Possible functional scaffolds for periodontal regeneration

Hidetoshi Shimauchi a,*, Eiji Nemoto a, Hiroshi Ishihata a, Masatsugu Shimomura b

a Division of Periodontology and Endodontology, Graduate School of Dentistry, Tohoku University, 4-1 Seiry-u-machi, Aoba-ku, Sendai 980-8575, Japan
b Organized Polymer Material, Institute of Multidisciplinary Research for Advanced Materials Laboratory (IMRAM), Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan

Received 9 March 2013; received in revised form 29 April 2013; accepted 22 May 2013

Summary Periodontal diseases are the most prevalent infectious diseases with the irreversible loss of tooth supporting apparatus. To reestablish the stable health of periodontal tissues after healing, the concept of regenerative surgical procedures has been developed over the past three decades, including various grafting materials, guided tissue regeneration (GTR), and the use of enamel matrix derivatives (EMD) in a clinical setting. More recently, tissue engineering strategies have also been applied and developed for periodontal regeneration: (1) stem cell therapies; (2) recombinant human growth factor therapies; (3) combined use of cell and growth factors with matrix-based scaffolds. However, the complete and predictable reconstruction of healthy periodontal tissues still remains a challenging field. To overcome therapeutic limitations and develop stem cell-based strategies, it is necessary to optimize cell—scaffold combinations by understanding the cellular events during periodontal wound healing and regeneration. We reviewed: (1) the current status and strategies for periodontal regeneration; (2) a possible biomaterial design for the scaffold used in periodontal tissue engineering; (3) a possible interaction between scaffold materials and periodontal tissue cells.

© 2013 Japanese Association for Dental Science. Published by Elsevier Ltd. All rights reserved.

1. Introduction ................................................................. 119
2. Current strategies for periodontal regeneration ................................................................. 119
3. Periodontal tissue engineering ................................................................. 120
4. The desirable properties of scaffolds designed for periodontal regeneration ................................................................. 122
  4.1. Mimicking the stem cell niche to alter the regulation of stem cells ................................................................. 122

* Corresponding author. Tel.: +81 22 717 8333; fax: +81 22 717 8339.
E-mail address: simauti@dent.tohoku.ac.jp (H. Shimauchi).

1882-7616/$ – see front matter © 2013 Japanese Association for Dental Science. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jdsr.2013.05.001
1. Introduction

Periodontal diseases are the most common inflammatory diseases caused by plaque biofilm in the oral cavity. During the progression of periodontal diseases, the pathological change of gingivitis to periodontitis is characterized by the irreversible loss of tooth supporting apparatus, ultimately leading to the loss of the tooth. It includes the progressive destruction of whole periodontal tissue: the gingiva, periodontal ligament (PDL), cementum, and alveolar bone, as well as the connective tissue attachment between the root surface and alveolar bone [1]. The concept of conventional periodontal treatments is a “cause-related therapy” aimed at removing causative agents from the oral cavity, with a focus on eliminating bacterial plaque and calculus. This therapy commonly results in successful improvements in the inflammatory process of periodontal tissue; however, it only induces the unstable healthy condition of destroyed periodontal tissue in case of progressive periodontitis even after a successful result. The recovered healthy condition of destroyed tissue is easily broken by the reaccumulation of bacteria surrounding the teeth, leading to the recurrence of periodontitis. In the United States, a recent national survey (2009–2010 National Health and Nutrition Examination Survey; NHANES) revealed that an estimated 47.2% or 64.7 million adults aged 30 and over suffered from periodontitis, and prevalence rates increased to 70.1% in adults 65 and older [2]. The prevalence rates of periodontal diseases in Japan are also similar, indicating that periodontal diseases are one of the most common infectious diseases in the world. Furthermore, recent clinical research has indicated the close and bidirectional relationship between periodontitis and systemic disorders, such as diabetes, cardiovascular disease, and metabolic syndromes. Thus, it is important to reestablish the stable health of periodontal tissues after healing, as well as prevent periodontal diseases to maintain both systemic and oral health.

Why is periodontitis at high risk of recurrence? There are two major reasons: easily occurring bacterial re-accumulation and the formation of a long junctional epithelium (periodontal repair) after surgical/non-surgical procedures. Lindhe et al. [3] investigated the long-term effects of surgical/non-surgical treatment over a 5-year period, and concluded that the patient’s self-plaque control, but not treatment modality, was the critical determinant of a good prognosis in periodontal therapy. Both of the procedures induced healing with a long junctional epithelium that was easily broken by recurrent inflammation due to plaque accumulation [4]. To obtain good stability and predictability after therapy, periodontal regeneration of destroyed tissue, which is characterized by de novo formation of cementum, a functionally organized PDL, alveolar bone, and gingiva, is desirable. Clinical research has extensively shown that regeneration remains the favorable outcome over periodontal repair [5,6].

