Layered scaffolds for periodontal regeneration

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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

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

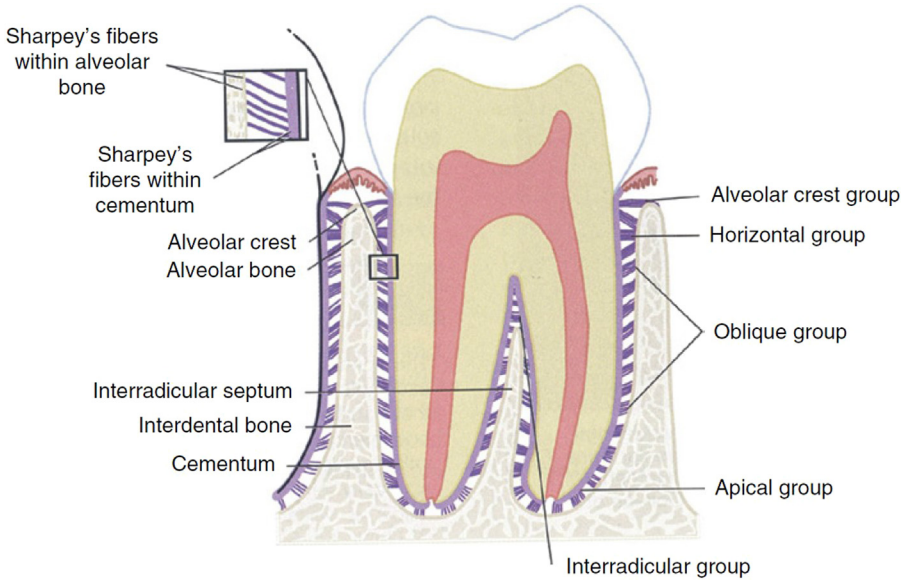

Figure 17.1 Schematic illustration of normal periodontium.
Reproduced from Bath-Balogh, M.M., Fehrenback, M.M., 2006. Dental Embryology, Histology, and Anatomy. Elsevier Inc. by permission from the publisher.

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.

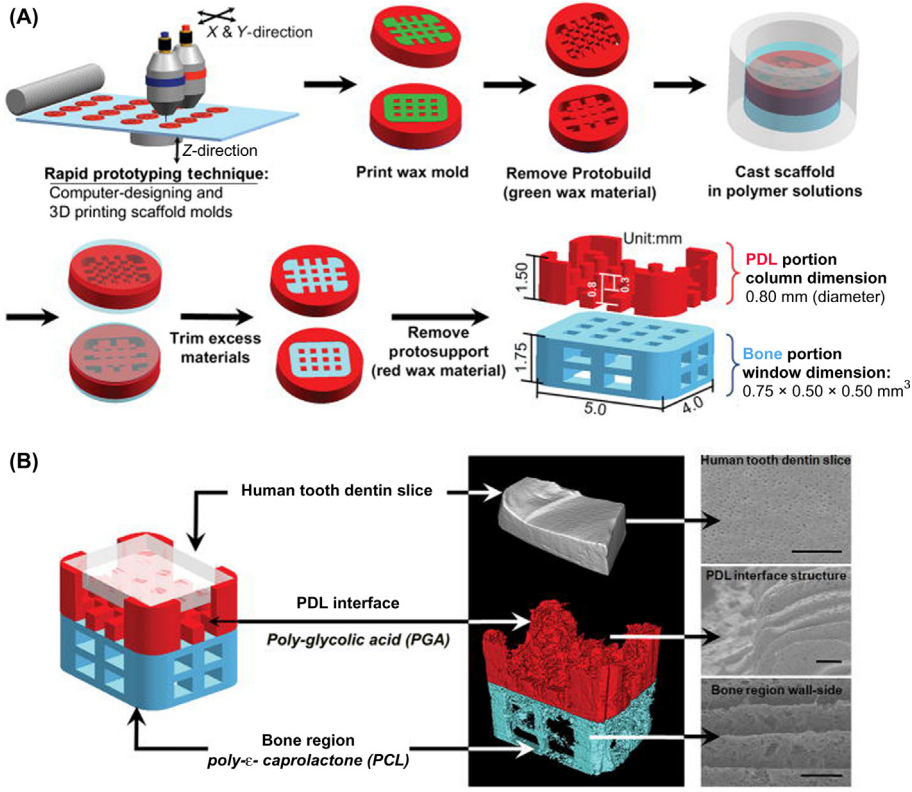

Figure 17.2 Schematic illustration of the 3-D wax printing system.
Reproduced from Park, C.H.C.H. et al., 2010. Biomimetic hybrid scaffolds for engineering human tooth-ligament interfaces. Biomaterials 31(23), 5945−5952. Available at: http://dx.doi.org/10.1016/j.biomaterials.2010.04.027 by permission 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.

