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## Cell- and Gene-Based Therapeutic Strategies for Periodontal Regenerative Medicine

Hector F. Rios<sup>\*</sup>, Zhao Lin<sup>†</sup>, BiNa Oh<sup>\*</sup>, Chan Ho Park<sup>‡</sup>, and William V. Giannobile<sup>\*‡</sup>

<sup>\*</sup>Department of Periodontics and Oral Medicine, School of Dentistry, University of Michigan, Ann Arbor, MI

<sup>‡</sup>Department of Biomedical Engineering, College of Engineering, University of Michigan

### Abstract

Inflammatory periodontal diseases are a leading cause of tooth loss and are linked to multiple systemic conditions, such as cardiovascular disease and stroke. Reconstruction of the support and function of affected tooth-supporting tissues represents an important therapeutic endpoint for periodontal regenerative medicine. An improved understanding of periodontal biology coupled with current advances in scaffolding matrices has introduced novel treatments that use cell and gene therapy to enhance periodontal tissue reconstruction and its biomechanical integration. Cell and gene delivery technologies have the potential to overcome limitations associated with existing periodontal therapies, and may provide a new direction in sustainable inflammation control and more predictable tissue regeneration of supporting alveolar bone, periodontal ligament, and cementum. This review provides clinicians with the current status of these early-stage and emerging cell- and gene-based therapeutics in periodontal regenerative medicine, and introduces their future application in clinical periodontal treatment. The paper concludes with prospects on the application of cell and gene tissue engineering technologies for reconstructive periodontology.

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Tissue engineering and regenerative medicine are part of an emerging multidisciplinary and interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biologic substitutes; they have the potential to revolutionize the way health and quality of life are improved for millions of people worldwide by restoring, maintaining, or enhancing tissue and organ function.<sup>1</sup> Applied to periodontal biology, tissue engineering has the potential to improve the regeneration of lost tooth-supporting structures in a more predictable manner than conventional periodontal treatments (Fig. 1). Periodontal engineering uses advanced engineering and life science technologies to restore the structure and function of alveolar bone, periodontal ligament (PDL), cementum, and gingival tissue, recreating the proper periodontal interface complex characterized by multitissue integration and proper tissue cell orientation.

A successful outcome of periodontal tissue engineering requires the following essential factors: appropriate cells, signals, scaffolds, blood supply, mechanical loading, and pathogen control. Cells provide the machinery for new tissue growth and differentiation, whereas growth factors and other molecules modulate the cellular activity and provide stimuli for cells to differentiate and support tissue neogenesis. A three-dimensional template structure is provided by scaffolds to support and facilitate these processes that are critical for tissue

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Correspondence: Dr. William Giannobile, Michigan Center for Oral Health Research, University of Michigan School of Dentistry, 1011 N. University Ave., Ann Arbor, MI 48109 1078., Fax: 734/763-5503; william.giannobile@umich.edu.

<sup>†</sup>Currently, Division of Periodontology, Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, MA; previously, Department of Periodontics and Oral Medicine, School of Dentistry, University of Michigan.

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regeneration.<sup>2</sup> New vascular networks promoted by angiogenic signals provide the nutritional base for tissue growth and homeostasis, whereas appropriate mechanical loading is essential for the development of highly organized, functional PDL fibers. Finally, because of the microbial load at the periodontal lesion, strategies to control infection and host response are required to optimize periodontal regeneration.

This paper reviews the concepts and available information in tissue engineering concerning early-stage cell and gene delivery for periodontal therapy. The discussion focuses primarily on three important aspects: 1) cell-based therapy, 2) gene therapy, and 3) scaffold fabrication technologies for delivering cells and genes to periodontal osseous defects.

## CELLS FOR PERIODONTAL REGENERATION

In general, cell therapy involves the treatment of disease or disorder by transferring new cells into a tissue. Cells are the center of new tissue growth and differentiation. In cell-based regenerative medicine, cells are delivered to a defect site with the goal of improving the regeneration process.<sup>3</sup> Cell delivery approaches are used to accelerate periodontal regeneration through two primary mechanisms: to use cells as carriers to deliver growth or cellular signals, and to provide cells that are able to differentiate to multiple cell types to promote regeneration. The use of cells as vehicles to deliver growth factors can stimulate an endogenous regeneration process.<sup>1</sup> This strategy has been intensively investigated in both soft and hard periodontal tissue regeneration. Stem cell research has soared in the past few years and the effects on healing and regenerative potential have been extensively studied.

Mesenchymal stem cells (MSCs) are self-renewable and can differentiate into a variety of cell types that form mesenchymal and connective tissues.<sup>3,4</sup> Bone marrow stromal cells are the most widely investigated MSCs because they are easily accessible. Bone marrow stromal cells were initially isolated and described nearly 50 years ago based on their ability to adhere to plastic substrates of cell culture plates.<sup>5</sup> Since then, this simple protocol has been widely used to isolate MSCs from many tissues, such as adipose tissue, muscle, liver, pancreas, and cartilage.<sup>6</sup> MSCs have a tremendous potential in regenerative medicine because of their multipotency and capability to form a variety of tissues, including the periodontium (Table 1).<sup>7-21</sup> Regarding periodontal tissue engineering, both extraoral and intraoral stem cells can be harvested and then subjected to enrichment and expansion techniques (Fig. 1). Within this context, multiple sources of stem cells have been evaluated for the treatment and regeneration of the periodontium.<sup>22</sup>