The desire to induce the complete regeneration of periodontal tissue has inspired the introduction of tissue engineering technology into dental clinics [7,8]. Tissue engineering is defined as a multi-disciplinary field of medicine, chemistry, physics, engineering, and biology [9]. The triad for conventional cell-based tissue engineering involves cells, signaling molecules, and scaffold/supporting matrices [10]. In this triad, the role of the scaffold is the “niche” of cells, and facilitates the attachment, migration, proliferation, and three-dimensional (3D) spatial organization of the cell population. However, recent progress in material sciences has provided bioactive properties to scaffold materials for transmitting specific signals to cells that will decode these into biochemical signals. Topography, chemistry, and physical properties are considered to be critical parameters for directing cell fate [11]. This review has focused on the following topics: (1) the current status of and strategies for periodontal regeneration; (2) a possible biomaterial design for the scaffold used in periodontal tissue engineering; (3) a possible interaction between scaffold materials and periodontal tissue cells.

2. Current strategies for periodontal regeneration

For more than three decades, periodontal research has been attempting to discover clinical treatment regimens that can regenerate periodontal tissues with good predictability. These trials successfully developed two types of strategies with the combined use of biomaterials and grafts shown in Table 1.

A number of animal and human trials demonstrated that the combined use of bone grafts/implant materials with flap surgery successfully stimulated alveolar bone regeneration. These grafts/materials include: (1) autogenous grafts; (2) allogeneic graft; (3) xenogeneic grafts, and (4) alloplastic materials [12]. The use of grafts/biomaterials presumably served as a scaffold for bone formation and contained the bone-forming cells and bone-inducing substances that finally resulted in bone formation. The biological performance of bone grafts can be divided into three interrelated, but not identical, rationales: osteogenesis (the formation of new bone by stem cell lineage derived from graft material); osteoinduction (bone growth by the surrounding immature cells recruited by graft material); and osteoconduction (bone growth on the surface of a material with fabrication) [13]. Autogenous grafts only contain self-bone forming cells.
that can induce osteogenesis, and still serves as the “golden standard” of bone grafts.

The second strategy aimed at regenerating a 3D arrayed structure of lost periodontal tissue including root cementum, alveolar bone, and the PDL with the connective tissue attachment. The biological rationale of this strategy was based on the “Melcher hypothesis” [14], which proposed that the nature of the attachment in periodontal healing depended on the origin of cells (epithelial, gingival connective, bone, PDL) repopulating the area adjacent to the root surface. The hypothesis was successfully demonstrated in a series of animal experiments, and the principal of Guided Tissue Regeneration (GTR) was established [15,16]. The cell occlusive membrane of GTR functions to isolate the periadicular bone and root surface wound area from the rest of the tissues to maintain a space for the repopulation of cells originating from the PDL. For this purpose, different barrier materials have been used, both non-resorbable and resorbable (biodegradable).

The concept of the GTR technique only awaits the repopulation of PDL cells in the wound healing area and does not interfere with the growth/differentiation speed of these cells. Recent biological approaches have attempted to mimic/enhance the cellular events leading to the normal development/wound healing process of periodontal tissues [17]. Enamel Matrix Derivative (EMD), the active component of Emdogain®, was the first signaling molecule that could regenerate periodontal tissue. Emdogain® is prepared from a piglet’s unerupted teeth and has been clinically used throughout the world after approval for the market in Europe (1995), the United States (1996), and Japan (1998) [18]. Amelogenin proteins that dominate 90% of EMD are capable of periodontal regeneration, but the contaminated ameloblastin functioned synergistically [19]. Better understanding of the wound healing/regeneration process has driven periodontal research to evaluate the role of growth factor, which is endogenously secreted during the wound healing/regeneration process. It is also meritorious that advances in molecular cloning have made unlimited quantities of recombinant human proteins available for tissue engineering.

Various recombinant growth factors were studied for their periodontal regeneration ability in vitro and in vivo. Among them, platelet derived growth factor (PDGF; GEM215®) and bone morphogenetic protein-2 (BMP-2; Infuse®) have already become commercially available for clinical use in the United States [20]. Furthermore, fibroblast growth factor (FGF)-2 is being investigated in a large clinical trial (Phase III) in Japan, and its clinical efficacy for periodontal regeneration has already reported [21–23].

In either approach, there is a therapeutic limitation due to the morphology of alveolar resorption to obtain a predictable outcome. Both strategies are only applicable to angular bony defects, and not horizontal resorption. The classification of infrabony defects that referred to the number of surrounding bony walls also exerted a significant influence on the success and failure of regeneration [24,25]. To overcome these limitations and achieve complete regeneration, the introduction of modern tissue engineering technologies is anticipated in this field.

