Current Advances in Oral and Craniofacial Tissue Engineering

Editors

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Oral tissue engineering involves the study of current approaches for \textit{in vitro} regeneration of soft and hard tissues located into the oral cavity. In this context, recent approaches involve the use of innovative biomaterials to replace the lost or damaged human oral tissues. Recent discoveries in material science and nanotechnology are drastically changing the traditional approach to dentistry by the design of innovative devices supporting more efficiently the natural regeneration process. The objective of this book is to highlight current progresses in tissue engineering for various dental hard/soft tissues including enamel, dentin, pulp, alveolar bone, periodontium, gum and oral mucosa, by emphasizing the role of materials and their specific applications.

The book aims to offer a large but timely overview of the current state of art in biology and clinical surgery applied to oral and craniofacial tissues.

The book includes 14 chapters, divided into three different subsections. An introductory and general section is mainly aimed at focussing upon the consolidated approaches used in tissue repair and regeneration (Chapter 1), also taking into account basic regulatory aspects (Chapter 2) and future targets in cell (Chapter 6) and gene therapy (Chapter 12) and clinical use (Chapters 13–14). The second part singularly addresses the recent discoveries on the use of biomaterials such as polymers (Chapter 3), ceramics (Chapters 4–5) and their composites (Chapters 8–9) for the design of innovative devices for repair and/or regeneration of hard and/or soft tissues in the oral or craniofacial compartments. Lastly, the third part explores the current applications of the biomaterials based devices as a function of the specific tissue target, including bone (Chapter 9), gengiva and periodontal tissue (Chapter 10), Pulp dentin complex (Chapter 11).

In each section, the peculiar role and the relevant impact of biomaterial features on the development of innovative treatments for \textit{in vivo} surgery is strongly emphasized.
Preface

1. Introduction to Oral and Craniofacial Tissue Engineering
   María Verónica Cuevas González, Eduardo Villarreal-Ramírez, Adriana Pérez-Soria,
   Pedro Alberto López Reynoso, Vincenzo Guarino and Marco Antonio Álvarez-Pérez

2. Translation of Tissue Engineering Approach from Laboratory to Clinics
   Daniel Chavarría-Bolaños, José Vega-Baudrit, Bernardino Isaac Cerda-Cristerna,
   Amaury Pozos-Guillén and Mauricio Montero-Aguilar

3. Polymer Materials for Oral and Craniofacial Tissue Engineering
   Iriczalli Cruz Maya and Vincenzo Guarino

4. Calcium Phosphate and Bioactive Glasses
   Osmar A. Chanes-Cuevas, José L. Barrera-Bernal, Iñigo Gaitán-S. and David Masuoka

5. From Conventional Approaches to Sol-gel Chemistry and Strategies for the
   Design of 3D Additive Manufactured Scaffolds for Craniofacial Tissue Engineering
   Gloria A., Russo T., Martorelli M. and De Santis R.

6. Mesenchymal Stem Cells from Dental Tissues
   Febe Carolina Vázquez Vázquez, Jael Adrián Vergara-López Núñez,
   Juan José Montesinos and Patricia González-Alva

7. Composite Materials for Oral and Craniofacial Repair or Regeneration
   Teresa Russo, Roberto De Santis and Antonio Gloria

8. Biomimetic Approaches for the Design and Development of Multifunctional
   Biodegradable Layered Scaffolds for Dental Regeneration
   Campodoni Elisabetta, Dozio Samuele Maria, Mulazzi Manuela,
   Montanari Margherita, Montesi Monica, Panseri Silvia, Sprio Simone,
   Tampieri Anna and Sandri Monica

9. Craniofacial Regeneration: Bone
   Laura Guadalupe Hernández Tapia, Lucia Pérez Sánchez, Rafael Hernández González
   and Janeth Serrano-Bello

10. Gingiva and Periodontal Tissue Regeneration
    Avita Rath, Preena Sidhu, Priyadarshini Hesaraghatta Ramamurthy,
    Bennete Aloysius Fernandes, Swapnil Shankargouda and Sultan Ömer Sheriff
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.</td>
<td>Dentin-Pulp Complex Regeneration</td>
<td>Amaury Pozos-Guillén and Héctor Flores</td>
<td>159</td>
</tr>
<tr>
<td>13.</td>
<td>Injectable Scaffolds for Oral Tissue Regeneration</td>
<td>Suárez-Franco, J.L. and Cerda-Cristerna B.I.</td>
<td>197</td>
</tr>
</tbody>
</table>

Index                                                                                      229
Introduction to Oral and Craniofacial Tissue Engineering

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Introduction

Oral and craniofacial tissues are important in several physiological functions such as mastication, speech, facial aesthetics, and the most important in the quality of health in life. In this fundamental role, teeth are essential to these functions relying on its unique combination of hard tissues—including enamel, dentin, root cementum and alveolar bone—and soft tissues—including periodontal ligament, gingiva and dental pulp (Orsini et al. 2018a, b). The development of all organs which form from the ectodermal and endodermal sheets lining the embryo is regulated by the communication between the epithelium and underlying mesenchyme. Teeth are unique, greatly specialized organs, in which studies of classic tissue recombination in the developmental biology field established that the tooth shape, dental cells and tissues, takes place through a strict series of well-defined regulated stages that involve this kind of communication (Luukko and Kettunen 2016). This process in tooth development is characterized by complex reciprocal interactions between the epithelial and mesenchymal tissue, which occur in a stepwise process classically described, from early to late as: Lamina, Bud, Cap and Bell stages (early and late stage), in which each phase is discernible by specific histomorphological and cellular features (Yildirim et al. 2011; Mitsiadis and Harada 2015; Thesleff 2014). In recent years, with a better understanding of molecular biology and cell-tissue signaling, the process of tooth development could be broadly described as a sequential differentiation process mediated by the conserved signaling pathways, such as FGF, BMP, Hedgehog, EDA, and Wnt, where the basal cells of the dental lamina (dental epithelial tissue) undergo proliferation and form a horseshoe-shaped band that invaginates into the underlying mesenchymal tissue (this process is called epithelium invagination). The mesenchymal

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tissue, derived from neural crest cells, proliferated and differentiated to ameloblasts for depositing the enamel tissue, meanwhile mesenchymal tissue responds to dental epithelial signaling beginning with the process of differentiation into cementoblasts, periodontal ligament, odontoblasts and other dental pulp cells (including neurons, endothelial cells and fibroblasts) (Catón and Tucker 2009; Balic 2018; Xiao and Nasu 2014). Thus, tooth-specific tissues originating from the two principal sources gives the complete anatomy of soft and hard tissues in teeth, including dentin, dental pulp, alveolar bone and periodontal ligament.

In dentistry, it is well known that in humans these soft and hard tissues could be lost due to damage of tissue by trauma, dental caries, periodontal disease or a variety of genetic disorders that combined with age could lead to suffering a physical and mental dramatic event that compromises an individual’s self-esteem and quality of life (Batista et al. 2014; Kassebaum et al. 2014). It is, therefore, necessary to develop innovative approaches for the repair/regeneration of damaged or missing alveolar bone and dental tissues (Caton et al. 2011). Recently, with the recognition of the molecular and cell biology based on the development of dental tissues structure, more and more efforts have been focused on applying the knowledge gained to design therapies to promote the dental tissues regeneration because modern dentistry is not limited to maintenance of dentition but has many subspecialties encompassing diagnosis and treatment of conditions affecting the oral and maxillofacial structures. Thus, regeneration of lost dental tissue by the rehabilitation of patients has been the ultimate dream of every clinician and dental healthcare researcher. Due to the unique, diverse role of the teeth and associated structures, there has been a sustained effort to replace the missing dentition over many centuries. This effort could be seen by several examples of proposed approaches to engineer biological dental tissues including tissue engineering scaffolds; cell-tissue recombination, gene-manipulated cell for improve tooth regeneration, dental mesenchymal stem cells co-cultures, 3D tissues culture strategies and 3D scaffold design by polymer printing, bioprinting and CAD-CAM technology (Moioli et al. 2007; Jahangirian et al. 2018; Saratti et al. 2019; Werz et al. 2018).

This chapter describes the strategies for the regeneration of oral and craniofacial tissues. First, to address the principles of the specific area related to dental tissue engineering, second to describe the components of the extracellular matrix and what strategies are used to try to mimic the natural extracellular matrix of tissue structures by using of scaffolds and, finally, what are those strategies that are being used to try to understand how to achieve the regeneration of the oral and craniofacial complex.

**Dental Tissue Engineering**

Oral and craniofacial tissues have a limited ability to correctly auto regenerate when the original tissue integrity has been severely damaged. Moreover, this limitation on the structural integrity of the damaged tissue is because of several common conditions requiring craniofacial and dental treatment including caries pulp, odontogenic infections, periodontal disease, tooth impactions, tissue dysfunction and malocclusion disorders are constant challenges in the dental health area (Gurtner et al. 2008; Duailibi et al. 2008). Moreover, with the high expectation of life, and with the increasing number of cases of dental pathologies related to the traumatic, inflammatory and neoplastic origin, congenital anomalies and degenerative diseases affecting the principle of oral and craniofacial structures, a new area that is getting relevant as a strategy to address these problems and developing new biological therapies to face this wide range of dental issues is dental tissue engineering.

