J.H. Kim^{1,2†}, C.H. Park^{1,2†},
R.A. Perez^{1,2†}, H.Y. Lee^{1,2}, J.H. Jang³,
H.H. Lee^{2,4}, I.B. Wall^{1,5},
S. Shi^{1,6}, and H.W. Kim^{1,2,4*}

¹Department of Nanobiomedical Science & BK21 PLUS NBM Global Research Center for Regenerative Medicine, Dankook University, Cheonan 330-714, Republic of Korea; ²Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan 330-714, Republic of Korea; ³Department of Biochemistry, Inha University School of Medicine, Incheon 400-712, Republic of Korea; ⁴Department of Biomaterials Science, College of Dentistry, Dankook University, Cheonan 330-714, Republic of Korea; ⁵Department of Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, United Kingdom; and ⁶Center for Craniofacial Molecular Biology, Ostrow School of Dentistry, University of Southern California, Los Angeles, CA, USA; [†]authors contributing equally to this work; *corresponding author, kimhw@dku.edu

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ABSTRACT

Periodontitis is an inflammatory disease that causes loss of the tooth-supporting apparatus, including periodontal ligament, cementum, and alveolar bone. A broad range of treatment options is currently available to restore the structure and function of the periodontal tissues. A regenerative approach, among others, is now considered the most promising paradigm for this purpose, harnessing the unique properties of stem cells. How to make full use of the body's innate regenerative capacity is thus a key issue. While stem cells and bioactive factors are essential components in the regenerative processes, matrices play pivotal roles in recapitulating stem cell functions and potentiating therapeutic actions of bioactive molecules. Moreover, the positions of appropriate bioactive matrices relative to the injury site may stimulate the innate regenerative stem cell populations, removing the need to deliver cells that have been manipulated outside of the body. In this topical review, we update views on advanced designs of biomaterials—including mimicking of the native extracellular matrix, providing mechanical stimulation, activating cell-driven matrices, and delivering bioactive factors in a controllable manner—which are ultimately useful for the regenerative therapy of periodontal tissues.

KEY WORDS: tissue engineering, biomaterials, biomimetic materials, regenerative medicine, biological factors, stem cells.

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Advanced Biomatrix Designs for Regenerative Therapy of Periodontal Tissues

OVERVIEW

Structure of Periodontal Tissues

Periodontal tissue surrounds and supports the tooth structure and consists of 4 components: gingiva, cementum, alveolar bone, and periodontal ligament (Fig. 1). The gingiva is a mucous membrane covering the tooth-bearing border of the jaw. The crown enamel and the root cementum meet at the marginal gingival (cemento-enamel junction), and the space between these structures is termed the gingival sulcus. The cementum is a hard tissue that covers the root dentin and anchors one side of the periodontal ligament (PDL) fibers. The other side of the PDL interfaces with the alveolar bone. Alveolar bone, which has properties similar to those of bone elsewhere in the body, supports the teeth and the gingival tissues. The root side of the alveolar bone consists of a thin layer of dense compact bone, appearing as a thin radiopaque line surrounding the root, called the lamina dura, into which the Sharpey's fibers of the PDL insert deeply (Nanci and Bosshardt, 2006). The PDL consists of functionally oriented collagen fibers (primary), elastic fibers (secondary) that have a vaguely anarchic orientation arranged around vessels, reticulin and oxitalan fibers, blood and lymphatic vessels, and nerves. As a functional unit, the PDL provides tooth fixation in the socket *via* dense connective tissue and is usually exposed to mechanical forces during occlusion and mastication. The functional stimulus of the mechanical forces to PDL and alveolar bone play an important role in the biological properties of periodontal cells to maintain healthy physiology (Weidenhamer and Tranquillo, 2013). Recently, several studies have reported that mechanical forces affect the synthesis of numerous mechanoresponsive osteotropic cytokines and growth factors, which are beneficial in mediating cellular and molecular events (Marchesan *et al.*, 2011).