### 3. Periodontal tissue engineering

Periodontal regeneration is one of the earliest clinical disciplines that has achieved the therapeutic application of tissue engineering-based technologies. As well as the clinical application of growth factors (signaling molecules), preclinical studies of cell therapy targeted for periodontal regeneration have been carried out using various sources of somatic stem cells (Table 2) [9]. Somatic stem cells were obtained from both dental and non-dental tissues: periodontal ligament-derived stem cells (PDLSC), dental follicular cells (DFC), bone marrow stem cells (BMSC), and adipose stem cells (ASC). These stem cells have the capability of periodontal tissue formation as well as bone formation [26,27]. In addition to adult/somatic stem cells, induced pluripotent stem (iPS) cells were successfully generated from gingival fibroblasts by the introduction of four factors (Oct3/4, Sox2, Klf4, and c-Myc), suggesting the potential for its clinical application in regenerative dentistry [28]. It is also

| Table 1 Proposed strategies clinically available or tested for periodontal tissue reconstruction. |
|----------------------------------------|-----------------------------------------------|
| 1. Targeted for alveolar bone repair |
| (1) Autologous bone grafts (autografts) |
| Intra-oral grafts |
| Extra-oral grafts |
| (2) Allogenic bone grafts (allografts) |
| Freeze-dried bone grafts |
| Demineralized bone grafts |
| (3) Xenogenic bone grafts (xenografts) |
| Bovine mineral matrix |
| (4) Alloplastic bone grafts |
| Hydroxylapatite |
| β-Tricalcium phosphate |
| Bioactive glass |
| Coral-derived calcium carbonate |
| (5) Polymer and collagen sponges |
| Collagen |
| Hard-tissue replacement polymer |

important to provide a large amount of cells at the site of regeneration. For this purpose, PDL cell sheet technology has been developed and successfully regenerated both new bone and cementum connecting with collagen fibers in canine periodontal defect models [29]. However, none of these cell transplantation models showed the complete regeneration of horizontally lost periodontal tissues.

Therefore, periodontal tissue still remains one of the most difficult tissues to achieve complete regeneration because many clinical and biological variables may affect the regeneration process. Firstly, this tissue is always in contact with the outside environment of the oral cavity, and is at risk of infection during the regeneration process. Secondly, several types of mechanical stresses are also loaded onto periodontal tissue: one is from occlusal forces and the other is the tension stress from gingiva and mucosal membranes, which disrupts horizontally resorbed periodontal tissue. As shown in Fig. 1, it may be difficult to overcome these environmental factors and induce complete periodontal regeneration without the “golden” triad of cell/signaling molecule/scaffold for conventional regenerative medicine. It is important to maintain the space of horizontally lost tissues and protect regenerating tissues from infection and mechanical stresses.

The last component of the tissue engineering triad is the scaffold. Table 3 shows the scaffold materials used for growth factor therapy aimed at periodontal regeneration [30]. Polymeric scaffolds commonly have porous and biodegradable structures fabricated from natural/synthetic materials.

### Table 2 Preclinical application of cell therapies for periodontal regeneration.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cell origin</th>
<th>Experimental model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMSC</td>
<td>Auto</td>
<td>Dog</td>
<td>Class III defects</td>
</tr>
<tr>
<td></td>
<td>Auto</td>
<td>Dog</td>
<td>Fenestration</td>
</tr>
<tr>
<td></td>
<td>Auto</td>
<td>Dog, Rat</td>
<td>Osteotomy</td>
</tr>
<tr>
<td>ASC</td>
<td>Auto</td>
<td>Dog</td>
<td>Palatal defects</td>
</tr>
<tr>
<td>PDL cells</td>
<td>Auto</td>
<td>Rat</td>
<td>Fenestration</td>
</tr>
<tr>
<td></td>
<td>Auto/Xeno</td>
<td>Human</td>
<td>Fenestration</td>
</tr>
<tr>
<td>PDL cell sheet</td>
<td>Auto</td>
<td>Dog</td>
<td>Class II defects</td>
</tr>
<tr>
<td></td>
<td>Auto</td>
<td>Dog</td>
<td>1-Wall defects</td>
</tr>
<tr>
<td>PDLSC</td>
<td>Auto</td>
<td>Nude rat</td>
<td>Ectopic</td>
</tr>
<tr>
<td></td>
<td>Auto</td>
<td>Dog, Rat</td>
<td>Fenestration</td>
</tr>
<tr>
<td>CM</td>
<td>Allo</td>
<td>Rat</td>
<td>Ectopic</td>
</tr>
<tr>
<td>DFC</td>
<td>Allo</td>
<td>Rat</td>
<td>Ectopic</td>
</tr>
</tbody>
</table>

Modified from Rios HF et al., J Periodontol 82:1223—1227, 2011 [9].

BMSC = Bone marrow stem cells; ASC = Adipose stromal cells; PDL cells = Periodontal ligament cells; PDLSC = Periodontal ligament stem cells; CM = Cementoblasts; DFC = Dental follicle cell; Auto = Autograft; Allo = Allograft; Xeno = Xenograft.