### Extraoral MSCs for Oral and Periodontal Engineering

There is strong potential for the use of MSC sources from outside the oral cavity for transplantation to the oral, periodontal, and craniofacial complex.<sup>6,9,23</sup> Kawaguchi et al.<sup>7</sup> showed that bone marrow MSC transplantation promoted periodontal regeneration in experimental Class III defects in dogs. The treatment promoted up to 20% of cementum and bone regeneration. Using a cell-labeling technique, it was shown that these cells differentiate into cementoblasts, PDL fibroblasts, and alveolar bone osteoblasts in vivo.<sup>8</sup> In a subsequent small clinical trial, autologous, expanded bone-marrow stromal cells mixed with atelocollagen were transplanted into periodontal osseous defects at the time of periodontal surgery, and positive clinical results were obtained. Furthermore, the use of MSCs and platelet-rich plasma (PRP) demonstrated a reduction in intrabony defect depth and resolution of bleeding and tooth mobility.<sup>24</sup>

Bone marrow stromal cells have also been shown to promote bone healing and dental implant osseointegration.<sup>25</sup> In a series of studies, Yamada et al.<sup>10-12</sup> used PRP as an autologous scaffold with in vitro expanded bone-marrow stromal cells to increase

osteogenesis in dental implant surgery. This “autogenous injectable bone treatment” resulted in higher marginal bone levels, better bone implant contact, and increased bone density compared to PRP alone and particulated cancellous bone and marrow control groups. Recently, it was shown that cells harvested from the bone marrow can be driven down MSC pathways via a single-pass perfusion process to promote bone regeneration in tooth extraction socket and sinus floor augmentation procedures.<sup>26</sup>

Adipose tissue is another extraoral and non-craniofacial source of MSCs for oral and periodontal tissue engineering. Its greatest advantage is ease of access. Tobita et al.<sup>13</sup> demonstrated in rats that, mixed with PRP, adipose-derived stromal cells could promote periodontal regeneration.

### Oral and Craniofacial MSCs for Periodontal Engineering

Just as PDL is essential for the osteogenesis and cementogenesis during development and remodeling, cells derived from this tissue are supportive for the healing response to injury.<sup>27</sup> Transplantation of PDL cells has shown the potential to regenerate periodontal attachment apparatus in vivo.<sup>14,28</sup> Akizuki et al.<sup>15</sup> developed a PDL cell sheet using temperature-responsive cell culture dish technique and hyaluronic acid (HA) carrier. After the transplantation of PDL cell sheets in small and large preclinical animal models, significant cementum formation and anchoring PDL fibers were observed together with new alveolar bone formation. Using specific labeling techniques, Lekic et al.<sup>16</sup> showed that transplanted PDL cells integrate and differentiate into newly formed periodontal tissues.

Specific cell types derived from the periodontium have also been examined for their potential and roles in periodontal regeneration. Cementoblasts have been shown to induce mineralization in an ex vivo model<sup>29</sup> and in vivo in periodontal wounds.<sup>21</sup> By contrast, periodontal healing was inhibited when less-differentiated dental follicle cells were delivered in a similar fashion.<sup>21</sup> Similarly, progenitor cells isolated from dental follicle failed to form dentin, cementum, or bone in vivo, although they expressed high levels of bone sialoprotein, osteocalcin, and alkaline phosphatase.<sup>30</sup> Such results are suggestive of selective behaviors of different cell types in periodontal regeneration. PDL stem cells (PDLSCs) have recently been isolated and they express several mesenchymal stem cell markers, such as STRO-1 and CD44, and exhibit osteogenic, adipogenic, and chondrogenic characteristics under defined culture conditions.<sup>3,22</sup> Implanted PDLSCs generate cementum and PDL-like structures similar to native periodontal apparatus.<sup>17</sup> In a porcine periodontal defect model, PDLSCs were shown to regenerate new bone, cementum, and PDL, and the height of the new alveolar bone was significantly greater than that of a ceramic carrier group.<sup>28</sup> Combining PDLSCs with another stem cell population from the root apical papilla of human teeth (stem cells from apical papilla), Sonoyama et al.<sup>31</sup> generated a “bio-root” structure encircled with PDL tissue. Most recently, Gault et al.<sup>32</sup> have taken autologous PDLSCs from extracted teeth, grown them in tissue bioreactors, and delivered them onto titanium implants as a cell transplantation approach. These implants form ligamentous attachments anchoring bone-to-implant surface and are termed “ligaplants.” These ligaplants demonstrated functional loading for extended periods of time as shown in a case series of human participants.<sup>33</sup> The use of cell therapy approaches as hybrid living, implantable biomaterials offers another avenue for oral tissue-engineering strategies.<sup>33</sup>

Besides stem cell delivery, other strategies have been developed based on the concept that transplanted cells promote regeneration by secreting growth factors via autocrine and paracrine pathways. Allogenic foreskin fibroblasts have been shown to be safe and are able to promote keratinized gingiva formation at gingival recession defects.<sup>34</sup> A tissue-engineered living cellular construct composed of viable neonatal keratinocytes and fibroblasts was reported to achieve comparable clinical outcome as gingival graft<sup>35</sup> with

strong potential to promote tissue neogenesis through the stimulation of angiogenic signals.<sup>36</sup>

Supporting the significant potential of cell-based therapy to form a variety of periodontal tissues, these investigations support the use of this approach to deliver important cues that drive the regenerative process.