Periodontal Diseases/Damage and Current Surgical Approaches

Periodontitis is an inflammation with destruction of the elements of the periodontium. It is characterized by the formation of a periodontal pocket by (i)

Figure 1. Structure of periodontal tissues. (A, B) Schematic illustrations of periodontal complexes, including cellular, vascular, and extracellular matrix elements. (C, D) Light-microscopic images of highly vascularized network in periodontal ligament (PDL) interface and gingival region. (E) Scanning electron microscopic images of rich vascular network (12x), with PDL, alveolar bone (AB), and inferior alveolar artery (IAA), (F) Volkmann's canals (VC; 50x), and (G) alveolar bone and PDL (150x) (Marchesan *et al.*, 2011).

irreversible destruction of the junctional epithelium from the tooth surface, (ii) loss of periodontal attachment between the cementum and alveolar bone, and (iii) pathologic deepening of

the gingival sulcus (Pihlstrom *et al.*, 2005). The ultimate goal in the treatment of periodontal disease is to use periodontal-regenerative techniques to achieve restoration of all periodontal attachment apparatus, including soft (gingival attachment and periodontal ligament) and mineralized (alveolar bone and cementum) tissues, to their original form, function, and consistency. Many researchers have provided evidence that most periodontal diseases can be treated with a broad range of interventions, but thus far, periodontal regeneration has not been satisfactory in humans (Chen *et al.*, 2010).

Bone regeneration, fibrous connective tissue formation, and new attachment of connective tissue from the tooth root surface to bone are critical for successful periodontal regeneration. For the past few decades, some effective surgical techniques have been developed for periodontal regeneration. These include bone grafting, root biomodification, guided tissue regeneration (GTR), and a combinatory approach with biological factors (Darby and Morris, 2013). While those periodontal therapies have been widely used and documented, the clinical outcome is still not optimal for many reasons.

Importance of Regenerative Approaches and Biomaterials for Periodontal Tissues

There is a huge conceptual shift from restoration to regeneration of tooth structure, including periodontal tissues. The discovery of stem cells, particularly mesenchymal stem cells (MSCs), spurred interest in regenerative therapies for damaged and diseased periodontal tissues (Mao *et al.*, 2006; Satija *et al.*, 2007). MSCs, residing in many adult tissues, are stimulated to migrate to injured sites and to acquire active biological functions for repair and regenerative processes, such as immune regulation, tissue-specific differentiation, and secretion of a cocktail of cytokines and growth factors (Lin *et al.*, 2009). This innate regenerative capacity *via* MSCs is often limited in the injured and diseased sites, particularly due to the severity of disease, large volume of defects, and the patient's health conditions (Kaigler *et al.*, 2013). Therefore, aiming to help MSCs function properly *in vivo* to regenerate tissues is a rational strategy, and this is possible through re-establishment of the microenvironments of MSCs.

Biomaterials play crucial roles in reconditioning the microenvironments of stem cells, providing key matrix cues for anchoring and spreading, to support self-renewal and appropriate differentiation (Horst *et al.*, 2012). As mentioned above, the grafting method is in fact the most clinically available technique utilizing matrices; it is simple, yet can provide suitable environments to support cell-mediated repair and regeneration. Motivated by technological advances and the regenerative concept, several functional biomatrix designs have been proposed. Control over the physical and chemical traits of the matrix surface determines cellular recognition to ligands and the consequent fate of cells (Lee *et al.*, 2014). A surface that mimics native tissue extracellular matrix (ECM) in terms of composition, topography, and rigidity is better recognizable to stem cells and more effective in driving them to differentiate into target tissues. Some recent technological advances have facilitated the development of tissue-mimicking designs of biomaterials (Jin

Table. Summary of Biomatrices Currently Available and Recently Generated by Advanced Approaches

Biomatrices for Periodontal Tissue Regeneration	Applicable Materials and Technologies	References
Pre-clinical and clinical applicable biomatrices	Bioactive ceramics	
	HA	Dorozhkin, 2010
	β -TCP	Li <i>et al.</i> , 2009
	CaP-coated surface	Shue <i>et al.</i> , 2012
	Degradable polymers	
	Natural	
	Collagen	Sachar <i>et al.</i> , 2014
	Gelatin	Kaigler <i>et al.</i> , 2013
	Chitosan	Suh and Matthew, 2000
	Synthetic	
	PCL, PGA, PLA	Liu and Ma, 2004
	PCL/ β -TCP	Costa <i>et al.</i> , 2014
	Composites	Lickorish <i>et al.</i> , 2004
		Perez and Ginebra, 2013
	Autologous biomatrices	
Advanced Biomatrices in Development	Platelet-rich plasma (PRP)	Creeper <i>et al.</i> , 2009
	Platelet-rich fibrin (PRF)	Thorat <i>et al.</i> , 2011
	Allograft biomatrices	Piemontese <i>et al.</i> , 2008
	Composition-mimicking biomatrices	Lee <i>et al.</i> , 2014
		Nudelman <i>et al.</i> , 2010
	Structure-mimicking biomatrices	
	ECM-mimicking	Jin <i>et al.</i> , 2003
	Periodontium-mimicking	Park <i>et al.</i> , 2012
	Cell-driven biomatrices	
	Demineralized bone matrix (DBM)	Miron <i>et al.</i> , 2013
	Cell sheet engineering	Iwata <i>et al.</i> , 2009
	Mechanically stimulated biomatrices	Kraft <i>et al.</i> , 2010
	Biofactor-delivering biomatrices	Chen <i>et al.</i> , 2009b