Figure 1 Required “pieces” for complete periodontal regeneration.
have been tailored to films, fibers, sheets, gels, and sponges [31]. Inorganic materials also have been used for periodontal regeneration to specifically regenerate alveolar bone defects, including hydroxyapatite (HA) and calcium phosphates (CaP). CaP materials such as β-tricalcium phosphate (β-TCP) have excellent properties including: (1) a similar composition to bone minerals; (2) the ability to form bone apatite-like materials or carbonate HA; (3) the ability to stimulate cells, leading to the formation of bone-CaP; and (4) osteoconductivity [32]. HA is the major mineral component (approx. 60%) of human hard tissues, and is often clinically applied to fill alveolar bone defects (shown in Table 1). HA can eliminate donor site morbidity, but can lead to granular migration and incomplete resorption, resulting in long-term difficulties [33]. β-TCP has been shown to be an osteoconductive material, allowing for bone growth onto the scaffold adjacent to bony surfaces in both animal and human studies [34,35], and has been clinically applied in combination with PDGF [36].

Even if the material is a polymer or inorganic, it is expected to fill a specific anatomic defect to allow the localization of transplanted cells; to serve as scaffolding for the formation of new tissue; to provide a niche maintaining stem cell viability and proliferation; and to recruit host stem cells for subsequent homing to the site of injury [37]. The first aim of the scaffold should be to contribute to the maintenance of space for horizontally lost periodontal tissues; however, no material was found to fulfill this requirement and induce complete periodontal regeneration. Another point for successful periodontal regeneration is how to protect the regenerating tissue from infection by oral microorganisms. Periodontal tissue is the only apparatus in which soft and hard tissue attachments are exposed to the outside environment. In skin tissue engineering, the same property is also required; nanofibrous polymer materials were developed to release low molecular weight antibacterial agents during degradation [38,39]. GTR/ guided bone regeneration (GBR) procedures have been shown to fail when membranes were exposed to the oral cavity and became infected [40]. To avoid contamination, multiple studies were conducted to incorporate antibacterial agents such as tetracycline hydroxide and metronidazole [41]. However, no such trial has been carried out to develop an anti-infective scaffold specially designed for periodontal regeneration. Further exploration of scaffold-based infection control methods may contribute to the elimination of bacteria, resulting in the successful generation of new tissue.

### 4. The desirable properties of scaffolds designed for periodontal regeneration

#### 4.1. Mimicking the stem cell niche to alter the regulation of stem cells

The goal of tissue engineering and its application in generative medicine is the creation of functional tissues or organs. Therefore, it is important not only to generate hard and soft tissues in periodontal regeneration, but also to mimic the attachment of both tissues. Cell-based tissue engineering requires both cells from an appropriate origin to create the target organ and a 3-dimensionally designed scaffold that promotes cell—cell proximity and enhances self-assembly and tissue functions [42]. The ideal combination of cell and scaffold sources varies in relation to the target tissue to be created. Furthermore, recent advances in material science developed new feasible biomaterials and fabrication techniques, resulting in the creation of functional/bioactive scaffolds that can activate cellular functions.

---

**Table 3** Scaffold materials applied with/without signaling molecules for periodontal tissue regeneration.

<table>
<thead>
<tr>
<th>Scaffold</th>
<th>Growth factor (combination)</th>
<th>Experimental model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Naturally occurring polymers</td>
<td>PDGF or BMP-7gene</td>
<td>Mouse</td>
<td>Zhang et al. (2007)</td>
</tr>
<tr>
<td>Methylcellulose gel</td>
<td>FGF-2</td>
<td>Human</td>
<td>Kitamura et al. (2008, 2011)</td>
</tr>
<tr>
<td>Hydroxypropyl cellulose</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2. Synthetic polymers</td>
<td>rhBMP-2</td>
<td>Cat</td>
<td>Takahashi et al. (2007)</td>
</tr>
<tr>
<td>PLA-PGLA copolymer + gelatin sponge</td>
<td>TGF-b1</td>
<td>Sheep</td>
<td>Mohammed et al. (1998)</td>
</tr>
<tr>
<td>PLA + Fibronectin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3. Hydrogels</td>
<td>rhBMP-2</td>
<td>Dog</td>
<td>Chen et al. (2005)</td>
</tr>
<tr>
<td>Dex-GMA gelatin gel</td>
<td>TGF-b1</td>
<td>Monkey</td>
<td>Ripamonti et al. (1996)</td>
</tr>
<tr>
<td>Collagen gel</td>
<td>TGF-b1</td>
<td>Monkey</td>
<td>Takayama et al. (2001)</td>
</tr>
<tr>
<td>Gelatinous carrier</td>
<td>FGF-2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Calcium phosphate cement</td>
<td>TGF-b1</td>
<td>Dog</td>
<td>Tatakis et al. (2000)</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>rhPDGF-BB</td>
<td>Human</td>
<td>Giannobile et al. (2006)</td>
</tr>
<tr>
<td>b-Tricalcium phosphate</td>
<td>rhPDGF-BB</td>
<td>Human</td>
<td>Giannobile et al. (2006)</td>
</tr>
<tr>
<td>Hydroxyapatite (HA)</td>
<td>rhBMP-2</td>
<td>Dog</td>
<td>Emeron KB et al. (2012)</td>
</tr>
<tr>
<td>Bone allograft</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>


---
Recently, the term “biomimeticism” has been introduced in tissue engineering and refers to the creative initiation of various specific biological systems gaining inspiration from nature [43–45]. It is also the case for adult stem cell therapy because these cells reside in specialized niches that coordinate self-renewal versus differentiation in vivo [46,47]. Thus, the mimicking stem cell niche is considered to facilitate self-renewal (proliferation) and differentiation both ex vivo and in vivo after transplantation.

