 229–230
    pure collagen scaffolds, 228–229
  fibroblast-populated skin substitutes, 228
  full-thickness oral mucosa engineering, 225–232
    scaffolds, 226–231
  future trends, 237
  hybrid scaffolds, 231
    cells, 231–232
  naturally derived scaffolds, 227–228
    acellular cadaveric dermis, 227
    amniotic membrane, 227–228
  normal human oral mucosa, 223–224
  oral epithelium, 223–224
    connective tissue, 224
  split-thickness oral mucosa engineering, 224–225
  synthetic scaffolds, 230–231
Oral mucosal models of bacterial infection, 250–251
Oral nerve tissue repair and regeneration, 319
  applicable stem cells in oral and maxillofacial nerve repair, 327–328
  clinical applications and future trends, 330
  growth factors in oral and maxillofacial nerve repair, 329–330
  nerve tissue engineering principles, 321–327
    materials, 324–327
    techniques, 321–324
  peripheral neuroanatomy applicable to dentistry, 320
Oral squamous cell carcinoma (OSCC), 245–246, 248
Organic compounds coatings, 92
Organotypic skin model, 245
OrthoCAD, 458
Orthodontic tooth movement (OTM), 506, 512–514, 516, 519–520
Orthodontically induced inflammatory root resorption (OIIRR), 517, 519–520
Orthodontic-induced root resorption (OIRR), 516
Orthodontics, 454–455, 458
  clinical use of low-intensity pulsed ultrasound in, 456–457
Oseomalacia, 508
Osseointegration, 85–86, 89, 91–92, 166–167
Osteoarchitechture synthesis and resorption, 389
Osteoblasts, 9, 70–71, 169–170, 368, 389, 452, 507, 512, 516
Osteocalcin, 30, 206, 507
Osteoclasts, 283, 390, 452, 454, 506–507, 510
Osteocytes, 368, 389
Osteogenesis, 48–49, 91–92, 198–199, 387, 410–411, 431, 433, 450, 503, 506–507
Osteoinductivity of CPCs, 14
Osteoprogenitor cells, 9, 91, 389, 503, 513, 516
Osteoradionecrosis, 185
Outer enamel epithelium (OEE), 469
Oxytalan fibers, 452

P

Pamidronate disodium, 518–519
Pamidronate disodium pentahydrate, 518–519
Pamidronic acid, 518–520
Parathyroid hormone (PTH), 389, 504
Particle leaching, 284
Particulate-leaching technique, 34
PEGylated fibrin scaffold, 32
Penetrometer, 107–108
Peptide coating, 91–92
Perfusion bioreactors, 197
Peri-implant bone regeneration, 405, 414–416
Peri-implant hard tissue engineering, mesenchymal stem cells in, 414–416
Perineurium, 320
Periodontal and peri-implant hard tissue regeneration, 405
  conventional approaches, 406–412
    anatomic repositioning, 406–408
    bone augmentation techniques, 408–412
    dental prosthesis and preprosthetic surgery, 406
    guided bone regeneration, 410–411
    inferior alveolar nerve (IAN) lateralization, 408, 408*f*
    inlay bone grafting, 407, 407*f*
    onlay bone grafting, 411–412, 411*f*
    sinus augmentation, 410
    socket preservation, 409–410
  future direction, 418–419
  tissue engineering, 412–418
    coapplication of mesenchymal stem cells with growth factors, 416–417
    mesenchymal stem cells in peri-implant hard tissue engineering, 414–416
    mesenchymal stem cells in preprosthetic hard tissue engineering, 413–414
Periodontal ligament (PDL), 169–170, 281, 284
  PDL tissue engineering, 451–455
Periodontal ligament stem cells (PDLSCs), 186, 188, 473–474
Periodontal regeneration, layered scaffolds for, 279
  biological evaluation, 287–289
  challenges for, 290
  fabrication methods, 284–286
    individual layers, 284–286
    interface, 286
  in vitro and in vivo studies, 289*t*
  limitations, 290
  requirements of, 283–284
Periodontal regeneration
  via PLGA/calcium phosphate (CaP) construct, 172*f*
Periodontal soft tissue reconstruction, 257
  allogenic materials, 260–262
    acellular dermal matrix allograft (ADMA), 260–262
  alloplastic materials, 271–272
    three-dimensional printing scaffolds, 271–272
  autogenous materials, 267–268
    platelet-rich fibrin (PRF) membrane, 267–268
  autologous grafts, 257–259
  categories of, 259*t*
  general considerations for biomaterials in, 259
  tissue engineering, 268–271
    living cellular construct, 268–271
  xenogeneic materials, 262–267
    bilayer collagen matrix, 265–267
    extracellular matrix membrane, 262–265
Periodontal tissue engineering, 283, 290
Periodontal tissue regeneration, 173–177, 279, 483
Periodontium, 187, 257, 280*f*, 281, 283–284
  structure of, 280–283, 280*f*
Peripheral neuroanatomy applicable to dentistry, 320
Permanent teeth, 467–468, 468*f*
Pharmacological agents for bone remodeling, 503
  bisphosphonates (pamidronic acid, zoledronic acid), 518–520
  calcium, 504–506
  fibroblast growth factor, 516–517
  gonadal hormones, 514–516
  nonsteroidal antiinflammatory drugs (ibuprofen, aspirin) and acetaminophen, 517–518