Dental tissue engineering is a multidisciplinary approach that tries to the restore, maintain and enhance oral tissue function along with diagnostic and clinical applications. Dental tissue engineering works to combine the basic principles of physics, chemistry, tooth development, cell biology, nanotechnology, regenerative medicine and engineering to create a logical strategy approach to generate functional oral tissues at cellular and molecular levels that closely match with the physical
and mechanical properties of naturally formed oral and craniofacial tissues (Rai 2015; Bossu et al. 2014). However, dental tissue engineering could be summarized in three central components of this multidisciplinarity as specific source of living cells, because one of the biggest challenges in the repair/regeneration strategies is what kind of cells must be used? Or what kind of specific phenotype of source cells must be isolated? Considering that oral and craniofacial tissue have distinct functions and coupled with this problem, also a different extracellular matrix that could be regarded as soft or hard, implies the uses of biomaterials as an artificial structure. This artificial structure also assumes the understanding of how to develop new materials that meet the physicochemical, structural and mechanical properties to instruct cellular sources to respond to deposit a specific cellular tissue, in which the bioactive signals (peptides, growth factors or genes) finally enter that have to be integrated in the artificial structure to give biological cues and regulate cell functions (Fig. 1.1).

There is no doubt that in dental tissue engineering cells play a central role in developing innovative strategies for repair/regeneration of the oral and maxillofacial region. But today not all kind of cells can be used as a source for this purpose, only specific cells that are immature, unspecialized, that have self-replication and conserve their potency and cell plasticity to differentiate into a vast variety of cells populations including dental tissue have gained attraction and are particularly important for developing tissue engineering strategies in this area. These unspecialized cells called dental mesenchymal stem cells could be identified and isolated based on their adherence to tissue-culture-treated plastic when being cultivated in vitro, for their rapid expansion, for showing a fibroblastic morphology, and for having a positive signal and expressing CD105, CD73 and CD90 surface markers and lacking the expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules. Dental mesenchymal stem cells isolated from different tissues, for example from oral mucosa, pulp, periodontal ligament and gingiva represent an excellent source for dental cell therapy and regeneration of damaged oral and maxillofacial tissues. This option as a source is because they display multipotency when specific factors are used for inducing the differentiation showing the capacity to give rise to osteo/odontogenic cells, chondrocytes, adipocytes, neuronal cells, muscle cells,

Figure 1.1. Schematic representation of the three principal components of dental tissue engineering involved in the challenge for designing new strategies for tissue repair/regeneration.
cardiomyocytes, endothelial cells, hepatocyte-like cells and islet-like cells (Bartold and Gronthos 2017; Han et al. 2014; Ge et al. 2012; Egusa et al. 2012).

A detailed chapter on this topic will focus on mesenchymal stem cells isolated from a different source of dental tissues and also address the subject of how the mesenchymal stem cells could be used as a cell-based therapy with its potential clinical applications. Moreover, these days new cell-based approaches for enhancing the cell response on regeneration strategies that deal with replacing the defective genes with their correct analogues to produce functional proteins will be addressed in the chapter focused on gene therapy that definitely becomes a potential and promising future treatment modality of a number of diseases especially affecting the oral and craniofacial tissues.

As a second point on dental tissue engineering, important elements for tissue regeneration and for the success and efficacy of the knowledge in the cell therapy not only involve genes and dental mesenchymal stem cells but also designing appropriate scaffolds that should be biocompatible, nontoxic, under biologically safe degradation and eventually resorbed by remodeling the new tissue, allowing it to acquire similar mechanical properties and withstand mechanical forces (Zafar et al. 2015).

The importance of this kind of artificial architecture or scaffolds will be addressed in more detail in various chapters of the book that try to focus on topics such as biodegradable polymers, calcium phosphates and bioactive glasses, metal organic and hybrids, composite materials and injectable scaffolds on the applications on the regeneration strategies of oral and craniofacial tissues. Moreover, an interesting point in the synthesis of biodegradable scaffolds is that surface chemistry cues presented in the form of micro- and nanoscale topographies are significant in regulating the behavior of cells because it must be instructive and this topic will be addressed in the chapter related to biomimetic approaches for the design and development of multifunctional bioresorbable layered scaffolds for dental regeneration.

Thus, the cell instructive property implies that topography of the scaffolds gives a specific signal that allows functionality to trigger the cascade of events and also the architecture of the scaffold has to mimic the scale of the extracellular matrix creating an artificial microenvironment that improve and may direct the differentiation of stem cells towards a precise fate and function leading to regeneration (Choi et al. 2018; Darnell et al. 2018; Liu et al. 2018). Thus, nanosurface scaffolds such as nanoparticles, nanoceramic, nanofibers and nanocomposites have gained increasing interest in dental tissue engineering. This larger interest is because topography including pore size and porosity also have a direct influence on control stem cell behavior as cell attachment, cell morphology, cell proliferation, cell differentiation that allow to guide tissue repair/regeneration (Bettinger et al. 2009; Mansouri and SamiraBagheri 2016; Skoog et al. 2018).

The mechanism by which the topography exerts its effect on stem cell response is by altering focal adhesion assembly and cytoskeletal stress, resulting in adaptive gene- and protein-level changes (Kolind et al. 2014). Thus, the surface topography features of sub-micrometers to 10μm in size act on the actin cytoskeleton, whereas features of ten to hundreds of nanometers act on integrin receptors (Miyoshi and Adachi 2014). However, the cell-surface interaction regarding the link between topographical cues and changes at proteomic, genomic and epigenomic levels are not completely understood.

Furthermore, topography of the scaffold could also be relevant for hydrophilicity and for specific protein adsorption, as shown by the selective take up of proteins relevant for cell attachment, such as fibronectin and vitronectin, on fibrous meshes with nanoscale fiber diameters (Bai et al. 2018).

Indeed, since most types of mesenchymal stem cells are dependent on anchoring and could die if there is not a favorable cell adhesion by the surface instruction of the scaffold; the search for functionalization of the scaffold for enhancing a favorable cell–material interaction are paramount for inducing a specific signaling of cellular respond at the cell–biomaterial interface. This could be achieved by integrating bioactive signals as cell adhesion peptides or growth factors or by specific adsorption of endogenous extracellular matrix proteins, as functionalization of the surface scaffold
Introduction to Oral and Craniofacial Tissue Engineering

by the different methods to synthesize the scaffolds as solvent evaporation, salt-leaching, molecular self-assembly, gas foaming, phase separation, emulsification/lyophilization, 3D rapid prototyping and electrospinning, for help to regulate cell function.

Finally, the third point in dental tissue engineering, signaling biomolecules as growth factors are the choice for functionalization because these proteins activate the cellular communications network and influence functions, such as cell adhesion, cell proliferation, matrix deposition and differentiation of tissues. Also, growth factors have been shown to play a key role on the induction of fracture and wound healing, formation and repair of all craniofacial tissues and there are at least six growth factors that could be involved in oral and craniofacial tissue engineering that have been used in vitro and in vivo as Platelet-Derived Growth Factor (PDGF), basic Fibroblast Growth Factor (bFGF), Insulin-like Growth Factor (IGF), Transforming Growth Factor beta (TGFb), Vascular Endothelial Growth Factor (VEGF), and Bone Morphogenetic Proteins (BMPs) (Mandla et al. 2018). Moreover, structural fibrous protein of the extracellular matrix provides adhesive ligands such as collagen, elastin, keratin, laminins, fibronectin and vitronectin that direct cell function and could allow a more precise regulation of cell function and tissue formation (Cruz-Maya et al. 2018).

Extracellular Matrix Mimicry Dental Tissue Engineering Scaffolds

The loss of tissues due to trauma, disease or congenital anomalies is a health care issue. Therefore, the development of materials that could be implanted or incorporated into tissues to regain function is of utmost importance. The global market for biomaterials was valued at US $ 66.2 billion in 2015 and is likely to expand to 14% by 2020 (Nagrath et al. 2018). A large part of this market is centralized on mineralized tissues such as orthopedic and craniofacial/dental implants. In dentistry, tooth loss is not seen as an aesthetic or psychological problem; this is definitively a problem with serious physiological consequences. Tooth loss should be considered as an imbalance in the masticatory system. Edentulism can lead directly to impairment, functional limitation, gum damage and alveolar bone resorption (Polzer et al. 2010). For many years, autologous implants were considered the gold standard. However, this requires a more significant discomfort for the patient. Here we try to describe the recent advances in dental tissue engineering which will likely produce alternatives to autogenous bone grafts that may well exceed existing clinical outcomes and replace traditional indications for its use (Misch 2010).

The alternative to the gold standard toward the fundamental understanding of structure-function relationships in normal and pathological oral and craniofacial tissues could be the development of biological substitutes to restore, maintain or improve tissue function (O’Brien 2011). This biological substitute named biomaterial scaffold could be defined as either naturally occurring materials in living tissues or materials designed, fabricated, tested, applied and synthetic to replace, and repair oral and craniofacial tissues, which can perform, enhance or replace a specific biological function (Stupp and Braun 1997; Ratner et al. 2013). Moreover, these biomaterial scaffolds should be able to interact with cells to perform their function with an appropriate host response in a specific clinical application (Williams 2014).