The microenvironment or niche surrounding stem cells is shown in Fig. 2. Cytokine/growth factor signaling regulates the proliferation and differentiation of stem cells as previously described. Cell–cell and cell–matrix interactions also transmit signals into stem cells, controlling stem cell functions. Cell–cell interactions occurred not only between stem cells, but also between stem cells and supporting cells that modulate stem cell retention and regulation. Several cell surface ligands are known for their association with stem cell activation, including cadherins and the Notch ligand [48,49].

The third pathway is cell–extracellular matrix (ECM) interactions, including matrix composition, stiffness, and topography. The ECM contains various proteins such as fibronectin and laminin, as well as proteoglycans (GAG), hyaluronic acid, and fibers (collagens and elastins) [50]. These components can regulate cell behavior as well as support cell growth because stem cells also have cell adhesion molecules, including integrins and CD44, initiate intracellular signaling, and associate with the cellular cytoskeleton [51]. Differences in the composition and crosslink density of the ECM in each organ and tissue have also been adapted for their mechanical properties of stiffness and topography. Cells can sense and respond to various signals, consisting of biochemical and biophysical cues provided by the ECM. In combination with the mechanical properties of cell membrane, matrix stiffness affects the proliferation and differentiation of stem cells [52–54]. Cells are exposed to a diverse topography including fibrous ECM and mineralized bone with a rough surface. The ECM presents various geometrically defined and 3D physical cues in the order of a micron and sub-micron scale, known as topographies [55]. Physical cues in a cell’s surrounding environment are integrated and converted to biochemical, intracellular signaling responses, leading to the modification of cell function through a process of mechanotransduction [56].

Oxygen gradients in the niche also affect stem cell function. Stem cell niches are known to be located in low oxygen tension and low pO2 regions, where the rate of cell differentiation is decreased and proliferative potential is increased [57]. Furthermore, oxidative stress was found to suppress the E-cadherin-mediated cell–cell adhesion of hematopoietic stem cells (HSC) to osteoblasts, inducing the exit of HSC from the niche [58].

Additionally, another cue should be added when we consider effective bone regeneration strategies. Bone is a biocompatible and self-remodeling tissue consisting of an organic phase (mainly collagen type I, <20%) and an inorganic phase (mainly carbonated hydroxyapatite, <60%) [32]. During bone metabolism, osteoclasts release Ca2+ and PO43− (Pi) derived from the matrix, causing a local increase in ion concentrations in the microenvironment, which plays a role in osteoblast proliferation and differentiation, as well as on subsequent bone formation. Increased extracellular Ca2+ concentrations are potent chemical signals for cell migration and directed growth [59,60], as well as homing signals that bring together the different cell types required for the initiation of bone remodeling [61]. Pi is also a regulator of osteoblast proliferation and differentiation [62], and the high concentration of Pi in the microenvironment induced osteoblast apoptosis in vitro [63] and the in vivo mineralization of the bone matrix [64]. Moreover, specific Ca2+ and Pi concentrations have been suggested to induce the higher proliferation and osteogenic differentiation of MSC [65]. CaP bioeceramics appear to be candidates for scaffold–aimed bone tissue engineering because they can release inorganic ions during dissolution.

![Figure 2](image-url)  
**Figure 2** Interactions of stem cells with microenvironmental factors within the niche Ca: Calcium ion, Pi: Phosphate ion.
4.2. Cell–scaffold interplay and scaffold designing

As discussed above, many factors are associated with mimicking of the cell niche/microenvironment to enforce regeneration by the application of tissue engineering. Not only scaffold properties affect stem cell functions; cells can also induce the deformation and degradation of scaffolds during the process of regeneration, leading to the altered mechanical properties of scaffolds (Fig. 3). Thus, the cell–scaffold interplay is bidirectional and involves a feedback loop of cells and scaffolds. Forces are generated in the context of cell adhesion to ECM/scaffolds, and mechanotransduction occurs to transduce them into intracellular signals that drive functional modulations in cells: proliferation, differentiation, migration, and apoptosis [55]. The actin cytoskeleton plays the most prominent role in these events [66]. Human MSC also express specific transcription factors of mechanotransduction and undergo tissue-specific cell fate switches when cultured on ECMS with mechanical stiffness mirroring the physical properties of respective specific tissue [67].