et al., 2003; Liu and Ma, 2004; Liu *et al.*, 2006). Cell-secreted biomatrices are also considered a promising platform, since they produce new biologic tissue similar to that of native ECM, ultimately enabling the *ex vivo* production of tissue-engineered constructs (Iwata *et al.*, 2009). Mechanical regulation of stem cells is another important yet often underestimated consideration, particularly in periodontal tissues, since they have a dynamic mechanical environment, which is key for maintaining tissue functions (Kraft *et al.*, 2010). Last, the biomatrices that exogenously deliver bioactive factors in a controllable manner will improve the regenerative capacity of tissues, due to a range of potential therapeutic actions including anti-inflammation, enhanced progenitor cell recruitment, angiogenesis, and tissue differentiation. The Table summarizes the biomatrices currently available and recently generated by advanced approaches, which will be discussed in detail in this review.

ADVANCED BIOMATRIX DESIGNS

Compositional Control to Mimic Native ECMs

While many different biomaterials are currently available for periodontal regeneration—including bioactive ceramics, biodegradable polymers, composites, allografts, and autologous materials (Appendix)—designing biomatrices that mimic native

tissue ECMs, particularly the composition, is of importance. The bone phase is particularly sophisticated, with many organic ingredients organized with inorganic mineral crystals. A primary route is the mimicking of mineral-phase hydroxyapatite (HA), which is enabled by the biomimetic synthesis of HA, including low-temperature solution-based methods (Tas, 2000; Moreau *et al.*, 2009). HA with poorly crystallized form has better bone bioactivity, such as protein adsorption, cellular osteogenesis, bone formation, and bone remodeling, due to its higher dissolution properties (Sawyer *et al.*, 2005). However, most synthetic routes involve only ionic-buffered medium in the absence of biological proteins. Since the mineralization of HA *in vivo* is mainly induced by cellular processes and the interactions with bone ECM proteins, such as collagen, osteopontin, and osteocalcin (Nudelman *et al.*, 2010), the biomimetic route to prepare HA needs to involve those kinds of proteins. Some studies have utilized protein molecules in the creation of HA minerals (He *et al.*, 2003). Moreover, *in situ* mineralization of HA within the collagen fibrils is an effective means of preparing bone-ECM-mimicking biomatrices (Lickorish *et al.*, 2004).

In addition to tailoring bulk properties of biomatrices, controlling the surface to mimic native bone ECM composition is an attractive approach because cells first recognize the surface to adopt subsequent behaviors like osteogenesis and bone formation. In fact, many types of bone ECM proteins (*e.g.*,

Figure 2. Biomaterials designed to mimic the native extracellular matrix (ECM) compositions. **(A)** Bone mineral apatite. (A-a) Two-dimensional cryo-TEM image. (A-b) Slice from a section of the three-dimensional volume. (A-c) Computer-generated three-dimensional visualization of mineralized collagen (scale bar, 100 nm). Cryo-TEM images of (A-d) stained, non-mineralized collagen, (A-e) mineralized collagen, and (A-f) stained fibril (mineralized) (scale bar, 50 nm). (A-g) Intensity profile of A-d; (A-h) intensity profile of A-e; and (A-i) histogram of the distribution of the number of nucleating crystals per staining band (Nudelman *et al.*, 2010). **(B)** Hydroxyapatite (HA)-collagen (COL) nanocomposites: SEM images of the HA scaffold coated (B-a) with non-cross-linked COL with compact COL; (B-b) collagen fibers; (B-c) with EDC/NHS cross-linked COL; and (B-d) heterogeneous COL distribution. (B-e) BMP-2 cumulative release profiles: HA scaffold (♦), HA scaffold coated with COL (■), and HA scaffold coated with COL/heparin conjugate (▲). (B-f) Concentration of BMP-2 retained: HA, HA scaffold with COL (HA XL COL) and HA scaffold with COL and heparin (HA COL HEP). Scale bars: 600 μm for B-a and B-c; 30 μm for B-b and B-d (Teixeira *et al.*, 2010).