In dental tissue engineering, the biomaterial scaffold basically acts as a template for cell growth and recolonization of injured tissue areas as a conductor tissue growth. Scaffolds not only provide a mechanical support, but they also may present biochemical signals like growth factors to encourage cell attachment, migration onto or within the scaffold, cell proliferation, cell differentiation and modulate cell behavior (Hutmacher 2001). Some scaffolds are frequently seeded with stem cells to accelerate cell recruitment and homing (Hussey et al. 2018). Scaffolds are made from several materials like polymers, metals, ceramics or composites. Therefore, before making the selection of the material for the scaffold design, it is necessary to understand the mechanical properties—structure relationships of the tissue to replace. The scaffolds are designed to replace a wide variety of soft tissues such as skin, tendons, blood vessels, among others, and they are generally composed of synthetic or natural
Current Advances in Oral and Craniofacial Tissue Engineering

polymers. Replacement of mineralized tissues such as craniofacial tissues, bone and tooth are usually designed scaffolds built up from metallic, ceramics, polymers and composites materials and must be designed to be permanently implanted. Nevertheless, long-term complications such as stress shielding, wear debris, loosening and mechanical or chemical breakdown of the material itself, encouraged scientists to develop new materials. Scaffolds, in addition, must provide high porosity, high surface area, structural strength, three-dimensional micro- and nano-structure, and biodegradability if needed (Vats et al. 2003). However, an important key to design a cellular scaffold and it should be pointed out as essential for the biological response is if the scaffold could replicate or mimic the extracellular matrix properties. To understand the challenge of replicating the extracellular matrix, of oral and craniofacial tissues we will briefly explain the extracellular matrix functions and mention their most representative members.

**Extracellular Matrix**

The extracellular matrix is a non-cellular structure present in all vertebrate tissues. The origin of multicellularity in metazoa is intimately related to the development of the extracellular matrix. The evolutionary transition from unicellular to multicellular organisms was a fundamental change in the history of living beings on Earth. At the beginning of cellular cooperation, the cells were able to work in a cohesive way to perform more complex and sophisticated tasks; this was due to the emergence of the proteins secreted by the cells and their particular structural arrangement (Czaker 2000).

Extracellular matrix dictates several cellular functions as migration, differentiation, proliferation, morphogenesis, growth, survival and maintains tissue homeostasis (Frantz et al. 2010; Bonnans et al. 2014). Extracellular matrix maintains a highly organized three-dimensional heterogenous fibrillar network structure with tissue-specific composition and topology, that provides an indispensable physical scaffolding for the cellular constituents and is under constant and highly coordinated remodeling throughout the entire lifetime, in accordance with physiological or pathological conditions (Theocharis et al. 2016).

The extracellular matrix is composed of water and macromolecules like fibrous proteins and proteoglycans (Frantz et al. 2010). The most abundant proteins in the extracellular matrix are collagens, elastins, fibronectin, tenascin, laminin and fibrillins. Besides, extracellular matrix contains significant contents of polysaccharides as glycosaminoglycan (GAG) chains covalently attached to a specific protein core (PG-proteoglycans), except for the hyaluronic acid (Rozario and DeSimone 2010). The GAG chains are generally long linear and negatively charged with disaccharide repeats. PG make up a hydrogel due to their extended conformations and remarkably hydrophilic character. This cross-linked biopolymer gel is needed to hold up high compressive forces (Kwansa et al. 2014). The PG more abundant in the extracellular matrix are perlecan, syndecan, decorin, aggrecan, glypican and lumican. The three principal families are Small Leucine-Rich Proteoglycans (SLRPs), modular proteoglycans and cell-surface proteoglycans (Schaefer and Schaefer 2010; Kresse and Schönherr 2001).

Collagens are the most abundant family of proteins in the animal kingdom with at least 45 different collagen genes that code for collagen polypeptides, and account for up to 30% of total protein mass present in the interstitial of the extracellular matrix (Rozario and DeSimone 2010). Moreover, collagen fibrillar and non-fibrillar proteins that can be assembled into supramolecular structures, to give organization and rigidity to the extracellular matrix. The mechanical properties of the extracellular matrix can be determined by the organization, distribution and density of the collagen fibers in the tissues (Birk and Brückner 2011). Mutations in the genes of collagen can be fatal in embryonic development, even embryos lacking collagen I that reach late stages of development suffer from abrupt rupture of the aorta due to lack of tissue compliance and it is striking that one collagen type can predominate overwhelmingly in tissues that are extremely diverse (Löhler et al. 1984).

All collagen members share common features. The collagen proteins have at least one triple alpha helix collagenous domain (COL), which is a rod-like structure to provide stiffness and the content
or number of repetitions of this COL domain depends on the specific type of the collagen. The alpha helix chains are supercoiled to form a triple helix. The COL domain frequently has a triplet sequence Gly-X-Y, where Gly corresponds to the glycine residues and generally by steric constraints occupies the central positions in the triple helix. Meanwhile, X and Y can be any amino acid but frequently are found in these positions to proline and hydroxyproline, respectively. Hydroxyproline residues are essential to triple helix stability (Birk and Brückner 2011).

In the extracellular matrix, there are mainly two types of elastic fibers, chemically and morphologically different: elastins and microfibrils. Elastin fibers comprise approximately 90% of all of them. Elastin fibers provide the properties of resilience and elasticity to the tissues preventing deformation when they are under repeated stretching (Mithieux and Weiss 2005). However, the elastin fibers stretch is limited due to its close structural association with collagen fibers. Poles apart to collagen family that is encoded by several genes, elastin is encoded by a single gene in mammals and secreted to the extracellular medium as a monomer with a molecular weight of 60–70 kDa, called tropoelastin (Frantz et al. 2010). In the extracellular space, the secreted tropoelastin molecules are processed to elastin fibers; this process is catalyzed by a member of the lysine oxidase family, > 80% of the lysine residues form covalent cross-links between and within the elastin molecules. The arrangement and size of elastin fibers vary according to the tissue, but elastin fibers are associated with different proteins such as fibulins and fibrillins, which are essential to preserving the integrity of elastin fibers (Muiznieks and Keeley 2013).

Another essential fibrous protein in the matrix for playing a crucial role in the organization of the interstitial of the extracellular matrix and for interactions with cells is fibronectin. Fibronectin is directly related to central cellular events related to the matrix, such as cell adhesion, migration, growth and differentiation. Fibronectin is a protein made up of a dimer that is held together by a pair of disulfide linkages in the carboxyl terminus, and each monomer has a molecular weight around of ~ 250 kDa. Similar to elastin, fibronectin is a protein encoded by a single gene. However, fibronectin may occur in different versions due to alternative splicing, generating up to 20 known isoforms. Fibronectin can be stretched several times over its resting length by a neighboring cellular traction force. Owing to the tensile strength over fibronectin, it undergoes to conformational changes to expose integrin-binding sites recognized by integrins on the cellular surface. Consequently, the adhesion of integrins to fibronectin is allowed and promotes fibronectin-fibril assembly, which implies that fibronectin is also a mechano-regulator of the extracellular matrix (Frantz et al. 2010; Pankov and Yamada 2002; Xu and Mosher 2011).

In contrast to the predominantly fibrillar structure of collagen and elastin, proteoglycans adopt highly extended conformations that are essential for hydrogel constitution (Mouw et al. 2014). The hydrogel molecules support the high compression forces in the extracellular matrix, around the cells and interstitial matrix. As stated above, the biological function of proteoglycans derives from the biochemical and hydrodynamic properties of the GAG macromolecules, which combine chemically with water to provide hydration and compression resistance. GAG has been classified into subtypes according to the function and structure of these carbohydrate chains, as well as the distribution, density and length of these chains concerning the core protein (Iozzo and Schaefer 2015). The most common GAG chains found in the ECM are heparin sulfate, chondroitin sulfate, dermatan sulfate, hyaluronan and keratin sulfate. The proteoglycans are encoded by a little less than 50 genes, besides several of them can be subject to alternative splicing, which shows the enormous variety of members of proteoglycans present in the matrix. They also have a vast range of functions such as cell adhesion, migration, proliferation, signaling, communication, morphogenesis and growth. In turn, proteoglycans also participate in angiogenesis, the inflammatory response to pathogens and injuries (Kresse and Schönherr 2001; Iozzo and Schaefer 2015). After having explained the unique features, functions and essential members of the extracellular matrix some techniques to replicate or mimic the extracellular matrix need to be shown.
Biomimetic Extracellular Matrix

The scaffolds designed to replicate the properties of the extracellular matrix must achieve several premises described above as morphology and mechanical properties. To set up a simple classification, they could be divided into two broads categories: organic scaffold materials and inorganic scaffold materials.

The inorganic scaffold materials can also be classified into different classes according to: (a) type and whole number of polymers constituent, (b) ability to be reabsorbed and replaced by the host tissue, (c) capability of chemical modification and bio-functionalization on their surface, (d) capability of covalent bond forming with some other materials (organic or inorganic), and finally, (e) type of manufacturing. The biodegradable polymers are preferred because they reduce the immune reaction to the foreign body, and also allow the recolonization of the damaged area allowing a more efficient regeneration. Polymers more frequently used for preparing inorganic scaffolds are poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(caprolactone) (PCL) and poly(lactic-co-glycolic acid) (PLGA) (Hussey et al. 2018; Stratton et al. 2016).