## GENE THERAPY FOR PERIODONTAL ENGINEERING

Gene therapy is defined as the treatment of disease or disorder by transferring genetic materials to introduce, suppress, or manipulate specific genes that direct an individual's own cells to produce a therapeutic agent.<sup>37</sup> This therapeutic concept has emerged as a promising strategy for the modulation of the host response triggered by periodontal microbe and the regeneration of periodontium during diseases. Gene therapy has several advantages over traditional treatments, such as those involving compounds and proteins: 1) greater sustainability than that of a single protein or compound application; whereas the half-lives of pharmaceutical compounds or recombinant protein usually range from several hours to several days, viral vector genes can be expressed *in vivo* from weeks to years; 2) gene delivery reduces technical challenges associated with *ex vivo* protein expression and purification, such as palmitoylation and glycosylation; 3) transient and controlled delivery of genetic sequences encoding a combinatorial group of regenerative factors could mimic the natural biologic healing response; and 4) coupled with tissue-engineering strategies, delivery of different genes in a spatially controlled and bioavailable fashion offers strong potential in regenerating tissues in three dimensions at the tooth-ligament–bone interface.

Applied to periodontal tissue engineering, a therapeutic gene can be introduced into a patient by either direct delivery through cell-based delivery method with or without a scaffolding matrix or indirectly through *ex vivo* approaches (Fig. 1). In general, the indirect or cell-based delivery approach is considered safer and a more controlled approach because the targeted cells are isolated and the therapeutic gene is delivered *ex vivo* under controlled conditions.

### Gene Delivery Methods

Specific conditions are an important consideration for gene transfer applications:<sup>38</sup> 1) the required duration of protein release (transient versus long-term expression); 2) target cells (dividing and non-dividing cells, targeted locale of cells); 3) host immune response to vectors in viral-based approaches; 4) route of gene delivery (*ex vivo* or *in vivo*); and 5) the anatomic constraints of the target site. For example, a one- or two-walled defect may require the use of a supportive carrier, such as a scaffold. Other, more contained defect sites, such as three- and four-wall defects, may be conducive to the use of an adenoviral vector embedded in a collagen matrix. A wide variety of viral and non-viral vectors have been developed for gene delivery. Examples of viral vectors are retroviruses, lentiviruses, adenoviruses (Ad), and adeno-associated viruses (AAVs). Non-viral vectors include plasmids, DNA polymer complexes, nanobubbles and microbubbles, and ultrasound (Table 2).<sup>39</sup>

Retroviral vectors are single-stranded RNA viruses that are replicated in a host cell through the enzyme reverse transcription to produce DNA from its RNA genome, and the resulting reverse-transcribed viral DNA is incorporated into the host cell's DNA strand by an integrase enzyme. When the genetically altered host cell divides, its descendants contain the viral DNA copy. These vectors have significant advantages for sustained and efficient transgene expression that is ideal for the treatment of life-threatening hereditary disorders, although most retroviruses can only infect dividing cells. Because the integrase enzyme may insert the DNA copy into an arbitrary position of the target cell DNA, endogenous gene

expression may be disrupted by insertional mutagenesis of a proto-oncogene or tumor suppressor, and carcinogenesis may occur.<sup>40</sup>

Lentiviruses, such as the human immunodeficiency virus, are a specialized class of the retrovirus family and are characterized by a long incubation period. Lentiviral vectors are one of the most efficient methods in gene delivery, being able to transfect both dividing and non-dividing cells. These vectors are also integrated into the host cell genome. Despite the evidence that the insertion sites of lentivirus are more restricted than other retroviruses, the carcinogenesis induced by insertional mutation is still a hurdle for clinical application. Additionally, their human immunodeficiency virus origin raises many concerns regarding the possibility that recombination events will lead to replication-competent viruses.<sup>41</sup>

Ad are non-enveloped icosahedral viruses composed of a nucleocapsid and a double-stranded linear DNA genome.<sup>42</sup> In contrast to lentiviruses, adenoviral vectors are attractive gene delivery vehicles because of a number of features: 1) Ad have high transduction efficiency in both dividing and non-dividing cells, 2) Ad do not induce apparent phenotypic changes in transduced cells, and 3) Ad fail to integrate into the host genome and remain episomal. These vectors may be advantageous in periodontal tissue engineering because the transient expression of growth factors may prevent the overgrowth of newly formed tissue. However, in large-size craniofacial defects, the short-term gene expression may be insufficient to induce complete tissue regeneration.<sup>43</sup> One major concern regarding Ad gene delivery is the strong host immune response to viral capsid proteins, and viral backbone modification for reduction of immunogenicity has been investigated.<sup>43</sup> Recently, several studies have provided supporting evidence indicating that local therapeutic Ad seem safe and efficient in diabetic foot ulcer treatment and periodontal regeneration.<sup>44,45</sup>

AAVs derive from the parvovirus family and are small viruses with a single-stranded DNA genome.<sup>39</sup> AAV has attracted considerable interest from gene therapy researchers because of its several significant advantages: 1) AAV is currently not related to any human disease, 2) AAV presents very low immunogenicity, and 3) AAV infects both dividing and non-dividing cells. It has the ability to integrate its genetic material into the host cell genome at a specific site in the human chromosome 19, which makes it more predictable than retrovirus.<sup>46</sup> However, random integration of AAV DNA into the host genome is low but detectable. A recent report raised concerns about the clinical use of AAV vectors when mice developed hepatocellular carcinoma after neonatal injection of an AAV vector, which is associated with the insertion in a 6-kb region of chromosome 12.<sup>47</sup> Types of recombinant AAV have been developed either to remain extrachromosomal or integrate into non-specific chromosomal sites.<sup>39</sup> One disadvantage of the AAV is that it is small and it can only carry target DNA usually <5 kb.<sup>43</sup>