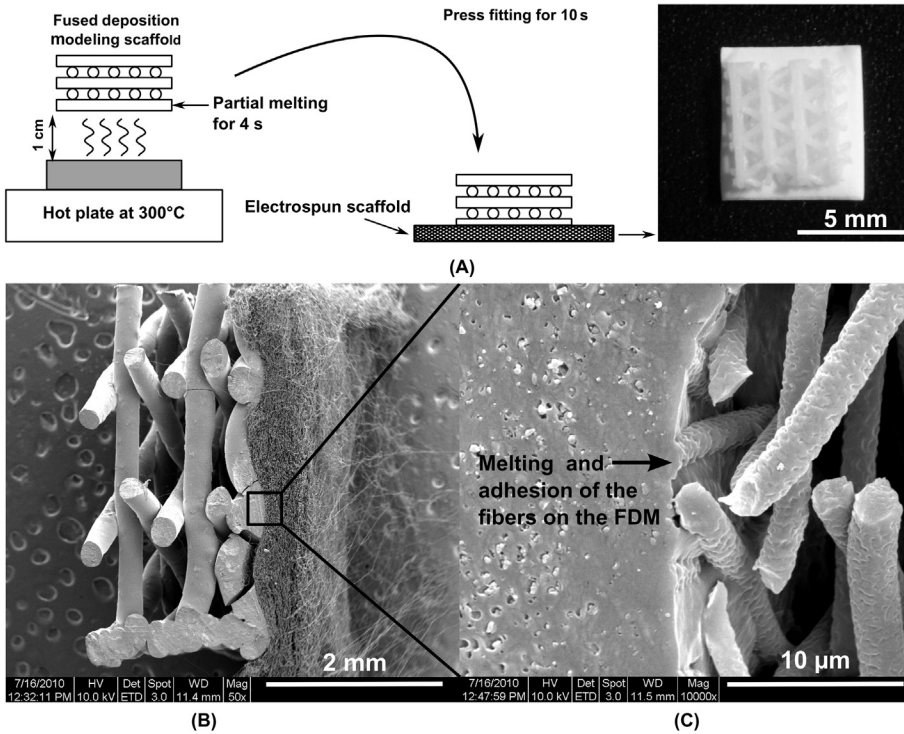

Figure 17.3 Fabrication of the biphasic scaffold. (A) Fabrication scheme, (B and C) cross-sectional views of the biphasic scaffold by scanning electron microscopy showing the fusion of the electrospun fibers (right hand side) onto the FDM component (left hand side). Reproduced from Vaquette, C.C. et 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)

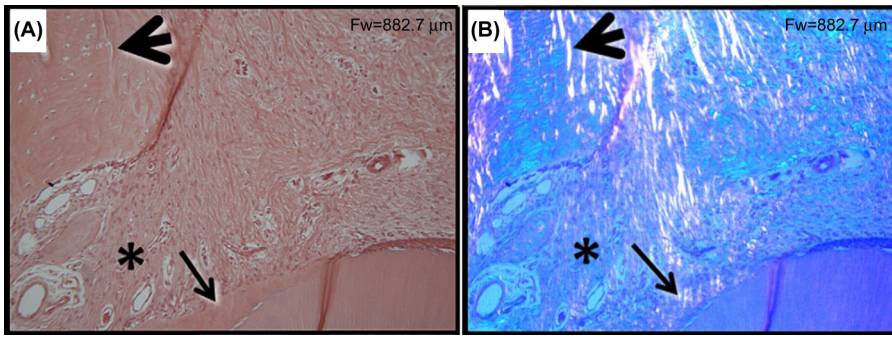

Figure 17.4 (A) New cementum (thin arrows) and periodontal ligament (*) formed along with the new alveolar bone (large arrows). (B) The collagen fibers of the periodontal ligament under polarized light, perpendicularly inserting in the new cementum, crossing the periodontal ligament and inserting in 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.

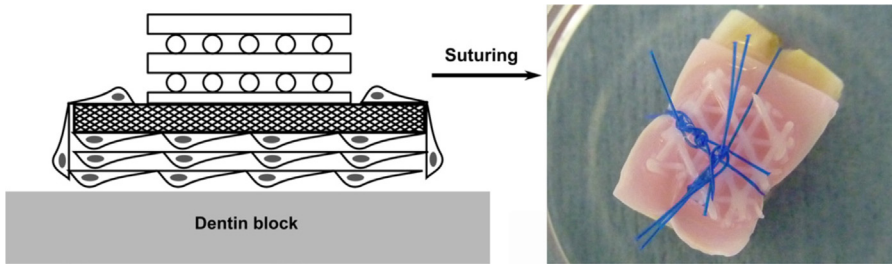

Figure 17.5 Description of biphasic scaffold assembling onto a dentin block.
Reproduced from Vaquette, C.C. et al., 2012. A biphasic scaffold design combined with cell sheet technology for simultaneous regeneration of alveolar bone/periodontal ligament complex. Biomaterials 33(22), 5560−5573. Available 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	–	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)
	Periodontal ligament cells			
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.