The Organic Scaffold Materials (OSM), on the other hand, are those formed of macromolecules from a natural source from a living being, sometimes purified or treated as an extract. OSM also can include macromolecules as proteins over-expressed and purified in a cell host. Organic scaffold materials have several advantages, producing a low foreign body reaction, which is why, in general, they have a moderate inflammatory response. Besides, organic materials are biodegradable by enzymes that are naturally found in the body. Moreover, organic materials can easily be mixed with inorganic materials to form composite materials with new physical and chemical properties. Among them, biopolymers more frequently used for preparing natural scaffolds are collagen, fibronectin, glycosaminoglycans, fibrin, silk proteins, alginate, chitosan, hyaluronic acid and cellulose (Mano et al. 2007; Guarino et al. 2012).

Nanofibers

One of the goals of dental tissue engineering is to mimic the extracellular matrix; to achieve this goal, different technologies have been developed including Air-Jet Spinning (AJS) and electrospinning (ES). Several studies have demonstrated that AJS and ES can be successfully used to fabricate biomimetic analogs able to respond to the fibrillar architecture of the extracellular matrix (Braghirolli et al. 2014; Stojanovska et al. 2016; Guarino et al. 2016).

Air-jet spinning (AJS) is a technique to produce spun microfibers and nanofibers of polymeric materials and composite materials. This technique is based on the use of air regulated pressure to apply as a jet or spray the polymer solution as thin fibers onto the surface and may also be passed or conducted under controlled pulsations directly through the nozzle. As the polymer solution leaves the AJS nozzle, the solvent evaporates during the formation and deposition of the fibers onto the surface. The system has many advantages such as: easy to use, not requiring sophisticated equipment, scalability, short period for manufacturing and low cost (Suarez-Franco et al. 2018; Lasprilla et al. 2012).

Electrospinning is a technique which uses the basic principles of AJS. However, the driving force to generate the fibers from a polymer solution consists of the high voltage electric field applied between a metallic nozzle and the grounded collector where the fibers will be deposited. Electrospinning shows an essential advantage related to the capability to reduce fiber size down to several nanometers in diameter, despite this some limitations still remain, such as low production rates and high dependency on polymer properties. However, this technique, i.e., AJS, is easy to use and with high adaptability to several conditions and different materials (Guarino et al. 2018; Sill and von Recum 2008). For these reasons, AJS and electrospinning have gained considerable attention in the past several years as technology for mimicking the fibrous structure and sizes of the extracellular...
matrix with several applications on oral and craniofacial tissue engineering. Due to the versatility, adaptability and easy handling of the fibers several studies were performed to increase the bioactivity and biofunctionality of fibers, by incorporating different dopants. Scaffold doping may be divided into two categories depending on whether this is non-covalent or covalent bonding between the fibers and the dopant (Ji et al. 2011). Non-covalent binding to the fibers could be guided by distinct forces as electronegativity, van der Waals forces, hydrogen bonds or the dopants could be adsorbed or wrapped by the polymer (Park and Ji 2011). The covalent bonding to the fibers is accomplished by chemical modification to produce a reactive functional group to immobilize a bioactive dopant to the polymer (Yoo et al. 2009). These processes can change the behavior of the fiber dramatically and improve the cellular response for healing.

Some non-covalent composite scaffolds are polylactic acid (PLA)-hydroxyapatite (HA), copolymer PLGA [PLA + polyglycolide (PGA)]-titania, poly(methyl methacrylate) (PMMA)-carbon nanotubes (CNTs) (Gloria et al. 2010; Luong et al. 2008). These composite scaffolds have different physical and chemical properties in comparison with the pure polymer scaffolds. PLA-HA shows a high surface free energy and low water contact angle, because this degradation rate was slow as compared with PLA scaffold. Also, fibers were doped with different drugs, and they even have added organic compounds such as proteins, peptides and growth factors to develop other types of non-covalent composite scaffolds. In a recent study using a syringe pump coupled to two gas-brush devices, demonstrated depositing PLGA fibers with proteins physisorbed and/or adsorbed as collagen and fibrin. This scaffold with deposited proteins shows capability of fibroblast migrations towards the fibers, arrangements of cells in similar conformations to the connective tissues and high expression of ECM proteins (Kaufman et al. 2018). However, several studies have demonstrated that surface structure, hydrophobicity and charge affect the protein structure when they are adsorbed to a solid-liquid interface. The most crucial step in protein absorption on the biomaterial surface is the release of water. In this way, the interface protein biomaterial surface is separated by two interfaces: on the one hand surface and water interface and the other hand protein and water interface. The process of protein adsorption over the surface could cause changes in the protein structure, which could induce the denaturalization of proteins (Ostuni et al. 2001). These structural changes on protein adsorption are due to the native folding of the protein that corresponds to the free energy minimum in solution, and does not compare to the free energy minimum once the protein is in contact with the surface (Rabe et al. 2011).

**Hydrogels**

Hydrogels are cross-linked polymeric structures, with three-dimensional morphology composed by covalent bonds produced by the reaction of one or more comonomers, physical cross-links are due to chain entanglements. Furthermore, there are chain interactions due to weak bonds such as hydrogen bonds and van der Waals forces. Hydrogels are also hydrophilic materials with high capacity to absorb a large amount of water or biological fluids up to more than 90% (Peppas et al. 2000). Classification of hydrogels can be made according to the preparation method, the overall charge, and the mechanical and structural characteristics (Van Vlierberghe et al. 2011). Gelation involves the formation of a three-dimensional network. Several stimuli can promote gelation of the hydrogel, such as ion concentration, UV exposure or temperature (Carballo-Molina et al. 2016). Hydrogels are frequently synthesized based on inorganic scaffold materials, such as Poly(Propylene Fumarate-co-Ethylene Glycol) [P(PF-co-EG)], Poly(Vinyl Alcohol) (PVA), Poly(Acrylic Acid) (PAA) and polyethylene oxide (PEO). However, methodologies have been developed to use biopolymers derived from the extracellular matrix to form a more biomimetic hydrogel. Among the most frequent biopolymers used for the formation of hydrogels are collagen, gelatin, elastin, silk fibroin and polysaccharides (Re et al. 2019). Hydrogels designed from biopolymers, unlike synthetic polymers, have a high affinity and ability to adsorb proteins from biological fluids or plasma. Also, hierarchical multiphase porous
can mimic the critical extracellular matrix properties, to provide mechanical support for tissues and
to regulate cellular behaviors, such as adhesion, proliferation and differentiation. Moreover, micro/
nanoscale-topography of the hydrogels can be degraded by enzymes such as metalloproteases, allowing
cell migration, colonization and growth (Zhu et al. 2019).

**Proteins and Peptides**

Proteins and peptides are used as primary constituents to construct biofunctional scaffolds with
properties to mimic the extracellular matrix (Tsomaia 2015). Protein-based biomaterials have been
modeled from the structures found within the extracellular matrix to replicate the three-dimensional
microenvironment of the native tissue. Advances in molecular biology and synthetic biology now
allow for precise control and manipulation of amino acid sequences and the modular architecture of
proteins (Tang and Lampe 2018). Besides, these macromolecules have self-assembly properties that
can be exploited to build supramolecular structures with micro- and nano-structured dimensions and
used to design three-dimensional nanofibrous scaffold. The modular nature of proteins could be used
to create chimeras with modified features to have a better performance or even create new functions
by swapping modules from their different parental proteins. Theoretically, proteinaceous biomaterials
could be understood as a protein LEGO set, which would allow controlling the supramolecular
properties from the macroscopic to nanoscopic properties (Sandhya et al. 2016). Generally, these
proteinaceous biomaterials present a structure like biopolymer-based hydrogels, which promotes
cytocompatibility, cell interactions and minimizes foreign body reaction. Furthermore, current
knowledge of the interactions between cells and extracellular matrix allows knowing which are the
signal transduction pathways of different cellular events such as cell adhesion, proliferation and
differentiation through an integrin-binding Arg-Gly-Asp (RGD) motif (De Santis and Ryadnov 2016).

**Molecular Dynamics Simulations as a Tool for Designing Scaffolds**

Most of the research on development scaffolds carried out around the world has the following
approach to develop new data and materials: (A) These researches are begun with a solid base
of current knowledge. (B) Proficient skills on the techniques for material synthesis. (C) A robust
material characterization in physical and chemical properties. (D) Extensive observation of the
biological response to the material in vitro and in vivo. Nevertheless, to improve our understanding
of the features of these materials, it is necessary to decipher the behavior at the atomic level and the
physicochemical interactions that occur in the biomaterial-water-ECM interface (Zhou 2015). Proteins
and biomacromolecules, biopolymers, hydrogels and other small solutes, are often interfacially active
and adsorb to a variety of interfaces.