prostaglandins, 510–512
thyroid hormone, 513–514
vitamin D, 506–510
Phase separation technique, 35*t*, 158–159, 324
Phosphate bioactive glass, 10
Phosphate-based glass fiber, 355
Phosphatidylinositol 3-kinase (PI 3-k), 206
Photofunctionalization, 91
Physical characterizations techniques, 102–108
atomic force microscopy (AFM), 103–104, 104*f*
contact angle measurement, 105–106, 105*f*
gas adsorption measurements, 108
mercury intrusion porosimetry, 106–108
scanning electron microscope (SEM), 102–103
transmission electron microscopy (TEM), 103
X-ray diffraction (XRD), 104–105
Placodal cells, 469
Plasma spraying, 89
Plasminogen activator inhibitor (PAI-1), 246–247
Platelet rich fibrin (PRF), 306, 408
Platelet rich plasma (PRP), 211, 306, 308
Platelet-derived growth factor (PDGF), 205–207, 416, 482–483
functions of, 212*t*
Platelet-rich fibrin (PRF) membrane, 267–268
Platelet-rich fibrin (PRF) tube, 408
Platelet-rich plasma (PRP), 211, 414–416
Pluripotent cells of pulp, 308
Polyacrylamide (PAM)-based hydrogel, 57
Polyacrylamide gels, 345
Polyamide (PA), 26*t*, 31–32, 57
Polycaprolactone (PCL), 56, 130, 162–163, 173–177, 271–272, 321, 326–327, 328*t*, 432–433, 478–479
Polycarbonate permeable membranes, 230–231
Poly-D,L-lactic acid (PDLLA) solution, 161
Polydimethylsiloxane (PDMS), 166–168
Polyethylene glycol (PEG), 26*t*, 32, 57, 126, 129, 344–345
Poly (ethyleneglycolterephthalate)–poly (butylenes terephthalate) (PEGT/PBT), 230–231
Poly(ethyleneimine) (PEI), 54
Poly ethylene oxide (PEO), 129
Polyglycerol sebacic acid (PGS), 26*t*
Polyglycolic acid (PGA), 26*t*, 28–29, 31, 56, 126, 159, 326–327, 344, 432–433, 478–479
Poly-glycolic-co-lactic acid, 126
Polylactic acid (PLA), 26*t*, 28, 31, 49, 56, 129–130, 326–327, 328*t*, 478
Poly lactic-co-glycolic acid (PLGA), 28–31, 33–34, 159, 162–163, 164*t*, 167, 230–231, 321, 328*t*, 344–345, 473, 478
Polylactide-co-glycolide acid (PLGA), 170
Poly L-lactic acid (PLLA), 29, 159, 326–327, 344, 432–433
Polymeric biomaterials, 126–131
natural origin polymers, 127–129
alginate, 128
cellulose, 128–129
chitin/chitosan, 127
collagen/gelatin, 127
glycosaminoglycan (GAGs), 128
synthetic polymers, 129–131
poly(caprolactone) (PCL), 130
poly ethylene glycol (PEG) and poly ethylene oxide (PEO), 129
poly-glycolic acid (PGA) and poly-lactic acid (PLA), 129–130
polypropylene fumarates (PPF), 130–131
polyurethanes (PU), 131
polyvinyl alcohol (PVA), 130
Polymers for oral and dental tissue engineering, 25
polymeric scaffolds, different types of, 25–34
biomimetic scaffolds, 32–34
composite scaffolds, 31–32
naturally derived polymers, 30–31
synthetic polymers, 28–30
scaffold fabrication, methods for, 34–39
complex 3-D scaffold development, recent advances in, 34–39
conventional scaffold fabrication techniques, 34