Non-viral alternatives can also deliver genetic material into a host's cell.<sup>42</sup> They include naked plasmid, cationic lipids, polymers, peptides, and physical methods (electroporation and ultrasound). A major disadvantage for non-viral delivery methods is that non-viral gene carriers consistently exhibit significantly reduced transfection efficiency. However, because of their low immunogenicity, lack of DNA insert size limitation, and potential for large-scale production, non-viral vectors will be given more consideration in the future, especially in the field of siRNA gene therapy.<sup>48</sup> In the past decade, a significant amount of research has focused on designing cationic compounds that can form complexes with DNA and can avoid both in vitro and in vivo barriers for gene delivery.<sup>49</sup> Because of the anatomic advantage, some non-invasive physical methods may have a great opportunity in delivering DNA to the periodontium. Chen et al.<sup>50</sup> reported that a gene transfer approach using ultrasound and nanobubbles and microbubbles leads to high gene expression in gingival tissue.

## Target Genes

**Platelet-derived growth factor**—Initially identified as a platelet-derived mitogen specific for cells of mesenchymal origin, platelet-derived growth factor (PDGF) is a member of a family of multifunctional polypeptide growth factors.<sup>51</sup> PDGF is considered a critical switch to initiate tissue repair process.

Initial studies showed that Ad-PDGF can effectively transduce cells derived from the periodontium, including osteoblasts, PDL fibroblasts, gingival fibroblasts, and cementoblasts, which led to enhancing mitogenic effect on these cells.<sup>52,53</sup> Further studies demonstrated that Ad-PDGF treatment prolonged the PDGF signaling effect.<sup>54,55</sup> Using in vivo optical imaging, sustained and localized gene expression in periodontal lesions for up to 21 to 35 days after direct gene targeting has been demonstrated.<sup>44,56</sup> Regarding the safety profile of Ad gene therapy, Chang et al.<sup>44</sup> demonstrated that Ad-PDGF-B delivered in a collagen matrix exhibits acceptable safety parameters for possible use in human clinical studies. Ad-PDGF-B was well contained within the localized osseous defect area without viremia or distant organ involvement. No significant histopathologic changes were observed. Although minor alterations in specific hematologic and blood chemistries were seen, most measures were within normal limits.<sup>44</sup>

It has been shown that Ad-PDGF-B transduction was able to enhance soft tissue defect fill by induction of human gingival fibroblast migration and proliferation in an ex vivo model. Jin et al.<sup>56</sup> demonstrated that by using collagen as carrier, direct in vivo gene transfer of Ad-PDGF-B stimulated tissue regeneration in large periodontal defects. Ad-PDGF-B treatment resulted in more cell proliferation, a four-fold increase in bridging bone, and a six-fold increase in cementum repair above vector alone, whereas bone fill was significantly less in Ad-PDGF-1308 (a loss-of-function mutant of PDGF) treated defects.<sup>56</sup> Similarly, in a dental implant model, Chang et al.<sup>57</sup> reported that Ad PDGF-B shows regenerative capabilities for bone tissue engineering and osseointegration in alveolar bone defects comparable to rhPDGF-BB protein delivery in vivo.

**Bone morphogenetic proteins**—In dentistry, bone morphogenetic proteins (BMPs) have been shown to be potent growth factors stimulating alveolar bone formation. Jin et al.<sup>20</sup> used an ex vivo strategy, where dermal fibroblast were transduced with BMP7 and transplanted into large mandibular alveolar bone defects. Ex vivo BMP2 gene delivery using autologous bone marrow MSC has been evaluated using animal models for periodontal regeneration. This approach has been shown to regenerate not only cementum with Sharpey fiber insertion, but also statistically significant quantities of bone, reestablishing a more normal relationship among the components of the regenerated periodontal attachment apparatus.<sup>58</sup> Gene delivery by BMP7 resulted in rapid chondrogenesis, with subsequent osteogenesis, cementogenesis, and predictable bridging of periodontal bone defects,<sup>20</sup> whereas BMP signaling knockdown by Ad-Noggin tended to inhibit bone repair and cementogenesis.<sup>20,59</sup> In a direct gene therapy approach, Ad-BMP7 delivery with a collagen matrix significantly enhanced alveolar bone defect fill, new bone formation, and bone implant contact in a dental implant model.<sup>60</sup> BMPs have been recently studied for a variety of other periodontal gene-delivery applications.<sup>58,61</sup>

**Wingless (WNTs)**—Wnts are a family of 19 secreted glycoproteins that are crucial for embryonic development and post-developmental physiology through regulation of cell proliferation, differentiation, and apoptosis.<sup>62</sup> In the last several years, the role of Wnt signaling in bone development, postnatal maintenance of bone mass, and tooth morphogenesis has been investigated, and several pharmaceutical targets in Wnt signaling pathway for skeletal diseases have been identified, such as leucine-responsive regulatory

protien 5 and sclerostin.<sup>63</sup> The role of Wnts in periodontal homeostasis and regeneration remains largely unknown, although recently two studies highlighted the promising future of regulating Wnt signaling pathway. Nemoto et al.<sup>64</sup> showed that canonical Wnt/ $\beta$ -catenin signaling has been shown to inhibit murine cementoblasts differentiation and enhance cell proliferation. In the other study, Chang et al.<sup>18</sup> transduced human periodontal mesenchymal cells with retroviral *Wnt-4*, a non-canonical *Wnt*, and transplanted them into the experimental periodontal defects. The results demonstrated that *Wnt-4* gene delivery promotes healing of alveolar bone wounds in vivo.