Unwanted adsorption is a primary complication for phenomena as wettability, bioactivity and
biocompatibility on implants (Firkowska-Boden et al. 2018). Ideally, the surface of biomaterials should
be precisely controlled and designed to obtain good coordination with water molecules (Liping et al.
2008), controlling protein adsorption and avoiding the integrity of secondary structure elements is
compromised upon adsorption (Ge et al. 2011). Molecular dynamics simulations are an alternative
method to directly explore the structural and dynamic properties of scaffolds at an atomic level and to
analyze their interactions with macromolecules and water. Therefore, MDS is a versatile tool to obtain
in silico data from platforms, and this serves to limit the experimental conditions in the laboratory.
By using molecular dynamics simulations it is possible to study different sizes, shapes and surface
chemistries of the scaffolds, these properties can vary the toxicity, gene regulation and clearance due
to the interatomic interactions between materials and biological molecules (Qun et al. 2015). The
spatial and temporal resolutions that computational techniques currently allow enable the investigation
of the specific interactions and dynamics that scaffolds induce in biological molecules (Zhou 2014).
Therefore, the use of computational methods is becoming more frequent to elucidate the fundamental aspects of intermolecular interactions within the biomaterial-water-ECM system (Fig. 1.2).

**Strategies for the Regeneration of the Oral and Craniofacial Complex**

The regeneration strategies for the complex craniofacial structures is a highly promising field and in recent years focuses mainly on the regeneration of oral tissues such as oral mucosa, periodontal, bone and dental pulp tissues.

**Oral Mucosa**

The mucosa is the most abundant tissue that lines the oral cavity, its damage or loss is mainly caused by different diseases, inflammatory, traumatic, neoplastic or congenital origin, that result in defects of the buccal mucosa, among which are included gingival recessions, vestibuloplasties, cleft palate, traumatisms and the removal of tumors. Although various surgical procedures have been proposed for the treatment of these defects, there remains a need to find functional, anatomical and aesthetically similar substitutes for the tissue to be replaced, as well as solutions that reduce the morbidity associated with obtaining tissue from donor areas, which represents a clinical challenge for periodontists and oral and maxillofacial surgeons. Most surgical techniques and even the replacement of affected tissue include the use of partial or full-thickness skin grafts or flaps and heterologous transplants or reconstruction techniques. However, these techniques are not an option when the patient requires more than one surgical procedure due to the extension of the injury. For this reason, dental tissue engineering has tried to venture into developing new oral mucosal substitutes that are aesthetic and functional at the same time. In this case numerous studies have allowed the development of cell culture

![Figure 1.2. Molecular dynamics to understand the interaction between biomolecules with polymer surface. (A) Collagen fiber deposited on polylactic acid (PLA) surface. (B) Fibrinogen protein deposited on a PLA surface.](image)
strategies or development of scaffolds to regenerate the function of the oral mucosa (Yoshizawa et al. 2012; Moharamzadeh et al. 2012; Scheller et al. 2009).

One of the first approaches was the use of monolayers of keratinocytes whose clinical applications have been used in palate wounds and mucogingival defects. However, among its drawbacks is the reduced resistance to mechanical stress, its fragility due to the absence of the connective tissue (Rheinwald and Green 1975). Another approach to cell culture strategies was tissue-engineered 3D culture systems of the oral mucosa, which provide an organizational complexity and several advantages that include a high degree of differentiation, the potential for histological assessment of the process under study and the potential for monitoring tissue growth or damage (Dongari-Bagtzoglou and Kashleva 2006). Therefore, a bilayer substitution has been proposed in which an extracellular matrix analog that serves as a scaffold is sought. Among the extracellular matrix analogues, one finds the use of fibrin, elastin, collagen, chitosan, agarose, gelatin in combination with several synthetic polymers as poly(ethylene terephthalate), polycarbonate membrane, electrospun membranes of poly(L-lactic) acid, polycaprolactone, polystyrene and polyethylene glycolic acid which seeded and cultured with gingival fibroblast, epithelial or keratinocytes generate an artificial equivalent of partial and total thickness that allow to obtain the biocomplexity of the tissue-engineered oral mucosa (Moharamzadeh et al. 2008; Peña et al. 2010; San Martin et al. 2013).

Periodontal and Bone Tissues

The periodontium involves all the tissues that are surrounding and supporting the tooth, and can be divided into union tissues composed by the Root Cementum (RC), Alveolar Bone (AB) and periodontal ligament (PDL), and dentogingival tissues (Posnick and Posnick 2014). The periodontium is composed of dynamic tissues because it is a mixed combination of mineralized and soft tissue that makes the periodontium a complex tissue in the field of dental tissue engineering and a challenge for the strategies in its regeneration for the mixed-function development.

Root Cementum (RC) is a mineralized tissue that surrounds the superficial root of the tooth; their function is to support the tooth through the PDL and alveolar bone (Yamamoto et al. 2016). Alveolar Bone (AB) is another mineralized tissue and is associated with the formation of membranous bone of both mandibular and maxillary tissues during the development of the first dentition, two components form this kind of bone, the first belong to the alveolar process, which in turn is composed by the cortical and cancellous bone tissue, the last one stores Haversian systems required for maintenance and remodeling of the bone; the second component is the alveolar bone itself which corresponds to the bone portion that covers the dental surface and serves as a union site to the Sharpey fibers from PDL (Chu et al. 2014). Periodontal ligament (PDL) is formed by collagen fibers which could be classified according to their localization of the fibers onto the alveolar crest, oblique, transseptal, horizontal, inter-radicular or apical (Maheaswari et al. 2015). The union of these fibers to the soft tissue provides a natural coupling of the roots of the tooth in the alveolus: the union of the PDL to the RC or the AB facilitates the transfer of loads of the teeth towards the bone, because the bone-cement/PDL-binding sites contain areas between 10–15 μm rich in biochemical gradients, which are known as enthesis sites that facilitate cell-cell interactions and communications (Lee et al. 2015).

The periodontal tissue can be affected by multiple factors destroying the connective tissue and alveolar bone triggering the loss of the dental organ. The periodontitis has been described in the last 4000 years by Egyptians and Chinese, who mentioned that the periodontal disease is an inflammatory condition (Loe 2000). At present, periodontal disease is a chronic inflammation, clinically characterized, in the early stages by gum inflammation, loss insertion to probing, periodontal pockets, bleeding to probing and loss of bone level which vary in size according to the degree of affectionation (Chapple et al. 2018; Papapanou et al. 2018). Moreover, a series of risk factors have been described for the development of periodontal disease, these factors are divided into modifiable factors such as tobacco, poor oral hygiene, hormonal changes, diabetes mellitus, medication and stress, on the other hand, non-modifiable factors include age and genetics; however their pathogenesis has
also been attributed to the presence of multiple diseases such as cardiovascular, metabolic diseases, rheumatoid arthritis, respiratory diseases, cancer among others (Kinane et al. 2017; Nazir 2017; Shewale et al. 2016).

Due to the above, periodontal disease has been considered a public health problem; therefore, the prevention, treatment and regeneration strategies have played a crucial role.

In the treatment of tissue defects caused by periodontal disease it is necessary to promote tissue regeneration with the main objective of restoring the lost or injured tissues structure and function of the periodontium. Within the methods used for periodontal regeneration, specific biomaterials such as bone grafts from diverse origins (allograft, xenograft, alloplastic) and cell-occlusive barrier membranes, are used in guided tissue regeneration (Tassi et al. 2017).

One of the first approaches for wound healing to target the restoration of tooth-supporting bone, periodontal ligament and root cementum has been the use of growth factors that have shown significant growth in the field of periodontal regenerative medicine. Advances in molecular cloning have yielded an unlimited availability of recombinant growth factors for applications in tissue engineering. The growth factors are a group of small polypeptides involved in the stimulation of different cellular signaling pathways through its association with specific membrane receptors and promoting its phosphorylation in tyrosine, threonine or serine aminoacidic residues which in turn activates a complex system of transcriptional regulation inside the cell. Growth factors associated with soft and hard tissue regeneration have been used at a preclinical and clinical level, based on scientific evidence: Platelet-Derived Growth Factor (PDGF), Fibroblast Growth Factor (FGF) and Bone Morphogenetic Proteins (BMPs).

**Platelet-Derived Growth Factor (PDGF)** is a soluble protein has reported four additional forms (PDGFα, PDGFβ, PDGFc, PDGFd) a cystine knit motif characterizes this family of proteins and its function depends on its self-association in homodimers (AA, BB, CC, DD) and heterodimers (AB). The signaling mechanism of the five isoforms of PDGF depends on the interaction with some of the two receptors PDGFRα and PDGFRβ. Platelets are composed of multiple storage granules, i.e., lysosomes and alpha granules which are delivered after its activation (coagulation), through this process of degranulation PDGF is released and performs its function locally in an autocrine fashion or in other tissues such as a paracrine mode. The cellular mechanism described for the PDGF associated with periodontium regeneration involves binding to the cell membrane tyrosine kinase receptors and subsequently exerts the activation of its effects on chemotaxis, cell proliferation, migration, extracellular matrix synthesis and anti-apoptosis via the Rac GTPase which modulates the actin cytoskeleton and the lamellopodia formation (Trofin et al. 2013; McGuire et al. 2006; Mellonig et al. 2009).

In a systematic review, Li noted that the use of 0.3 mg/ml of rhPDGF-BB (recombinant human platelet-derived growth factor-BB) has a positive impact on the bone fill of periodontal defects, linear bone growth, clinical attachment level gain and probing depth reduction. In patients treated with this growth factor, bone fill was 22.71% higher than the control patients. Regarding the clinical attachment level, there was a gain of 0.76% compared to the control groups (Li et al. 2017).