Polymers for oral and dental tissue engineering (*Continued*)
fused deposition modeling (FDM), 38–39
selective laser sintering (SLS), 38
stereolithography (SLA), 38
Poly-methyl methacrylate polymer, 126
Polymethylmethacrylate (PMMA), 68–71, 173–177
Polypropylene (PP), 38–39
Polypropylene fumarate (PPF), 26*t*, 31, 56–57, 130–131
Polypyrrole (PPy), 327
Polyurethanes (PU), 131
Polyvinyl alcohol (PVA), 130, 173–177
Poly-ε-caprolactone (PCL), 28, 30
Porogen, 322–323
leaching, 322–323
Porosity, 106, 265
Porphyromonas gingivalis, 250
Postnatal and embryonic dental cells, 472
Premaxillary deficiency, patient with, 406*f*
Preprosthetic hard tissue engineering, mesenchymal stem cells in, 413–414
Preservation and storage, of biomaterials, 146–147
acellular biomaterials, 147
cell-containing products, 146–147
Prevascularization, 369–370
Processing of biomaterials, 142–145
animal and human-derived biomaterials, 142–144
hard tissue biomaterials, 143–144
soft tissue biomaterials, 142–143
natural scaffolds and synthetic biomaterials, 144–145
Progenitor cells, 452
Prostaglandins, 510–512
antagonists, 510–511
function, 510
pharmacological properties, 510
prostaglandin E_2, 512
Protein kinase B (PKB/Akt), 206
Proton beam therapy, 341–342
Pulp therapy, 298
nonvital pulp therapy (NPT), 298
vital pulp therapy (VPT), 298
PuraMatrix, 168, 309
Pure collagen scaffolds, 228–229
Puros allograft, 261–262, 261*f*

Q
Quality management system (QMS), 148–149

R
Radiation of the salivary glands, 340
Radio frequency (RF) transmitter, 101
Radiotherapy-induced oral mucositis, 247
Raman spectroscopy, 99
Rapid prototyping (RP), 34–39, 158, 321, 330
Recombinant human bone morphogenetic protein 2 (rhBMP-2), 187
Recombinant protein therapy, 368–369
Recommended daily (dietary) allowance (RDA), 505
Reconstituted human oral epithelial (RHOE) models, 249
Regenerative dentistry, 169
Regenerative endodontic procedures (REP), 297–312
case studies, 301*t*
clinical protocols of, 310–312
intracanal barrier, 311–312
intracanal medication, 311
irrigation, 310
in different apical cases, 306–308
avulsed teeth, 307–308
closed apices, 307
open apices, 306–307
key components of, 308–310
cells, 308
growth factors, 310
scaffolds, 308–309
Regenerative therapy, 299–300, 306, 308
biological basis of, 300*f*
Regulatory issues, 147–149
European regulators, 148–149
international regulations, 147–148
United Kingdom regulatory requirements, 149
United States regulatory agencies, 148
Removal torque tests, 85
Replica molding (RM), 166–167

Resin-modified glass ionomer, 71–72
Resorbable bioceramics, 7–8
Resorptive cells, 452
Reverse transcription-polymerase chain reaction (RT-PCR), 111
RGD (arginine-glycine-aspartic acid tripeptide), 54
Rickets, 508
Rocking platform bioreactors, 197
Root canal therapy (RCT), 297