To instruct stem cells and modify their fates, scaffold biomaterial should provide informative microenvironments mimicking a physiological niche of the target tissue. Biomaterials can have a suitable design to transmit specific signals to cells that can be decoded into biochemical signals depending on its composition and processing methods. Both biophysical and biochemical cues have been involved in cell–ECM interactions in nature. Hence, topography, chemistry and physical properties are involved and are critical for determining cell fate [68]. To accomplish the desirable interplay between cells, biomaterial designing should include parameters within (a) surface, (b) mechanical, (c) morphological, and (d) electrical properties (Fig. 3). Surface topography and chemical composition were able to drive cell adhesion, proliferation, migration, and differentiation [55,69,70]. Stem cells can also respond to the mechanical properties of the substrate, which were enhanced by the combination of polymers and organic/inorganic fillers [33,71], and osteoinduction without the supplementation of growth factors has recently been reported [72]. Conductive nanostructures, such as metal nanoparticles and carbon nanostructures, may modulate the electrical properties of biomaterials, affecting stem cell functions [73,74]. Scaffold morphology, e.g., pore size and shape, is critical for cell–biomaterial interactions in terms of mimicking the morphology of surrounding tissue. High porosity and an adequate pore size in particular are key conditions for increasing the surface area available for cell attachment and growth. In the following sections, the interactions between PDL cells and each parameter of the scaffold design discussed above will be covered in detail using our findings.

4.3. Application of a honeycomb microarray film for PDL cell sheet engineering

Cell sheet technology is a concept of tissue engineering that provides a mass of cells to the site of regeneration by the transplantation of an in vitro cultured multilayered cell sheet. This technology has already been applied to the regeneration of many organs in clinical settings, including corneal surface reconstruction, endoscopic treatment of esophageal ulceration, and myocardial tissue reconstruction [75]. The cell sheet was successfully prepared from PDL cells, and scaffold-free sheet transplantation could regenerate a complete periodontium in a canine model [29]. Cell sheet engineering is also applicable to 3D-tissue reconstruction of tissue by applying an appropriate scaffold material to increase the number of cell layers [76]. Skin reconstruction is another medical field applicable to cell sheet-based tissue engineering. The honeycomb biodegradable collagen scaffold has been shown to be suitable for 3D cell cultures [77] because the honeycomb structure has many advantages, such as mechanical stability under various physical conditions, the capability to exchange nutrients and waste products through the honeycomb pores, and the ability to retain its structure without a deformity or collapse until its biodegradation [78]. However, the pore size of natural polymers such as collagen is difficult to control.

The honeycomb pattern was also prepared on various types of polymers by a simple casting method [79–81]. Briefly, the hydrophobic polymer solution was casted on a basal plate, water droplets self-assembled on the polymer solution with the addition of humid air, and the honeycomb

---

**Figure 3** Cell–scaffold interplay and the components of scaffold designing.
pattern was observed on the polymer film after drying. We could control the pore size of the honeycomb structure and fabricate uniform pores using the casting method. This casting method is applicable for biodegradable polyester films made from poly(lactic acid) and poly(caprolactone) (PCL) [82], which is useful as a cell culture substrate. We cultured PDL cells on the honeycomb-patterned polymer films fabricated by the casting method with different pore sizes. PDL cells were obtained from extracted molars with a healthy periodontium, and subjected to experiments during 3–5 passages. PDL cells were cultured on flat (control) and honeycomb PCL films with 5- and 10-μm pores for 72 h. Scanning electron microscopy (SEM) images were shown in Fig. 4. PDL cells seemed to be spread on flat films (Fig. 4A), although they firmly caught the pillar structure of the honeycomb on the 5 μm-pored film (Fig. 4B). Interestingly, PDL cells migrated through the pores of the honeycomb structure of the 10 μm film (Fig. 4C). The schematic illustrations of PDL cell behavior cultured on 5 and 10 μm-pored honeycomb films were given in Fig. 4D and E, respectively.

The 3D orientations of PDL cells in the honeycomb films were further observed using confocal laser scanning microscopy after a long-term culture (28 days; Fig. 5). Fig. 5A shows the 3D-constructed image of PDL cells cultured on the 10 μm-pored film. PDL cells were seen inside the film and spread their bodies horizontally into the contiguously lined pores. PDL cells constructed multi-layered cell sheet-like structures after 28 days, and the shapes of cells on the upper cell layer, on the surface, and inside of the film were separately presented in Fig. 5B–D. The shapes and forms of the cells were markedly changed by moving between the outside and inside of the honeycomb films. PDL cells seemed to be desperate to move through the honeycomb lumens and showed a dendrite-like morphology form. Our results clearly indicated that the pore size of artificial substrates has a marked effect on cell behavior, and the honeycomb structure is suitable for the construction of a multi-layered cell sheet. The topographical effects of the honeycomb film also have a significant impact on PDL cell differentiation. We measured the mRNA expression levels of the osteoblastic markers of PDL cells cultured for 4 weeks on the honeycomb film [83]. Osteopontin (OPN) and osteocalcin (OCN) expression levels were higher than those on flat films, suggesting differentiation into osteoblastic cells. This result was further confirmed by the formation of calcified nodules on 10 μm-pored honeycomb films (data not shown).