fibronectin and collagen) have been introduced to the surfaces of scaffolds made from biopolymers and bioceramics by either weak chemical interactions or tight covalent links (Teixeira *et al.*, 2010). One recent approach involving a fibronectin-osteocalcin fusion protein is noteworthy, where the fusion protein was designed to accelerate initial stem cell adhesion *via* fibronectin and subsequently to accelerate osteogenesis and mineralization by osteocalcin at a much later stage. Importantly, tethering of the fusion protein was possible thanks to the affinity binding of osteocalcin to the HA crystal lattice, *i.e.*, molecular recognition of carboxyglutamic acid sequences in osteocalcin to 5 calcium ions in HA (Lee *et al.*, 2014). Aside from bone, a recent attempt to construct a cementum-like biomineralized layer has also been carried out (Gungormus *et al.*, 2012), where amelogenin-derived peptides were used to control HA mineralization and to mimic ECM composition of cementum. The specific region identified showed the formation of a mineral phase that supported attachment of periodontal ligament cells. Fig. 2 depicts the aforementioned approaches to compositional mimicking biomaterials.

Advanced Technologies to Mimic the Nano-/Microstructure of Native ECMs

The main challenge in periodontal tissue engineering is the hierarchical formation of sub-micron-scaled, multiple interfaces with maturation of alveolar bone, PDL orientation, and tissue integration. Recently, architecturally mimicking or anatomically adaptable constructs have been highlighted for periodontal tissue regeneration. Technological advancements have led to the development of structural mimicry within 2 types of scaffolds; ECM-mimicking nanofibrous scaffolds, and periodontium-mimicking scaffolds with nanoscopic and microscopic focuses.

ECM is the natural cell-supportive platform with nanofibrous structures and different types of proteins to promote cell activities (Wei *et al.*, 2007). By different methods, including electrospinning, phase separation, and peptide self-assembly, a nanofibrous form of polymeric scaffolds with different compositions has been successfully generated (Jin *et al.*, 2003). The structurally mimicking ECM constructs are often combined with bioactive molecules on the surface or within the structure

Figure 3. Biomatrices designed to mimic the native extracellular matrix (ECM) micro/nano-structures. **(A)** Nanofibrous scaffolds. (A-a) SEM of nanospheres (scale bar, 500 nm); (A-b) macroscopic photographs of scaffolds (scale bar, 1 mm); SEM of nano-fibrous scaffolds before nanosphere incorporation at 100x (A-c) and 10,000x (A-d); and SEM of nano-fibrous scaffolds after nanosphere incorporation at 100x (A-e) and 10,000x (A-f). Scale bars: 200 μ m for A-c and A-e; 2 μ m for A-d and A-f (Wei *et al.*, 2007). **(B)** 3D printed scaffolds to mimic periodontal ligament (PDL) microscopic structure. (B-a) Micro-computed tomographic image of mandibular defect; (B-b) computer-designed scaffold; (B-c, B-d, B-e) computational adaptation of scaffold; (B-f, B-g) freshly sectioned images of the scaffold; and (B-h) type-I collagen fluorescence (yellow arrows: type-I collagenous fiber bundles). Scale bar: 50 μ m (Park *et al.*, 2014).

to improve cellular activities (Franceschi, 2005). The nanofibrous structured matrices supported cells related to periodontal tissues, including PDL cells, bone cells, and stem cells, and stimulated them to engage in proper ECM synthesis and maturation, even better than their densely structured and/or microfibrillar counterparts (Jin *et al.*, 2003).