S

Salivary glands, 337
  diseases and pathology, 339–341
    infections, 339–340
    medication-induced salivary gland dysfunction (MISGD), 341
    radiation damage, 340
    Sjogren's syndrome, 340
    tumors, 340–341
  function, anatomy, and histology, 338–339
  prevention and preservation of, 341–342
    organ-sparing radiotherapy, 341–342
    preventative medication, 342
    salivary gland tissue transfer, 342
  regeneration challenges, 345–346
  tissue-engineering approaches, 342–345
    cells and bioactive agents, 342–343
    scaffolds, 343–345
Salt leaching, 322–323
Scaffold fabrication techniques, 34–39, 158–168
  additive manufacturing (AM), 163–165
  complex 3-D scaffold development, recent advances in, 34–39
    rapid prototyping (RP)/solid free-form fabrication (SFF), 34–39
    3-D printing (3-DP), 37–38
  conventional scaffold fabrication techniques, 34
  electrospinning, 159–162
    effect of parameters, 161–162
    important principles and methods, 160
    materials properties, 161
  freeze drying, 159
  fused deposition modeling (FDM), 38–39
  gas foaming, 159
  microfabrication approach, 165–168
    micromolding in microcapillaries (MIMIC), 167–168
    replica molding (RM), 167
    soft lithography, 166–167
  microsphere sintering, 162–163, 162*f*, 164*t*
  phase separation, 158–159
  selective laser sintering (SLS), 38
  self-assembly approach, 168
  solvent casting and leaching, 158
  stereolithography (SLA), 38
Scaffolds in tissue engineering of teeth, 477–482
  natural materials, 480–482
  synthetic materials, 478–479
Scaffolds' applications in oral tissue engineering, 173–177
Scanning electron microscope (SEM), 102–103, 161
Secondary ion mass spectrometry (SIMS), 102
Selective laser sintering (SLS), 38, 163–165, 390–391
Self-assembling nanofibrous scaffolds, 309
Self-assembly approach, 168
Self-assembly systems, 35*t*
Self-cell repair, 185
Serum-free culture medium, 232
Sialadenitis, 339
Silicate bioactive glass, 8–9
Silk fibroin scaffolds, 344
Silks, 52
Silorane material, 78–79
Silver nanoparticles, 71
Sinus augmentation, 410
Sjogren's syndrome, 340
Skin sensitization tests, 121–122
SkinEthic laboratories, 225
SkinEthic model of oral epithelium, 251
SMAD proteins, 208–209
Small interfering RNAs (siRNAs), 128
Socket fillers, 409–410
Socket preservation, 409–410
Sodium hyaluronate/chitosan complex, 32
Sodium hypochlorite, 310
Soft lithography, 166–167
Soft tissue biomaterials, 142–143
Sol–gel methods, 11–12, 89

Solid free-form fabrication (SFF), 34–39, 163–165
Solvent casting and leaching, 158
Solvent-casting particulate-leaching, 35*t*
Solvent-casting technique, 34
Spinner-flask bioreactor, 197–199
Split-thickness oral mucosa engineering, 224–225
Spreading parameter, 106
Staphylococcus aureus, 51, 339
Stellate reticulum, 469
Stem cells, 47, 58, 446–447, 453–454
  adipose-derived stem cells (ASCs), 415–416
  bone marrow-derived mesenchymal stem cells (BMMSCs), 413–416
  from dental and nondental tissues, 472–477
    adult stem cells, 472–475
    embryonic stem cells, 475–476
    induced pluripotent stem cells (iPSCs), 476–477
  dental pulp stem cells (DPSCs), 412–413
  mesenchymal stem cells (MSCs), 354–355, 412
    coapplication of, with growth factors, 416–417
    in peri-implant hard tissue engineering, 414–416
    in preprosthetic hard tissue engineering, 413–414
  from oral and maxillofacial tissues, 185
    application of clinically relevant stem cells, 187–188
    dental follicle progenitor cells (DFPCs), 187
    future trends, 188–189
    human exfoliated deciduous teeth, 186
    periodontal ligament stem cells (PDLSCs), 186
    stem cells from apical papilla (SCAPs), 186–187
  umbilical cord mesenchymal stem cells (UCMSCs), 415–416
Stem cells from apical papilla (SCAPs), 186–187
Stem cells from human exfoliated deciduous teeth (SHEDs), 186, 447
Stem cells of apical Papilla (SCAP), 307, 310
Stem/progenitor cells, 344, 354–355
Stereolithography (SLA), 38, 163
Sterilization of biomaterials, 145–146
Stratum intermedium, 469
Streptococcus mutans, 71
Sublingual glands, 338
Surface hydrophilicity, 89
Surface modification methods, 90–93
  additive methods, 88–90
    biochemical and organic compound coating, 90
    ceramic coating methods, 89
    fluoride-modified surface, 89
  antisclerostin immunoglobulin coating, 92
  discrete crystalline deposition (DCD), 90
  extracellular matrix protein coating, 91
  laser ablation, 90–91
  nanotechnology coatings, 92–93
  organic compounds coatings, 92
  peptide coating, 91–92
  photofunctionalization, 91
  subtractive methods, 86–88
    acid etching, 87
    anodized surface implants, 88
    blasting and acid etching (SLA), 87
    dual acid etching (DAE), 87
    grit blasting, 87
    laser etching and microarc oxidation, 88
Synthetic alloplastic materials, 173–177
Synthetic biocomposites, 68–69
Synthetic biodegradable three-dimensional scaffolds, 355
Synthetic biomaterials, 344–345
Synthetic cells, 452
Synthetic hydrogels, 56–58
  GelMA, 57–58
  polyacrylamide (PAM)-based hydrogel, 57
  polyethylene glycol (PEG), 57
  polyglycolic acid, 56
  polylactic acid, 56
  polypropylene fumarate (PPF), 56–57
Synthetic materials, 478–479
Synthetic polymers, 26*t*, 28–30, 29*t*, 129–131, 326–327, 432–433
  poly(caprolactone) (PCL), 130