4.4. Possible induction of cementogenesis by modulation of the extracellular ionic microenvironment using bioactive ceramic scaffolds

To accomplish the restoration of the original architecture of the periodontal apparatus, it is important to promote cementogenesis rapidly on the root surface after root planning/conditioning because the cementum is the only hard tissue that can insert PDLs and assists in anchoring the tooth to the surrounding alveolar bone [84]. Cementoblasts express alkaline phosphatase (ALP), runt-related gene 2, type I collagen, noncollagenous proteins, bone sialoprotein (BSP), and OCN in a similar manner to osteoblasts [84,85]. According to the anatomical location, which is in proximity to osteoblasts/alveolar bone, but is separated by a PDL, cementoblasts may be under a specific microenvironment resembling bone with higher extracellular Ca²⁺ and Pi concentrations in part related to osteoclast-mediated bone resorption during alveolar bone remodeling. Thus, these cells are physiologically and/or pathologically confronted with alterations in the concentrations of extracellular inorganic ions. Calcium phosphate ceramics are widely used as bone substitutes or scaffolds in dental fields because of their excellent

Figure 4  SEM images of PDL cells cultured on honeycomb (HF) films. Periodontal ligament (PDL) cells were cultured for 72 h on (A): a flat PCL film, (B): a 5 μm-pored honeycomb film (HF), and (C): a 10 μm-pored HF. D and E show the schematic illustration of PDL cells on 5 μm- and 10 μm-HF, respectively.
Osteoconductivity [86]. These ceramics are classified as non-resorbable and resorbable, and can release or exchange Ca\(^{2+}\) and Pi ions into their surroundings after implantation [87] as discussed in the previous section. Whether cementoblasts could sense extracellular Ca\(^{2+}\) and Pi ionic concentrations and altered cell functions such as cementogenesis and cytokine production remained unclear [88]. An immortalized murine cementoblast cell line (OCCM-30), established by the isolation of tooth root-surface cells from transgenic mice containing a SV40 large T-antigen under the control of the OCN promoter [89], was used for the following studies. OCCM-30 cells were stimulated with 10 mM CaCl\(_2\) for 24 h because basal [Ca\(^{2+}\)] was 1.8 mM. The expression of COX-2 and PGE\(_2\) was significantly increased in a time-dependent manner and subsequently increased Fgf-2 mRNA (Fig. 6). OCCM-30 expressed all EP1, EP2, EP3, and EP4 receptors to PGE\(_2\). Only an EP4 receptor agonist synergistically enhanced CaCl\(_2\)-induced Fgf-2 gene expression (data not shown). We finally concluded that the exposure of cementoblasts to CaCl\(_2\) activated NF-kB signaling and induced the expression of Cox-2 as well as Ep4, which led to the sequential activation of PGE\(_2\)/EP4 signaling and increase in Fgf-2 expression levels (Fig. 7). COX-2/PGE\(_2\)/EP4 signaling may function as a positive regulator for FGF-2 induction in cementoblasts. We previously reported that increased extracellular Ca\(^{2+}\) increased BMP-2 mRNA expression in human PDL cells as well as in the dental pulp (DP) cells [90]. Furthermore, hDP and PDL cells expressed Na-dependent Pi transporters (Pit-1, Pit-2) and

![Confocal laser microscopy images](image)

**Figure 5** Confocal laser microscopy images of PDL cells cultured for 28 days on a 10 \(\mu\)m-pored honeycomb film. (A) Three-dimensional reconstructed image of vertical slices, (B–D) PDL cells on the top of multilayered cells (B), center of cell layers (C), and inside of the honeycomb film (D), respectively.

**Figure 6** Elevated concentrations of extracellular Ca\(^{2+}\) induced Cox-2 mRNA expression and PGE\(_2\) production, and subsequently up-regulated the expression of Fgf-2 mRNA.
sensed extracellular Pi, resulting in the up-regulation of BMP-2 mRNA [91]. Taken together, periodontal and dental pulp cells can respond to changes in extracellular inorganic ion concentrations, resulting in the signal transduction that leads to proliferation/differentiation.

**Figure 7** Increased extracellular Ca\(^{2+}\) synergistically up-regulated FGF-2 expression via COX-2/PGE\(_2\)/EP4 signaling, COX-2: cyclooxygenase-2, EP4: EP4 receptor.