**Transcription factors and regulators**—In addition to growth factors, other genes that are critical transcription factors and regulators of osteogenesis, such as *Runx2*, Osterix (*Osx*), and LIM domain mineralization protein (LMP), may hold promise in periodontal tissue engineering, especially in alveolar bone augmentation. *Runx2* is a master transcription activator of osteoblast differentiation. Ex vivo *Runx2* gene delivery is able to induce bone marrow stromal cells and dermal fibroblasts to form mineral tissue in vivo.<sup>65–67</sup> *Osx* is a zinc-finger-containing transcription factor that works downstream of *Runx2* in osteoblast differentiation. *Osx* gene knockout results in impaired osteoblast differentiation and absence of bone formation. Tu et al.<sup>68</sup> reported that overexpression of *Osx* in bone marrow MSCs by retroviral vectors stimulates healing of critical-sized defects. Using the same ex vivo strategy, the same group further showed *Osx* gene therapy increases bone density and elevates bone–implant contact in a titanium implant model.<sup>69</sup> LMP-1 is an intracellular protein that is highly upregulated at the early stage of osteoblast differentiation. It has been shown that gene delivery of LMP-1 or a truncated version LMP-3 induces efficient bone formation in vivo in heterotopic and orthotopic (spine fusion and bone fracture healing) sites.<sup>70–73</sup> The value of LMPs shows potential in the modulation of periodontal progenitor cells but still requires further investigation for periodontal tissue engineering.<sup>74</sup>

### Gene Delivery for Host Modulation of Periodontal Disease

Acknowledging that tissue regeneration alone is not the only answer to predictably securing a long-term stable treatment for the patient with a history of periodontal disease is very important. Host modulation therapies are therefore rising as an important aspect in the control of periodontal diseases and tissue reengineering.<sup>75</sup> Future approaches for tissue engineering may benefit from dual delivery of tissue regenerative molecules with either antimicrobial or host modulatory factors. Based on the understanding that the host response against pathogenic bacteria is a major cause of periodontal tissue destruction, new strategies have been developed to target these factors, such as MMPs, cathepsins, and other osteoclast-derived mediators of bone resorption.<sup>75</sup> Gene therapy has also been investigated for the possibility of long-term maintenance of therapeutic proteins. Cirelli et al.<sup>76</sup> used AAV to deliver the tumor necrosis factor receptor-immunoglobulin Fc (TNFR:Fc) fusion gene to experimental *Porphyromonas gingivalis*–lipopolysaccharide-mediated bone loss. Gene therapy resulted in sustained therapeutic levels of serum TNFR protein for 3 months, and *P. gingivalis*–lipopolysaccharide-mediated bone loss volume and density was inhibited after AAV2/1-TNFR:Fc administration. Tristetraprolin, a key cytokine-regulating RNA-binding protein, downregulates inflammatory cytokines by transferring mRNA transcripts to degradation machinery.<sup>77</sup> Patil et al.<sup>77</sup> showed that tristetraprolin overexpression by an adenoviral vector significantly reduces the expression of interleukin-6, TNF- $\alpha$ , and prostaglandin E<sub>2</sub> in vitro and protects inflammation-induced bone loss and inflammatory infiltrate in an experimental periodontitis model. Most recently, Yu et al.<sup>78</sup> demonstrated the potential of mitogen-activated protein kinase phosphatase 1 to prevent alveolar bone loss in vivo. These findings suggest the possible application of gene delivery strategies in the modulation of periodontal disease progression.

The potential of transferring genetic materials to modulate specific genes that direct an individual's own cells to produce a therapeutic effect is the idea behind treating a disease by gene therapy. Dictating the local spatial and temporal distribution of the gene-therapy effect has been enhanced by their incorporation into natural and synthetic scaffolds.

## SCAFFOLDING MATRICES USED TO DELIVER CELLS AND GENES FOR PERIODONTAL REGENERATION

Scaffolding matrices are used in tissue engineering to provide an environment where space is created and maintained during a period of time for cellular growth and tissue in-growth. These matrices serve as three-dimensional template structures to physically support and facilitate periodontal tissue regeneration when combined with cell- or gene-based tissue engineering (Fig. 1). During the past two decades, scaffolds have been extensively developed, studied, and used. Several fundamental requirements for scaffold design have been proposed.<sup>79</sup> In their application to tissue engineering, they should 1) provide a three-dimensional architecture that supports a desired volume, shape, and mechanical strength; 2) consist of a high porosity and surface-to-volume ratio with a well-interconnected open pore structure to promote high seeding density and embrace bioactive molecules; 3) be biocompatible; and 4) degrade at a controlled rate and pattern that allows sufficient support until tissue defects are fully regrown. Scaffolds can also be engineered to serve as supportive carriers that conduct a sustained release of bioactive factors, thereby inducing stimuli for tissue formation. Transplantation of cells can be carried via tissue-engineered scaffolds<sup>79,80</sup> that provide adhesion and anchorage for interacting stem cells to control the presentation of adhesion sites, thereby improving cell survival and participation.<sup>81,82</sup> By furthering the pattern of tissue structure formed by stem cells, a new mandible was formed in a patient by using a metal and polymer scaffold seeded with stem cells and BMPs.<sup>83</sup> Bioactive molecules, such as growth factors, may also be encapsulated into nanoparticles and microparticles embedded into the matrices to aid in their sustained release from scaffolds. Other approaches include mimicking stem cell niches to regulate daughter cell proliferation, differentiation, and dispersion into surrounding tissue or by attracting useful cells to a desired anatomic site.<sup>1</sup>