For the transportation matrix, PDGF-BB has been used in combination with an allograft, such as FDBA (freeze-dried bone allograft) and β-TCP (β-tricalcium phosphate). The latter showed positive outcomes and a mean gain of 4.1 mm in the 50 treated periodontal defects (Rosen et al. 2011).

An optimal dose is warranted so that growth factors can exert the appropriate clinical effect. The 0.3 mg/ml dose of rhPDGF-BB results in a mean bone gain of 2.6 mm, compared to 1.5 mm with the 1.0 mg/ml dose, according to a multicenter study. The results suggest that PDGF high doses might reduce the tissue healing outcomes due to feedback inhibition of the local delivery high doses (Nevins et al. 2005).

According to Khoshkram’s systematic review and metanalysis, topical delivery of PDGF resulted in a statistically significant high linear bone fill in periodontal defects (0.95 mm or 20.17%) than control groups (Khoshkram et al. 2015).
Bone Morphogenetic Proteins (BMP) are a group of proteins involved in multiple development processes which include skeletal formation, embryogenesis, hematopoiesis and neurogenesis. These proteins belong to the Transforming Growth Factor Beta superfamily, and over 20 members have been characterized. Four groups are formed to classify these proteins based on its amino acid sequence similarity: BMP2/4, BMP5/6/7/8a/8b, BMP9/10, and BMP12/13/14. These proteins are synthetized as a large precursor from 400–500 aa, which has three main domains, N-terminal secretion signal, a prodomain and a C-terminal region that constitutes the mature protein. Most of BMPs have seven cysteine residues in the C-terminal region, which are involved in its self-assembly and is known as a cysteine knot. BMPs functioning depends on the structural arrangement as homo- or heterodimers, which in turn are associated with specific membrane serine/threonine receptors denoted as type I and type II to trigger two main signal pathways: Smad (mothers against decapentaplegic) dependent pathway and Mitogen-Activated Protein Kinase (MAPK) pathway. This BMPs-MAPK signal pathway has shown its potential as an inductor of mesenchymal stem cells differentiation into osteoblasts, this extracellular signal is transduced inside the nucleus via the activation of ERK1/2, p38, JNK 1/2/3 cascades which activate specific transcriptional factors (RUNX2, DLX5, and Osterix) related with the osteoblastic commitment and initiate the production of bone matrix proteins, leading to bone morphogenesis (Ripamonti 2019; Anusuya et al. 2016).

For craniofacial indications, the bone development induction exerted by rhBMP-2 has been assessed in rat calvaria critical defects, segmental mandibular canine defects, peri-implant defects and sinus augmentation in non-human primates. Several studies have indicated BMP-2’s effectiveness to correct infraosseous, supra-alveolar, furcation and fenestration defects in canine models, resulting in bone and root cementum formation, as well as the formation of fibrovascular tissue. In humans, it has shown significant roles in periodontal regeneration, bone healing, acceleration in osseointegration, oral surgery applied to orthodontics, repair as a sequel of bone pathology and distraction osteogenesis. The long-term evaluation of rhBMP-2 has displayed an increment in bone density after functional loads (Batool et al. 2018; Carreita et al. 2014). From a clinical standpoint, the new formation of autologous bone upon the rhBMP-2 application has been accomplished in sinus augmentation procedures, from a 1.6 mm baseline height to a final bone height of 10–12 mm (combining the remaining bone and new bone), in relation to the ridge baseline measurements for the implant placement protocol. In this study, it was also demonstrated that the dose-dependent effect of the protein of 1.5 mg/mL shows better results than 0.75 mg/mL. Thus, better outcomes have been noted in sinus bone augmentation procedures (De Frietas et al. 2015; van Hout et al. 2011; Wikesjö et al. 2009).

Fibroblast Growth Factor (FGF) is a family of proteins consisting of 23 members with related structural characteristics. These proteins are characterized by its capacity to bind to the Heparin-like glycosaminoglycans, FGFs trigger different cellular functions which include cellular proliferation, adhesion, migration, and differentiation of different target tissues. Specifically, members involved with FGFs play a role in PDL regeneration by enhancing cell proliferation, inhibition of alkaline phosphatase and angiogenesis through the modulation of the FGF-1 and FGF-2. Moreover; FGF-2 is the most extensively studied in regenerative medicine and periodontal tissue regeneration. This protein has been used in the treatment of ulcers and bone fractures due to its potential to facilitate revascularization.

Additionally, in vivo studies have shown that FGF-2 promotes osteoblast proliferation and bone formation acceleration.

Moreover, animal studies have confirmed that the local application of FGF-2 significantly enhances periodontal regeneration compared to control sites. Among its effects, FGF-2 promotes endothelial cell proliferation and possesses a potent angiogenic and mitogenic activity on mesenchymal cells within the periodontal ligament (Li et al. 2017; Suarez-López del Amo et al. 2015).

FGF-2 has not been thoroughly studied yet in the field of periodontology. However, several animal studies have shown that this factor is useful in terms of periodontal regeneration improvement in class II furcation defects. Other pre-clinical studies have shown that FGF-2 induces the formation of
new root cementum in periodontal regeneration, with Sharpey’s fibers functionally oriented towards the alveolar bone. In human periodontal regeneration studies, it has been observed that FGF-2 has improved the bone fill percentage compared to control groups. It has been noted that with different FGF-2 concentrations, the bone fill percentage is higher in FGF-2 treated sites at 36 weeks than controls without growth factors. Specifically, the 0.3% dose of rhFGF-2 shows a significant positive impact on the bone fill percentage and in the linear bone growth. As it can be noted with preliminary data of this concentration, a clinical attachment level gain is also noteworthy (Murakami 2011; Khoshkam et al. 2015).

A second approach for tissue regeneration has been subsequently introduced to the periodontal community as active biomaterial for natural and synthetic scaffolds. For example; collagen membranes, fibrin nanofibers or synthetic materials (i.e., poly(lactic acid), polycaprolactone, polyglycolide, polylactide polymers and copolymers). These materials may also be designed with nano- or microstructure that can mimic the stem cell niche to alter the regulation of stem cells or to release molecules to induce and accelerate the periodontal regeneration cascade events (Skoog et al. 2018; Sheikh et al. 2017).

The reconstruction of critical size bony defects remains a challenge in oral and maxillofacial surgery, and the strategies have been focused on the use of xenografts, allografts and autografts (Johnson et al. 2011). The approach is called guided bone, and periodontal tissue regeneration and its concept advocate the reconstruction of defective periodontium tissues using occlusive membranes that exclude undesirable cell types (fibroblasts or epithelial cells). The membrane acts as a physical barrier to prevent rapidly growing tissues (fibrous or epithelial) from invading the defect space, maintaining space and guiding the success of the regeneration of defective tissues. The structural integrity of the membrane must be maintained during the maturation of the newly formed tissue and varies according to the application. Moreover, the membranes used as a barrier could be non-resorbable and resorbable where the last could be of natural or synthetic materials (Elgali et al. 2016).

Within the non-absorbable membranes are polytetrafluoroethylene (PTFE) and titanium mesh. These membranes offer an effective barrier function in terms of biocompatibility, can maintain the space for a sufficient period, have a more predictable clinical behavior, have a lower risk and are easy to manipulate. However, one drawback in the use of this type of membrane is the need for its removal with a second stage of a surgical procedure (Naung et al. 2019; Rakhmatia et al. 2013).

Polytetrafluoroethylene is considered a stable polymer, chemically and biologically inert, and able to resist enzymatic and microbiological attack based on its structure; there are two different types of membranes named as high-dense polytetrafluoroethylene (n-PTFE) and expanded-polytetrafluoroethylene (e-PTFE). The e-PTFE membranes have a higher porosity range (5–30 µm) which is believed to enhance regeneration by improving wound stability, support and isolation of soft tissues, creation of a space occupied by the clot, exclusion of non-osteogenic cells and improving bone formation. In studies carried out in animals and humans, histological analysis indicates that no inflammatory, epithelial cells or foreign body reactions have been found, unlike the presence of highly calcified osteoid matrix, invasion of fibroblasts cells, thin collagen fibers and small capillaries. However, the drawbacks of e-PTFE are that an early bacterial infection can occur and require soft tissue coverage or primary closure to prevent soft tissue ingrowth. Meanwhile, the n-PTFE have low porosity ranges (0.2–0.3 µm), non-expanded, non-permeable and is not necessarily the primary closure of the flaps, there is no need of additional surgical intervention because the membrane could be extracted with a clamping from the site, impairs bacterial penetration and also prevents cell adhesion (Soldatos et al. 2017; Greenstein and Carpentieri 2015; Carbonell et al. 2014).

Nowadays, cell-based approaches in combination with growth factors and lyophilized bone, membranes or metallic prostheses are probably the most abundantly researched and demonstrate the formation of healthy lamellar bone, without complications up to 6 months after treatment.

Finally, as a third approach and the most recent advance in regeneration strategies is to explore the manipulation by gene therapy that could be used in combination with polymeric scaffolds or
fuse biomaterials for improving the gene expression and allowing to regenerate healthy tissue. Gene therapy has generated an alternative parallel to cell therapy, since its main component is the delivery of genes, which encode mainly for growth and transcription factors, allowing the activation of the differentiation process of undifferentiated mesenchymal cells present at the site of injury to the corresponding cell lineage inducing the deposition of collagen, hydroxyapatite, osteogenic peptides according to clinical needs.