poly ethylene glycol (PEG) and poly ethylene oxide (PEO), 129
poly-glycolic acid (PGA) and poly-lactic acid (PLA), 129–130
polypropylene fumarates (PPF), 130–131
polyurethanes (PU), 131
polyvinyl alcohol (PVA), 130
Synthetic scaffolds, 230–231, 309

T

Temporomandibular joint (TMJ), 447
Tetracalcium phosphate, 412–413
N,*N*-3,5-Tetramethylaniline, 79
3D printing (3-DP), 37–38, 163, 285, 330, 390–391
3D printing scaffolds, 173–177, 271–272
3-D wax printing system, 286*f*
Thyroid hormone, 513–514
in experimental bone remodeling, 513–514
pharmacological properties, 513
Time-of-flight SIMS (TOF-SIMS), 102
Tissue engineering bone grafts, 431–435
cell sources for bone and cartilage tissue engineering, 434–435
scaffolds for bone and cartilage tissue engineering, 432–434
Tissue-engineered models of oral soft tissue diseases, 245
bisphosphonate-related osteonecrosis, 247–248
drug delivery systems, 251–252
future trends, 252
imaging and spectroscopic diagnostic techniques, 248
in vitro models of dysplasia and oral cancer, 245–247
oral candidiasis disease modeling, 249–250
oral mucosal models of bacterial infection, 251
immune response studies, 251
radiotherapy-induced oral mucositis, 247
Tissue-engineered nasal cartilage, 437–438
Titanium, 85–87, 93*t*
Titanium dioxide, 91, 162–163
Tooth formation, principal stages of, 470*f*
Tooth germ cell-conditioned medium (TGC-CM), 475
Tooth tissue engineering, 467
biological structure of teeth, 468–469
developmental process of tooth formation, 469–471
future trends, 489–490
growth factors, 482–485
bone morphogenetic proteins (BMPs), 483–484
insulin-like growth factor, 485
platelet-derived growth factor, 482–483
transforming growth factors, 484–485
postnatal and embryonic dental cells, 472
scaffolds in tissue engineering of teeth, 477–482
natural materials, 480–482
synthetic materials, 478–479
stem cells from dental and nondental tissues, 472–477
adult stem cells, 472–475
embryonic stem cells, 475–476
induced pluripotent stem cells (iPSCs), 476–477
whole teeth regeneration: Recent strategies and techniques, 485–489
TR146 cells, 249, 251
Trabecular bone score (TBS), 505
Transforming growth factors, 484–485
TGF-β, 205, 208–209, 212*t*, 476
Transmission electron microscopy (TEM), 103
Treated dentin matrix (TDM), 473
Tricalcium phosphate (TCP), 7–8, 15–17, 131
Trifluroethanol (TFE), 173–177
Tri-iodothyronine (T3), 513
Trypsin, 264
Tumors of salivary glands, 340–341

U

Ultraviolet–visible spectroscopy, 100–101
Umbilical cord (UC), 475
Umbilical cord mesenchymal stem cells (UCMSCs), 415–416
United Kingdom regulatory requirements, 149
United States Pharmacopeia (USP), 109, 109*t*