Mimicking perpendicular or oblique orientations of PDL in the 200- to 300-micron PDL interface has been challenging by conventional methods. The spatially oriented PDL plays a significant role in mechanical functioning against regular occlusal or masticatory loading. Recently, however, computer-designed 3D printing technology made possible the creation of microscopically tailored scaffolds to anchor tooth roots to alveolar bone surfaces (Park *et al.*, 2014). The developed scaffolds microscopically mimicked the architecture of natural PDL, and successfully guided the orientation of regenerated fibrous connective tissues, perpendicularly and obliquely. The image-based, fiber-guiding scaffolds resulted in: (i) bone tissue formation; (ii) PDL orientation with cementogenesis on the tooth root surface; and (iii) functional regeneration of periodontal complexes, highlighting the advancement of current state-of-the-art technology

to mimic hierarchical periodontal tissue structure (Park *et al.*, 2014). The aforementioned approaches to structural mimicking biomatrices are illustrated in Fig. 3.

Cell-driven Biomatrices

Among the sources of cell-driven biomatrices, demineralized freeze-dried bone matrix (DFDBM) has been widely utilized for bone formation and periodontal treatments, pre-clinically and clinically, due to its clinical safety and capacity for bone formation, osteoconductivity, and osteoinductivity (Gurinsky *et al.*, 2004; Piemontese *et al.*, 2008; Banjar and Mealey, 2013). Moreover, commercialized DFDBMs have been combined with various biological factors for periodontal tissue regeneration, including enamel matrix derivative (EMD) (Gurinsky *et al.*, 2004; Miron *et al.*, 2013), BMP-2 (Schwartz *et al.*, 1998), platelet-rich plasma (PRP) (Piemontese *et al.*, 2008), and platelet-rich fibrin (PRF) (Bansal and Bharti, 2013).

Demineralized dentin was developed similarly. The demineralized dentin matrix promoted periodontal ligament cell proliferation and differentiation, since it contains a large amount of

Figure 4. Biomaterials driven by cells. **(A)** Demineralized freeze-dried bone matrix (DFDBM). (A-a, A-b) SEM images of DFDBM; (A-c, A-d) blood-coated DFDBM particles; and (A-e, A-f) enamel matrix derivative (EMD)-coated DFDBM particles. Scale bars: 100 μ m for A-a, A-c, and A-e; 50 μ m for A-b, A-d, and A-f (Miron *et al.*, 2013). **(B)** Cell-sheet engineering. (B-a) Schematic illustration of periodontal defects and three-layered cell-sheet transplantation. (B-b) Surgically created intrabony defects. (B-c) Transplantations of three-layered periodontal ligament (PDL) cell sheets and β -TCP. (B-d, B-e, B-f, B-g) Micro-CT analysis of periodontal defects after six-week period of healing. Photomicrographs of contralateral defect sites receiving β -TCP (B-h) and PCL cell sheet/ β -TCP (B-i). Polarized light-microscopic images (B-j, B-k). Scale bar: 500 μ m (Iwata *et al.*, 2009).

growth factors, and when exogenous growth factors such as BMP were combined, the demineralized dentin matrix enhanced periodontal regeneration, leading to a cementum-like tissue formation (Miyaji *et al.*, 2010).

Cell-sheet engineering has great promise as a tissue-engineering strategy (Iwata *et al.*, 2009). The engineered PDL cell sheets were layered and placed on the periodontal defect site in a canine model for periodontal complex regeneration (Tsumanuma *et al.*, 2011). The cell culture plates were chemically modified by means of a temperature-responsive polymer, poly(N-isopropylacrylamide) (pNIPAAm). During cell culture at 35° to 37°C, cell-cell and cell-matrix interactions were generated to form a mono-layered sheet. However, when the polymer-coated surface was incubated at 32°C, polymer chains became hydrophilic and too swollen to push the cultured cell sheet from the surface. For the periodontal complex, three-layered PDL cell sheets were placed on the exposed tooth-root surface, and then β -tricalcium phosphate (β -TCP) was filled in the canine defect (Iwata *et al.*, 2009). The methodology demonstrates surgical simplicity, effective dimensional compartmentalization (bone-PDL-tooth), and interfacial isolation for individual tissue growth and maturation, with high clinical relevance (Iwata *et al.*, 2009).