Understanding that proper periodontal regeneration is supported by important factors that include multiple tissue integration and cell-tissue directionality is critical for the optimization and further development of emerging scaffold technologies.<sup>84,85</sup> Today, the feasibility to establish a three-dimensional polarity and a customizable micro scaffold and macro scaffold architecture is one step forward to the ability to create biomimetic scaffold surfaces that are amenable for gene- and cell-therapy strategies.<sup>86</sup> Several scaffold fabrication technologies as applied to periodontal tissue engineering have developed during the past 10 years including conventional prefabricated scaffolds, such as particulated, solid form, and injectable scaffolds that are adapted or administered into a periodontal defect, and novel image-based designs that result in a three-dimensional printed scaffold that is custom fit to a defect (Fig. 1).

### Prefabricated Scaffolding Matrices

Conventional scaffolds used to regenerate tissue *in vivo* are prefabricated, and many techniques have been described that produce both natural and synthetic polymeric scaffolds. Naturally derived scaffolds include autografts, allografts, and xenografts. Other naturally derived scaffolds that have been adapted for tissue regeneration but remain unexplored as carriers in gene and cell therapy approaches for periodontal engineering include the coral-derived matrices and the hydroxyapatite and chitosan carriers.<sup>87,88</sup> Alloplasts and other



polymers are synthetically engineered materials consisting of bioactive molecules serving a purpose similar to that of natural scaffolds (Table 3).<sup>7,15,16,29,32,44,80,89–97</sup>

Synthetic polymers have been studied extensively as gene therapy delivery systems because they provide greater freedom for property modification, such as control of macrostructure and degradation time, compared to naturally derived scaffolds.<sup>98</sup> Furthermore, the release mechanism and exposure duration of bioactive molecules, such as growth factors, can be controlled.<sup>39</sup> By acting as a localized gene depot, synthetic polymer scaffolds have the ability to maintain the therapeutic level of encoded proteins that limit unwanted immune response and potential side effects.<sup>99</sup> Such polymers as poly(lactic-co-glycolic acid) have drawn much attention for its excellent properties for encapsulation of genes.<sup>100</sup> Poly(lactic-co-glycolic acid) microspheres have been used previously to deliver antibiotics, as an occlusive membrane for guided tissue regeneration, as growth factor carrier for periodontal regeneration, and for cementum and complex tooth structure engineering.<sup>29,101–105</sup> Microsphere systems have demonstrated promising results in the past; however, more novel approaches to microtechnologies today are focusing on nanosized particles.<sup>106</sup> Nanotechnology has been attracting much attention for therapeutic agent and gene delivery, and a number of studies and reviews have delineated its contribution and capability to meet challenges of current regeneration therapy.<sup>100,106,107</sup> Nanoscaled fibrillar structure of collagen shows promising effects on cellular biologic activities, and therefore, the potential of a synthetic polymer scaffold that mimics the nanofibrous structure of collagen.<sup>108</sup> Furthermore, a recent study has developed macroporous polymer scaffolds with varying pore wall architecture to enhance the environment for induction of cellular activity and provide guidance for three-dimensional regeneration.<sup>109</sup> Therefore, a delivery scaffold can provide a suitable environment for targeted cells and tissues and controlling the dynamic release of entrapped biologics. Periodontal therapy based on these systems, however, remains in its infancy.

The use of HA in the dental field has been demonstrated to restore periodontal defects and to carry and deliver growth factors, such as BMPs and fibroblast growth factor-2.<sup>110</sup> Although no clinical or in vivo studies have used HA for gene and cell therapy strategies for periodontal engineering purposes, a recent in vitro study has shown an HA and collagen combination scaffold to be a suitable environment for the growth of human PDL cells, therefore indicating its potential for periodontal tissue engineering.<sup>111</sup>

Inorganic calcium-phosphate-based materials have also been used as delivery systems. Such materials as  $\beta$ -tricalcium phosphate are synthetic scaffolds that can be used to repair osseous defects around teeth or dental implants by acting as a bone substitute or as a carrier for growth factor delivery and cells.<sup>90</sup> Gene-and cell-therapy tissue-engineering methods have used  $\beta$ -tricalcium phosphate as a carrier for bone reengineering approaches but its value for periodontal regeneration remains to be explored.<sup>112,113</sup>

Hydrogels, formed by the cross-linking or self-assembly of a variety of natural or synthetic hydrophilic polymers to produce structures that contain over 90% water, are obtained from natural materials, such as collagen chitosan, dextran, alginate, or fibrin. They are favorable for tissue engineering because of their innate ability to interact with and mediate degradation by cells.<sup>106,114–116</sup> Vector release from hydrogels is dependent on the physical structure and degradation of the hydrogel and its interactions with the vector.<sup>116</sup>

### Computer-Based Applications in Scaffold Design and Fabrication

Computer-based applications in tissue engineering are some of the more recent developments in scaffold design and fabrication for cell and gene delivery.<sup>117</sup> This type of technology, image-based design, has been used in recent years to define virtual three-

dimensional models for surgical planning by using data from computed tomography and magnetic resonance imaging. Specifically, in tissue engineering, computed tomography or magnetic resonance imaging data are used to define the three-dimensional anatomic geometry of a defect that can be used to create a template for a scaffold on a global anatomic level. This three-dimensional printed scaffold, because it is produced from the three-dimensional model, precisely fills the defect space (Fig. 2). Furthermore, the architecture of the scaffold can be defined to design the heterogeneous internal structure in a way to create region-specific variations in porous microstructures and scaffold surface topography, thereby altering material and biologic properties in specific regions of the scaffold, such as modulus, permeability, and cell orientation.<sup>118</sup> This new generation of scaffolds is addressing specific periodontal functional requirements, such as PDL fiber orientation and tissue integration of cell- and gene-based technologies (Fig. 3).<sup>86</sup>

Various novel delivery scaffolding systems are being extensively studied and fabricated and are demonstrating capabilities to meet the challenges of current regeneration therapy. There are several techniques and technologies that have been developed and applied to fabrication of scaffold matrices. Only through further research and development in this area, along with cell-based and gene therapy, can tissue engineering continue to advance.