The primary limitations that this methodology faces are associated with the type of vectors used for the release of the gene or genes of interest. The genes used in gene therapy are released through the use of viral vectors such as adenovirus, adenoassociated viruses, retroviruses and lentiviruses. These vectors can integrate stably and randomly to the host genome (lentivirus and retrovirus), generating mutations that can result in severe genetic disorders. On the other hand, in the adenoassociated virus and adenoviruses that have a very low integration efficiency to the host genome, they generate an immune response when producing viral proteins (Jooss and Chirnule 2003).

For decades the role of the RNA molecule had been considered merely as a transition element between DNA and proteins, however, the discovery that double-stranded RNA molecules (dsRNA) could lead to the silencing of genes post-transcriptionally in the Caenorhabditis elegans model, led to the development of a molecular tool known as interference RNA (RNAi), which later proved its usefulness in the manipulation of mammalian cells (Fire et al. 1998; Caplen et al. 2001). In general, this post-transcriptional control system works by means of the initial synthesis of a primary micro RNA called pri-miRNA in the nucleus, which acquires a secondary structure of stem-loop type, later this pri-miRNA is processed by the DROSHA-DGCR8 protein complex, generating a short double-stranded RNA (shRNA) called pre-miRNA from ~ 20–30 bp, which binds to the protein Exportin 5, which in turn translocates it to the cytoplasm of the cell where it is released and associated with the proteins DICER/TAR RNA-binding protein (TRBP) which remove the terminal loop of the pre-miRNA, allowing the assembly of the RISC silencing complex (RNA-induced silencing complex) that include the protein argonaute. The RISC complex finally selects one of the strands of pre-miRNA, which will act as a guide for the silencing of the target transcript.

The RNAi system has been considered a therapeutic promise in multiple fields of medicine, however, it is a system composed of various components controlling their functioning at the molecular level, its use in humans has been limited to animal models. Recently in 2018 the FDA approved the use of patisiran (Onpattro; Alnylam Pharmaceuticals), a siRNA that acts on the liver for the treatment of hATTR (hereditary transthyretin amyloidosis with polyneuropathy) (Setten et al. 2019).

The therapeutic use of RNAi involves a series of strict controls regarding its design: Avoiding toxicity of RNAi-based drugs, immunogenic reactions to dsRNA, toxicity of excipients, unintended RNAi activity, and on-target RNAi activity in non-target tissues (Setten et al. 2019). In the tissue regeneration field, particularly in the dental tissue engineering area, the use of RNAi has been proposed as a means of controlling cellular processes that affect the natural process of tissue repair. In particular, its use has been intended against genetic targets involved in the process of inflammation, cell proliferation and apoptosis (Intini 2010). At the molecular level, RNAi silencing studies on BMP inhibitors such as nogging and chording have been extensively studied in bone repair and regeneration. As an additional example silencing by RNAi of the gremlin glycoprotein (antagonist of the BMP protein), had shown the activation of the signaling cascade mediated by BMP-2 in osteoblastic cells, which led to the expression of bone differentiation markers such as Runx2 and osteocalcin (Gazzerro et al. 2007). However, even though this has proved the efficiency of using RNAi to induce cell differentiation of dental stem cells to bone tissue, one of the main challenges of this methodology is the release of siRNAs from the extracellular space into the cytosol of the cell and the ensuing formation of the RISC complex. These methods include the use of naked siRNA, coupled ligands to siRNA which allow it to be internalized into the cell (GalNAc), lipid nanoparticles, nanohydrogels, aptamer-siRNA conjugates (Ghadakzadeh et al. 2016). Moreover, a separate chapter of this book focuses on periodontal and bone tissue engineering and gives a more indepth approach to these topics.
Dentin-Pulp Complex

In cases of severe pulpitis, the capability of self-regeneration or repair is limited because the odontoblasts that produce reparative dentin are destroyed, therefore root canal treatment is the treatment of choice. However, regeneration of pulp tissue instead of its total elimination, is the current priority, giving rise to the birth of the ‘biological endodontics’ that try to replace the necrotic pulp with scaffolds, healing promoting factors and cell therapies with the aim of regenerating new pulp and dentine within the root canal system (Rosa et al. 2013; Gotlieb et al. 2008). This strategy promises excellent versatility in the design of constructs, depending on the needs of each patient (Chandrahasa et al. 2011; Demarco et al. 2010). Several authors argue that the next therapy will be ‘regenerative endodontics’, which works in the neoformation of vascularized tissue very similar to pulp tissue. They suggest that such a treatment is the most fundamental approach for clinical translation (Hecksher et al. 2018; Galler et al. 2011). Both strategies are promising, need to refine some aspects such as the construct insertion level, the irrigant type that better promotes cellular maintenance, the acceptable pulp tissue remotion amount and antibiotic therapy. However; a separate chapter will give an in-depth approach to this exciting topic of regeneration of the dentine-pulp complex with revitalization/vasculization therapy.

Brief information of some strategies for the regeneration of the oral and craniofacial complex are described. Two specific chapters in the book will focus on the clinical progress in regenerative dentistry and how the results from different points of view address the regeneration of dental tissue engineering strategies that could be used for translation of the tissue engineering approach from laboratory to clinical applications.

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References


Current Advances in Oral and Craniofacial Tissue Engineering


Williams, D.F. 2014. There is no such thing as a biocompatible material. Biomaterials 35: 10009–10014.


option will play a new role as observers and ‘anonymous evaluators’. They will report possible adverse effects or will even contribute by publishing clinical cases sharing their experience with 3D-SCAFF. Some users and researchers may also get involved in a pragmatic trial to fully understand the day-by-day behavior of the biomaterial. Curiously, with the new materials new clinical needs will also emerge, and maybe the cycle and the development of new materials will start up once again.

Conclusions

Tissue engineering provides a new era for therapeutic medicine; it is progressing very rapidly and extends to include all tissues in our physique. Three decades ago, tissue engineering was an idea and today it has become a potential treatment for numerous conditions. However, the road from the conception of new ideas to a fully developed biomaterial is complex, and all the right steps to achieve a good product cannot be avoided. Not all the ideas will become a tangible option, many will be part of the laboratory ‘experience-stock’, but definitely every attempt will contribute to the process.

References


Translation of Tissue Engineering Approach from Laboratory to Clinics


Conclusions

The use of polymers currently represents a valid practice to design scaffolds for hard and soft tissues replacement in oral implants. In this perspective, bioinspired approaches based on the use of biopolymers (i.e., proteins, polysaccharides) naturally present in specific compartments of the biological tissues are tracing new routes for the restoration/regeneration of natural tissues located in the oral cavity—from hard (i.e., bone, dentin) to softer ones (i.e., pulp, gum).

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References


Polymer Materials for Oral and Craniofacial Tissue Engineering


Changing due to introduction of new techniques and technological advances (Dorozhkin 2013). Advances in dentistry material sciences contribute significantly to study and find new potential materials. Basically, in dentistry, they are used for prosthetic dentures, filling bone defects, root repair and for apical retro fills (LeGeros 1988; LeGeros n.d.).

**Conclusion**

The development of bioactive ceramics at the regeneration of tissues has created an important field of interest, since their characteristics are those converted into the application of routine use in various medical areas. Here of some advances achieved in the field of bioactive ceramics are described. Not only did the knowledge of fundamental processes governing biomineralization grow tremendously, but their application as targeted delivery vehicles and as synthetic bone graft substitutes has demonstrated very important successes. They were present and have been used for many years clinically, these successes have been somewhat downplayed among other developments in the field of biomaterials. This, however, does not reflect the enormous diversity they have to offer both in terms of products and their applications. And these have not yet been explored to their maximum extent. Recent technological developments will bring bioactive ceramics research and development to another step further, that fits well in the search for largely available and affordable strategies for damaged and diseased tissues. For this reason the biomedical industry has great potential for better understanding these types of materials while supporting the continuous development of their potential biomaterials.

**References**


Calcium Phosphate and Bioactive Glasses

Current Advances in Oral and Craniofacial Tissue Engineering


for craniofacial tissue regeneration, combining the reverse engineering approach with the recent advances in the design for additive manufacturing and sol-gel chemistry.

Conclusions

The understanding of physiology, complex processes, molecular pathways and remodeling features are crucial for the regeneration of craniofacial tissues (Tevlin et al. 2014; Thrivikraman et al. 2017). Even though it results in difficulty to reproduce the nature and structure of complex tissues, current scientific and technological advances (i.e., sol-gel chemistry, CAD-based approach, additive manufacturing and reverse engineering techniques) provide a great potential for the design 3D customized scaffolds for craniofacial tissue regeneration, which are able to guide systemic and local biological processes.

Materials, geometry, architectural features and porosity, together with the ability to release specific biomolecules and to tailor drug release kinetics, clearly play a key role in the design of advanced and multifunctional scaffolds for craniofacial tissue regeneration. As an example, the possibility of combining growth factors or stem cell-based therapy with 3D porous scaffolds characterized by controlled architectural features could become one of the most promising strategies (Tevlin et al. 2014; Thrivikraman et al. 2017).

However, in this context 3D cell printing technology would also be an interesting approach enabling researchers to suspend and position cells embedded in materials such as hydrogels (Tao et al. 2019). 3D bioprinting could allow in obtaining specific mechanical properties, cell interactions and desired distribution of growth factors. The possibility of printing blood capillaries has been already reported and cell printing for craniofacial tissue regeneration would seem feasible, even if many studies are still ongoing (Tao et al. 2019).