**4.5. Application of nanofabricated hydroxyapatite for bone tissue engineering**

Hydroxyapatite (HA) is the prevalent form of CaP found in the bone; therefore, it has been used as the stable alloplast and scaffold for bone regeneration. However, HA has a lower dissolution rate at physiological pH (7.2—7.6) than the other types of CaP such as octacalcium phosphate (OCP) and tricalcium phosphate (TCP), resulting in poor biological responses. As this dissolution behavior has been associated with osteoinductivity, previous studies attempted to engineer CaP with an appropriately high solubility [87]. Combining the different scaffold fabrication technologies and different biomaterials can provide cells with mechanical, physicochemical, and biological cues at the macro- and micro-scale, as well as at the nano-scale. Due to size effects and surface phenomena at the nanoscale, nanosize HA (nano-HA) possessed unique properties over its bulk-phase counterpart. The high surface-to-volume ratio, reactivities, and biomimetic morphologies may make nano-HA more favorable in applications for bone tissue engineering [92, 93]. We investigated the effect of nano-HA on BMP-2 expression in human PDL cells [94]. Nano-HA selectively increased the expression of BMP-2 in dose- and time-dependent manners (Fig. 8A and B) at mRNA and protein levels, but not of BMP-4, -7, or -9 (Fig. 8C). However, concentrations of Ca\(^{2+}\) as well as Pi were not changed in culture supernatants (Fig. 9), suggesting that nano-HA functioned as a nanoparticle rather than as a possible source of Ca\(^{2+}\) and/or Pi extracellularly, which were

**Figure 8** Nano-HA stimulated BMP-2 expression at the gene level in human PDL cells. (A) Cells were stimulated with the indicated concentration of nano-HA for 72 h. (B) Cells were stimulated with 200 μg/ml nano-HA for the indicated times with a medium change every 3 days. (D) Cells were stimulated with 200 μg/ml nano-HA for 72 h. Total cellular RNA was extracted and transcripts were analyzed by real-time PCR.

**Figure 9** Nano-HA mediated increases in BMP-2 were not caused by extracellular Ca\(^{2+}\) or Pi. (A) Cells were stimulated with/without 200 μg/ml nano-HA, 10 mM CaCl\(_2\), or 3 mM Pi (as a mixture of Na\(_2\)HPO\(_4\) and Na\(_2\)HPO\(_4\), pH 7.4) for the indicated times. Total cellular RNA was extracted and transcripts were analyzed by real-time PCR. (B and C) Confluent cells were stimulated with/without 200 μg/ml nano-HA for the indicated times. The concentrations of Ca\(^{2+}\) and Pi in the supernatant were measured.
shown to also enhance the expression of BMP-2 in PDL cells. We further revealed that nano-HA-dependent BMP-2 expression was dependent on p38 MAP kinase, but not on ERK1/2 MAP kinase (data not shown). Thus, nano-fabricated HA may regulate the differentiation of hPDL cells via a mechanosensitive signaling pathway. This novel mechanism of the action of nano-HA may offer the promise of new strategies for bone and periodontal tissue engineering.

5. Conclusions and future prospects

This review focused on the cell—scaffold interaction possibly encountered when tissue-engineering approaches are applied to periodontal regeneration. Recent progresses in periodontal regeneration technology allowed the clinical application of cytokine therapies in dental clinics. However, as discussed in the previous sections, these cytokine therapies still have therapeutic limitations similar to those of Emdogain and the GTR technique. To overcome the limitations, researchers have extensively studied the application of stem cell therapy in this field, including autologous somatic stem cells from a dental origin and iPSCs. Current progress in tissue engineering approaches for periodontal regeneration has been summarized in Fig. 10. The biocompatible scaffold is another important strategy in tissue engineering technology that has been adopted for the creation of new tissue, whether stem cells are utilized or not. Scaffolds not only can contain stem cells and mimic the microenvironment suitable for these cells, but also can provide the controlled release of growth factors including gene delivery [95]. Furthermore, the scaffold should fill a specific anatomic space of the lost periodontal tissue including horizontally resorbed alveolar bone. Thus, a 3D version of biomimicry is the key for complete periodontal regeneration, although this research field is currently under intense exploration.

We discussed the possible periodontal cell—scaffold interactions that may be a powerful tool for developing the most suitable scaffold. To date, many cellular and molecular events involved in periodontal tissue repair/regeneration have been revealed. These advances in understanding may serve as the driving force toward a breakthrough for cell-based regeneration strategies in our research field. The rapid progress in material sciences and micro/nano-fabrication technology also pave the way for the development of new tissue engineering techniques for “complete” periodontal regeneration.

Conflict of interest

None of authors have a conflict of interest related to this review.

Acknowledgements

Portions of the author’s research discussed in this review were supported by A Grant-in Aid for Scientific Research (23390745 and 23659910 to H.S.). The authors would like to thank Drs. Sousuke Kanaya, Nagayoshi Iwama, and Mizuki Suto for their contributions in the research results shown here.

References

Possible Functional Scaffolds for Periodontal Regeneration


extracellular matrix is adaptive and can be restored by a transient change in Ca²⁺ level. PLoS ONE 2009;4:e7330. http://dx.doi.org/10.1371/journal.pone.0007330.