## FUTURE DIRECTIONS IN PERIODONTAL REGENERATION

Tissue engineering is making an important impact on periodontal therapy. The use of cell and gene therapy to enhance and direct periodontal wound healing into a more predictable regenerative path is being exploited in bioengineering efforts that aim at developing a therapeutic system to promote periodontal repair. However, numerous challenges remain. As discussed within the content of this review, there are a number of developing systems that have the potential to optimize tissue-healing biology. A major obstacle that remains today is how to maximize the use of cells and genes delivered to a passive or permissive environment where there is context for the type of cell needed, but in which some biologic signals are given to encourage normal cell function. The tissue-engineering field still needs to confront other hurdles, such as identifying cell sources and clinically relevant cell numbers, the integration of new cells into existing tissue matrices, and the achievement of functional properties of tissue equivalents using an expanded repertoire of biomaterials. Major constraints to the cell- and gene-transfer fields remain in the practical and regulatory requirements to apply these technologies to the clinical arena.

## CONCLUSIONS

Today, the available cell-based therapy strategies founded on tissue-engineering approaches have the most solid background for clinical application in human periodontal defects. Gene-based therapy is at the preclinical level at this point, and as such it may be several years before it enters the clinical arena. However, the cell-based, scaffold, and gene-therapy methods collectively interface and complement each other to enhance the potential to restore tissue function and structure in a predictable manner. The success and the future of periodontal regenerative therapy will thereby be supported by our understanding and ability to recognize those clinical situations that will benefit from one or more of these new emerging technologies.