Accordingly, although in recent years much progress has already been made in terms of interdisciplinary collaboration, a continuous and collaborative research among engineers, chemists, biologists and surgeons would be necessary.

References


Therefore, future research must address the safety of cell-based therapies, as well as the quality control in the production of MSCs, to ensure their reproducible and efficient effects when it is delivered to patients (Gao et al. 2016).

Besides, the type of patients who would benefit the most from MSCs therapies should be identified, and rapid translation of basic science into the clinic must be avoided because it could put the patients at higher risk.

MSCs derived from dental tissues have been intensively studied for a wide range of diseases. Notably, their therapeutic potential for immune disorders have shifted our appreciation for their use. Rather than unrealistically considering the D-MSCs as an immunological panacea, research has started to define new limits on cell-based therapies and correlating their efficacy. In the future, such restrictions will define the new therapeutic targets for cell-based therapies (English and Mahon 2011).

Although the extensive available research suggests that therapies exist with D-MSCs to treat many intractable conditions is possible, several issues are yet to be resolved. The immune-suppressive functions of D-MSCs and the degree of redundancy that exist among the many suppressive processes is an outstanding and growing field of research (Kichenbrand et al. 2019).

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References


fields, including biomaterials, showing a very high potential especially as customization, complexity and low weight design are concerned. It is not surprising that this approach has recently been applied to cranioplasty. 3D printing of biomaterials, without the use of a mould, represents the cutting edge technology in bone cranioplasty. This integrated approach combines reverse engineering and additive manufacturing. Recent advances in additive manufacturing technologies for direct production of implants avoids limitations related to the constraints in shape, size and internal structure. On the other hand, from a material point of view, medical-grade PEEK filaments represent the future trends in cranioplasty, and carbon fibre reinforced PEEK filaments provide further mechanical enhancement.

References


and edentulism, by designing smart biomimetic hybrid composites that have been identified as the perfect solution able to respect and fit the complexity of the human body. To induce tissue regeneration, it is essential to dispose the novel biomimetic scaffolds capable of providing chemical and mechanical cues to promote multiple specific interactions and orchestrate processes such as cell adhesion, migration, differentiation, matrix synthesis, mineralization and/or vasculogenesis. For the creation of new smart biomaterials, the indepth study and understanding of the tricks nature uses to create them in their original environment is fundamental to mimic and sometimes even improve them.

Starting from a wide and deep study and characterization of the target site, and through a biomimetic approach, it was possible to obtain new materials based on biopolymeric matrix and a biomimetic inorganic phase presenting the potential to act as a source of biochemical and topographical signals for the various cell lineages present in dental environment. This means that well-conceived biomimetic hybrid materials are able to recruit endogenous cells and promote tissue regeneration, exploiting the intrinsic regenerative potential of tissues.

Nevertheless, the development of biomaterials mimicking structure, composition and mechanical properties of biological tissues still presents important limitations. In fact, oral tissues engineering, as a multidisciplinary approach to build complex structures such as bone, teeth or soft dental tissues, remains a challenge that will require further significant development of materials chemistry, biochemistry and biology for the formulation of a multifunctional system. This means that, by interdisciplinary approaches and close communication between material scientists, biologists and clinicians, future dental treatment will be able to combine biomimetic scaffolds, stem cells, growth factors and antibacterial or anti-inflammatory molecules, to support the whole tissue regeneration instead of its replacement.

References


Acknowledgements

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References


combining the hydrogel microfabrication technology with cell culture platform, such as microfluidics devices, to provide nutrients and oxygen to the cells within the hydrogels, has not been investigated thoroughly to date. The progress in the fabrication of hydrogels and the development of methodology for cell cultivation will offer long term improvement of the biological functions in 3D tissue constructs. However, many questions remain to be answered until naturally occurring scaffolds can be used for clinically relevant tissue engineering, including the immunologic response of the host to such implants and the methods to modify their mechanical and physical properties. Thus, the ideal scaffold, which promotes angiogenesis of engineered tissue sufficiently, has not yet been determined.

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References


derived growth factor-B delivered to alveolar bone defects exhibits safety and biodistribution profiles favorable

cementum protein 1 from electrospun multiphasic scaffold for cementum regeneration. Int. J. Nanomed.
41: 3145.

Chien, H.W., Tsai, W.B. and Jiang, S.Y. 2012. Direct cell encapsulation in biodegradable and functionalizable
carboxybetaine hydrogels. Biomaterials 33: 5706e12.

41: 82–88.

Dabra, S., Chhina, K., Soni, N. and Bhatnagar, R. 2012. Tissue engineering in periodontal regeneration: A brief


JBJS 85: 82–88.


erials 17: 1659–1665.

effects of platelet derived growth factor-BB and insulin-like growth factor-I, individually and in combi-


Giorgio, I., Saeid, K. and Francesco, B. 2019. Biomaterials, current strategies, and novel nano-technological

237: 1588–1595.


Hauschka, S.D. 1988. Cell surface fibroblast growth factor and epidermal growth factor receptors are permanently


Dentin-Pulp Complex Regeneration

preventing its recurrence while favoring the repair or replacement of damaged structures of the dentin-pulp complex.

The European Society of Endodontology and the American Association for Endodontists have released position statements and clinical considerations for regenerative endodontics. In general, they consider that the degree of success of regenerative endodontics depends on three factors: (i) resolution of clinical signs and symptoms and bone healing; (ii) further root maturation; and (iii) return of neurogenesis, positive response to vitality testing. The results are variable for these objectives, and a true regeneration of the pulp/dentine complex is not reached (Feigin and Shope 2017; Kim et al. 2018).

The American Association of Endodontists has suggested the “AAE Clinical Considerations for a Regenerative Procedure. Revised 6-8-16” to help clinicians manage immature permanent teeth with necrotic pulp/apical periodontitis. These considerations should be seen as one possible source of information and, given the rapid evolving nature of this field, clinicians should also actively review new findings elsewhere as they become available.

Significant advances in regenerative endodontics are permitting a better understanding of factors that mediated regeneration and repair of the damaged dentin-pulp complex. However, the evidence from diverse histologic and clinical results suggests that the radiographic findings derive from successful cases of regenerative endodontic only imitate ectopic tissue development in the root canal space. Clinically, positive responses of vascularized teeth to pulp testing do not indicate that a more organized vital pulp tissue is formed and do not imply regeneration of the pulp-like tissue in the root canal space (Lin et al. 2014). No studies have shown that normal regeneration of human tissue or organ is possible if it is totally damaged; thus, tissue or organ transplantation is necessary. Based on the published evidence, true regeneration of the pulp and dentin-pulp complex has not been supported consistently.

Tissue engineering strategies for dental dentin-pulp complex regeneration in preclinical studies include transplantation of stem/progenitor cells or use of biological molecules (Kim 2017). Regenerative endodontic clinical management uses the endogenous stem/progenitor cells from periapical tissues and biological molecules released from dentin or evoked bleeding (Smith et al. 2016). On the other hand, numerous case reports, and series of regenerative endodontic cases have been published (Kim et al. 2013). Considering global evidence, although the pre-clinical animal models have provided significant scientific bases of the possible benefits associated with the use of techniques based on tissue engineering, these days, important challenges remain for these methodologies and results to be extrapolated to humans.

Continuous laboratory and clinical studies are necessary in order to elucidate the potential benefits of clinical application of tissue engineering principles for repair/regeneration of the damage dentin-pulp complex. Complete regeneration of pulp–dentin complex in empty root canal space with all laboratory or clinical approaches has not been achieved as yet. All current approaches of pulp regeneration are still in the development phase. Significant concerns should be considered, such as treatments, the education of clinicians and students, facilities for obtaining dental stem cells, production of scaffolds, laboratory and clinical studies (Hashemi-Beni et al. 2017). Long-term, well-conducted and controlled randomized clinical trials with adequate sample size are necessary to achieve a high level of evidence to demonstrate the success rate to regenerate the dentine-pulp complex in the root canal space.

References


Dentin-Pulp Complex Regeneration


Current Advances in Oral and Craniofacial Tissue Engineering


References


Glorioso, J.C. and Lemoine, N. 2017. Gene therapy—from small beginnings to where we are now. Gene Ther. 24(9): 495–496.


Gene Therapy in Oral Tissue Regeneration


to the experimentation with mesenchymal stem cells and cells with certain lineages, regenerative medicine still has challenges to face, because to date it has not been possible to replicate an organ that possesses the physiological properties of an organ in its native state. So for this purpose the harmonic and synergic interaction that only through its multiple signaling can grant. However, the advances are great and encouraging, since they have been synthesizing ever better materials which are biointelligent that can observe the interactions of cells, molecules and scaffolds, which reproduce the natural characteristics of the organ in question. These advances give hope to millions of patients who are waiting for a tissue or organ to restore their health and thus improve the expectation and quality of life for them.

References


based approach for generation of clinical materials and treatment of dental diseases. The challenges of introducing endodontic tissue engineered therapies are substantial; the potential benefits to patients and the profession are ground breaking. Better understanding of cell interactions and growth along with further research can make dental tissue engineering a reality in the near future.

References