United States regulatory agencies, 148
Urethane dimethacrylate (UDMA), 68–69
Urokinase-type plasminogen activator (uPA), 246–247
Usage tests, 123–124
  dental pulp irritation tests, 123
  endodontic usage tests, 124
  mucosa and gingiva usage tests, 124

V

Vacuum deposition, 89
Van der Waals forces, 108
Vascular endothelial growth factor (VEGF), 212, 212*t*, 368–370, 393–397, 416
Vascular inductive scaffold, 371–374
Vascular network, 368–370, 372, 375–376
Vascularization, 367
  clinical transplantation of engineered blood vessels, 369
  craniofacial and dental tissue-engineering approaches, 368–369
  current strategies to increment vascularization, 369–375
    decellularized matrix, 375
    growth factor and cytokines, 370–371
    mature and primary cell delivery, 371
    vascular inductive scaffold, 371–374
  key challenges and future trend, 375–376
Vasculature structure incorporation, 369–370
Vasculogenesis, 367
Vital pulp therapy (VPT), 298
Vitamin D, 504, 506–510
  clinical applications of vitamin D compounds, 508
  in experimental bone remodeling, 509–510
  pharmacological properties, 507
  specific characteristics, 508–509

W

Washburn equation, 107–108
Wet spinning, 284
Whole teeth regeneration, 485–489, 487*t*
Whole tooth tissue engineering (TE), 486*f*
Whole-organ decellularized scaffolds, 344

X

Xenogeneic materials, 262–267
  bilayer collagen matrix, 265–267
  extracellular matrix membrane, 262–265
    DynaMatrix, 263–265
    MucomatrixX, 262–263
Xenografts, 76, 408–409
Xerogels, 11
Xerostomia, 341–342
X-ray diffraction (XRD), 104–105
X-ray photoelectron spectroscopy (XPS), 99–100

Y

Yttria-stabilized zirconia (YSZ), 167–168

Z

Zinc oxide eugenol cements, 125–126
Zirconia hydroxyapatite composite, 69
Zoledronate, 519
Zoledronic acid, 518–520

Biomaterials for Oral and Dental Tissue Engineering examines the combined impact of materials, advanced techniques, and applications of engineered oral tissues. With a strong focus on hard and soft intraoral tissues the book looks at how biomaterials can be manipulated and engineered to create functional oral tissue for use in restorative dentistry, periodontics, endodontics, and prosthodontics.

Part 1 chapters review a wide range of biomaterial classes for oral tissue engineering. Further topics include material characterization and modification as well as biocompatibility and biotoxicity. Part 2 reviews strategies for biomaterial scaffold design whilst chapters in Parts 3 and 4 review soft and hard tissues.

Covering current knowledge of material production, evaluation, challenges, and applications as well as future trends, this book is a valuable resource for materials scientists and researchers in academia and industry.

Lobat Tayebi is an Associate Professor and Director of Research at Marquette University School of Dentistry. She received her PhD from University of California-Davis in 2011. She is a researcher in materials and regenerative medicine with multiple patents in the field. Her publication list comprises of more than 115 peer-reviewed articles including papers in Nature Materials and Advanced Materials. Her current research activities cover projects in smart materials, interfacial hard/soft tissue expansion, growth factor delivery, vascularization and stem cell seeding in patient specific 3D-bioprinted scaffolds, treatment of complex multi-tissue oral and craniomaxillofacial defects, and preservation of oral tissues. She enjoys interdisciplinary projects and has genuine believe in the effectiveness of multidisciplinary research.

Keyvan Moharamzadeh is a Senior Clinical Lecturer and Honorary Consultant in Restorative Dentistry at the University of Sheffield, United Kingdom. He is a registered specialist in Prosthodontics, Periodontics, Endodontics and Restorative Dentistry and has been closely involved with undergraduate and postgraduate teaching and research at the School of Clinical Dentistry. He is leading multidisciplinary internationally recognised research in the field of Dental Biomaterials and Tissue Engineering and has extensively published in the literature and given numerous presentations in the national and international conferences. His research activities comprise tissue engineering; biocompatibility of dental biomaterials; synthesis and analysis of polymers and composites in dentistry; and physical and optical properties of dental materials.

WP
WOODHEAD
PUBLISHING
An imprint of Elsevier
elsevier.com/books-and-journals

ISBN 978-0-08-100961-1