Recently, advanced combinatory technologies assembling cell sheets with 3D scaffolds have also been reported to construct periodontal complex mimics (Vaquette *et al.*, 2012, 2013). Constructs were composed of a bone compartment manufactured by fused deposition modeling (FDM), three-layered cell

sheets for PDL, and then an electrospun poly- ϵ -caprolactone (PCL) membrane between bone and PDL interfaces to provide physical stability to the cell sheets. The periodontium-mimicking complexes were transplanted to induce limited hierarchical periodontal tissue regeneration ectopically (Vaquette *et al.*, 2012). To improve osteoconductive properties, FDM PCL scaffolds were surface-modified with bone-mimetic calcium phosphate (CaP). These engineered scaffolds enhanced bone infiltration, vascularization, and fibrous tissue attachment to the mineralized tissue surface featuring tooth-supportive structures (Costa *et al.*, 2014). Combinatory technology thus offers improved spatially predictable and controllable tissue compartmentalization with micron-/nano-scaled, specific periodontal interfaces (Costa *et al.*, 2014). Fig. 4 presents the exemplar studies on creating cell-driven biomaterials.

Mechanically Stimulated Biomaterials

Recently, mechanical stimuli have been highlighted in the repair and regeneration of tissues, including periodontal tissues. The mechanical loads significantly alter the PDL and bone responses, activating tissue remodeling at the periodontal interfaces and leading to bone resorption through osteoclastic factor release (Mayahara *et al.*, 2007). *In vitro* mechanical shear stress has been shown to promote the osteogenic differentiation of dental stem cells, including those derived from pulp, alveolar bone, and PDL (Kraft *et al.*, 2010). However, most *in vitro* studies

have focused on osteogenic differentiation of different dental stem cells rather than on ligament or bone-ligament interfacial tissue regeneration. Because of complicated, sub-micron interfacial structures, periodontal tissue regeneration requires precise control of stem cell activation and integration into multiple tissues, which needs further study. Moreover, most studies on the effects of mechanical stimuli on stem cells have been performed in 2D culture dishes (Raab *et al.*, 2010), which do not accurately translate to 3D gel-like tissue environments. Therefore, further investigation into mechanical-cell interactions needs to be carried out under environmental conditions analogous to native tissue characteristics, such as 3D hydrogel matrices with matched stiffness (Raab *et al.*, 2010). Ultimately, the combinatory approach of biomimetic matrices with mechanical stimuli may deliver optimal culture conditions for stem cells to direct their fate into tissue-equivalent constructs *ex vivo*. The mechanical pre-stimulation and pre-differentiation of stem cells on biomimetic matrices prior to cell transplantation will be the next step to generate stem-cell-based engineered periodontal tissues that better mimic *in vivo* microenvironments. This remains a promising future technology for periodontal tissue regeneration.

Biofactor-delivering Biomatrices

While the immobile ECM molecules are the first design criterion producing biomimetic scaffolds, a cocktail of soluble bioactive molecules, such as growth factors and cytokines, should also be considered as critical in the regeneration of periodontal tissues. Therefore, strategies to exogenously deliver bioactive molecules provide a rational platform for the creation of therapeutic biomatrices. Above all, the bioactive molecules need to be protected from the *in vivo* environment and released at specific time points during the healing process.

Some possible elegant designs for this protective and controllable delivery system have recently been proposed. Fig. 5 summarizes the designs to produce therapeutic biomatrices for periodontal regeneration. In fact, the simplest approach is to tether the molecules to the surfaces of biomatrices. This approach is relevant when rapid release is needed, leading to direct contact with the cells and consequent initial cellular activation (Cooke *et al.*, 2006). While surface-tethering of bioactive molecules can be improved *via* stronger bonds to slow the release rate, a better approach is to incorporate within the biomatrices. However, many types of biomatrix do not allow for safe incorporation, mainly due to the necessary processing conditions, *e.g.*, the use of organic solvents and high-temperature processes,

Figure 5. Designs of therapeutic biomatrices to load and deliver bioactive molecules.

which limit the incorporation of bioactive molecules in native form (Chen *et al.*, 2010). *In situ* gelling natural polymers and self-setting inorganic cements are some candidates for this direct incorporation of bioactive molecules. Therefore, a more general and powerful method to incorporate bioactive molecules within the structure of biomatrices has been proposed. Micro-/nanoparticles encapsulating the molecules can be combined with biomatrices. Alginate microspheres preloaded with BMP-6 were incorporated into chitosan porous matrix, enabling more sustained release of the BMP-6 and stimulation of osteogenesis by MSCs for periodontal tissue engineering (Soran *et al.*, 2012). Along with the proteins, genetic molecules have also been delivered to cells *via* viral or non-viral vectors for periodontal regeneration (Chen *et al.*, 2009a). The target gene sequences are encoded and delivered by modified vectors for internalization of genetic information. Gene-transfected cells have already been reprogrammed for local production of the proteins desired for periodontal tissue regeneration (Taba *et al.*, 2005).

In addition to single-factor delivery, the delivery of multiple bioactive molecules is gaining great interest for the functional design of therapeutic biomatrices. Because the tissue repair and regeneration process is a complex series of events involving growth factors and cytokines that have temporal and dose-dependent activities, delivery of multiple factors in a controlled manner is a rational approach. Administration of 2 different growth factors often involves their partitioned incorporation within micro-/nanocarriers, enabling the simultaneous release of

2 different factors. More advanced is the use of different nano-/microcarriers, where the carriers have different release kinetics based on properties such as degradability, size, and permeability, enabling the independent/sequential release of molecules (Chen *et al.*, 2009b). For instance, scaffolds prepared by the incorporation of microspheres that contain BMP-2 or insulin-like growth factor (IGF)-1 facilitated prolonged release for both of the growth factors in an independent manner (Chen *et al.*, 2009b). A combinatory approach of using nano-/microcarriers loaded with one factor and scaffolding biomatrices loaded with the other factor is also possible, to allow for sequential delivery (Yilgor *et al.*, 2009). In such delivery systems, one factor is released rapidly, while the other factor is released much more slowly, enabling the bi-phasic delivery of factors. This sequential delivery concept can be profoundly utilized in periodontal regeneration *via* delivery of anti-inflammatory mediators in the initial phase, followed by regenerative molecules later, or angiogenic factors followed by osteogenic mediators at a later time, or chemotactic recruitment of stem cells initially, followed by stimulation of their tissue-specific differentiation (Yilgor *et al.*, 2009; Sundararaj *et al.*, 2013). For example, the use of 2 different nanocapsules with different degradation rates made of PLGA and PHVB allowed for a sequential release of BMP-2 followed by BMP-7 (Yilgor *et al.*, 2009). Additional recent work described an elegant design made of multilayer materials that allowed for the release of 4 different drugs in a degradation-dependent sequential manner (Sundararaj *et al.*, 2013). While multiple and sequential delivery strategy has not yet been fully realized for periodontal tissue regeneration, the concept can be potentially useful to achieve significant therapeutic effects, improving the regenerative capacity of damaged and diseased periodontal tissues.

CONCLUDING REMARKS

A regenerative approach utilizing stem cells holds great promise for the successful treatment of periodontal tissues, including periodontal ligament, cementum, and alveolar bone. The role of biomatrices is thus of special importance in guiding, altering, and regulating the behaviors of stem cells toward regenerative processes. Although clinically proven techniques, including bone grafting and guided tissue regeneration, use conventional available biomatrices, advanced biomatrices that ultimately function to engage in anti-inflammation, stem cell homing, angiogenesis, and bone/PDL integration are required for successful periodontal regeneration.

Biomimetic approaches to native periodontal tissue ECMs in terms of composition and tissue architecture will possibly create biomatrices that have mechanical and biological properties comparable with those of autologous tissues. In this way, technological advances contribute significantly to the design of sophisticated tissue architecture at the micro-/nanoscale. Not only are structural ECM components important, but also soluble molecules such as growth factors, which are essential components of microenvironments that promote tissue repair and regeneration. Multiple biological factors are involved and need to be delivered in the correct temporal fashion at relevant doses, which can be reflected in the design of biomatrices, to enable therapeutically active biomatrices

to perform. Conversely, designed biomatrices synthesized by living cells, *i.e.*, stem-cell-derived biomatrices, are considered to better mimic the native ECMs where the cells' innate capacity for ECM generation can be fully utilized, ultimately creating tissue-equivalent products. For this, biomimetic and therapeutic ECMs can better function in driving stem cell differentiation toward lineages of interest. Another consideration, important yet often ignored, is the mechanical-stimulating strategy, *i.e.*, to mimic the *in vivo* mechanically dynamic environments to achieve *ex vivo* functional tissues cultured with stem cells.

The designs and technologies for biomatrix engineering outlined herein offer considerable promise for clinical application in the future. However, further advancements are needed to unite fundamental science, pathophysiology, and engineering capabilities for the successful generation of complex periodontal tissue architectures for regenerative therapies.

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