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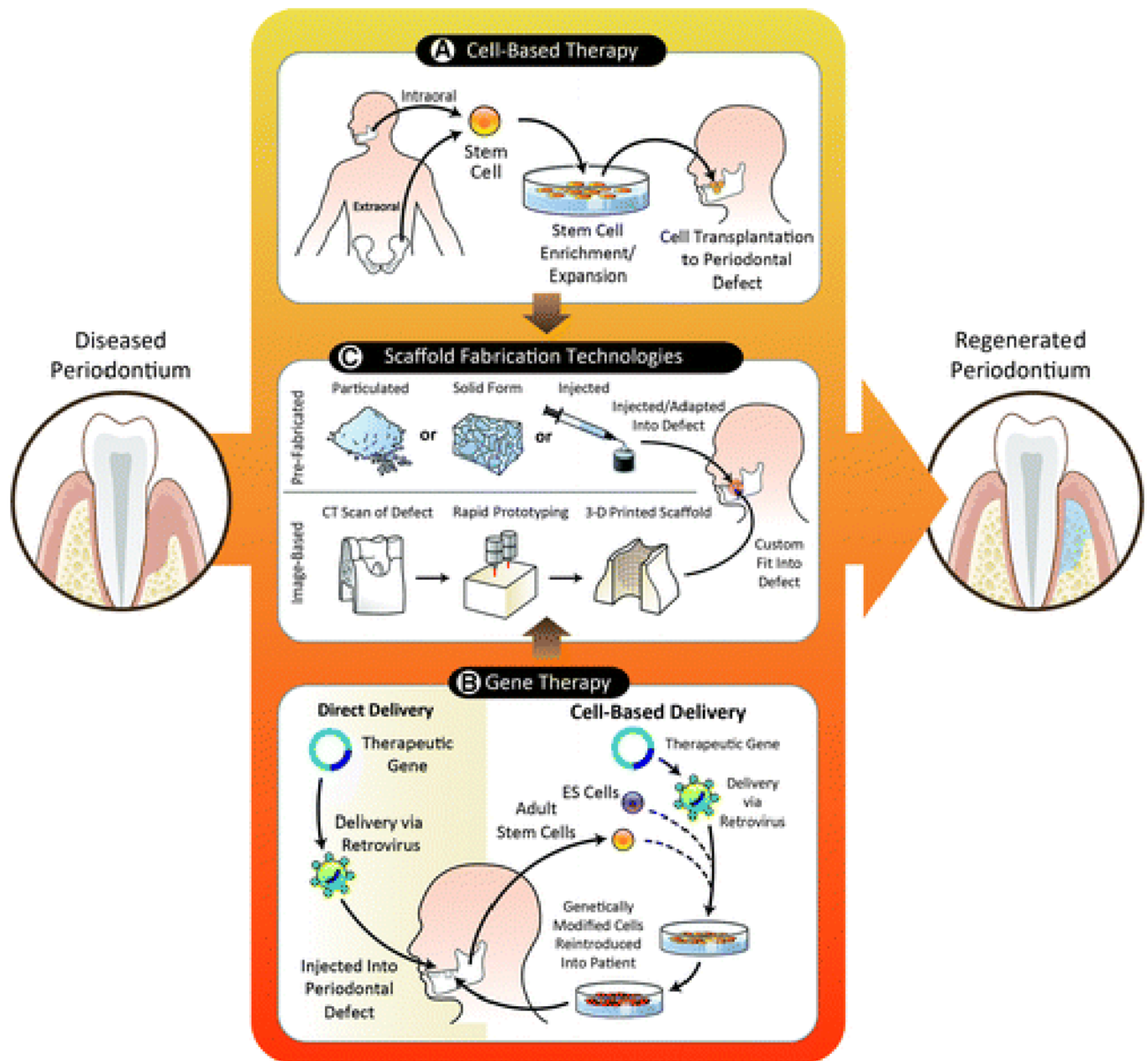
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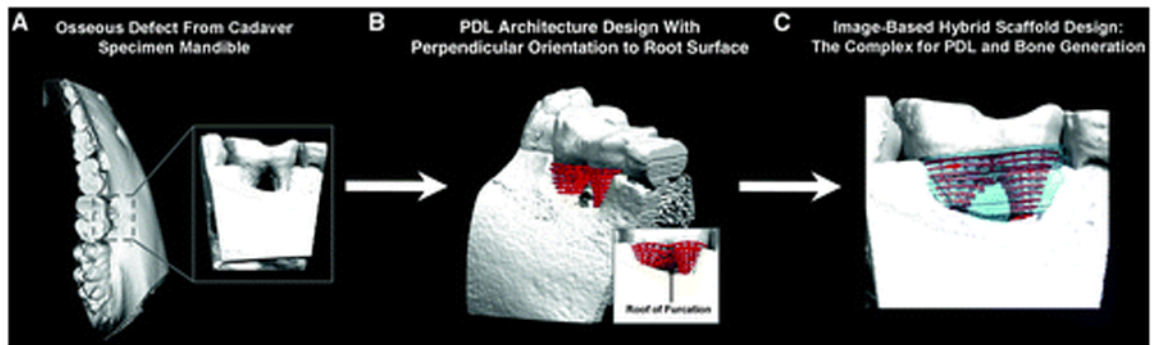
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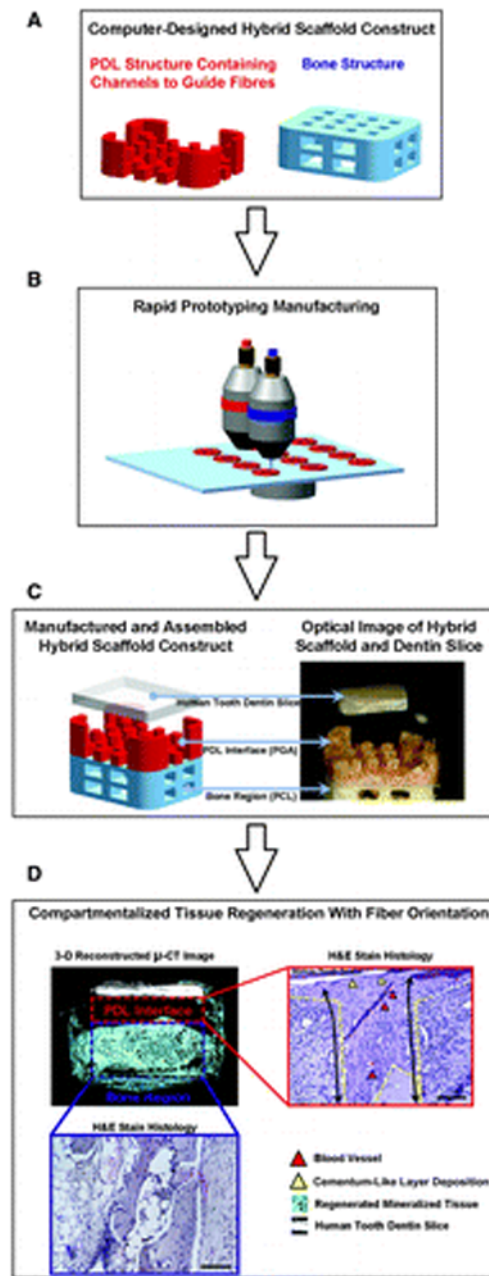
**Figure 1.** Cell- and gene-based technologies using scaffolding matrices for periodontal tissue engineering. **A)** Extraoral and intraoral stem cells represent a viable and accessible alternative source to harvest and expand multipotent colonies. Adequate cell density could be reached in vitro under a controlled environment and made readily available for reimplantation into a periodontal defect site. **B)** The available direct and cell-based delivery of a therapeutic gene has been shown to increase the regenerative potential and enhance the availability of important factors. The gene of interest is either injected directly into the periodontal defect via a retrovirus or alternatively could be incorporated into an embryonic stem cell (ES) or adult stem cell that is subsequently expanded and delivered into the area of interest. **C)** Prefabricated and image-based scaffolds are becoming an essential component in regenerative medicine. A defined supporting structure allows the localization and guidance of the appropriate cells and proteins and the establishment of a mechanically

competent environment. Currently, scaffolds for periodontal regeneration are available in particulated, solid, and injectable forms. New developing technology has allowed the customization of scaffolds that fit into the periodontal defect and include an external and internal architecture that enhances tissue orientation and regeneration. This figure highlights the potential of integrating the available tissue engineering strategies to enhance the outcome of periodontal regenerative therapy.



**Figure 2.**

Hybrid scaffolds for periodontal cell and gene delivery. **A through C)** Surgically created Class III furcation defect is observed in this figure. The image-based scaffold aims at generating a three-dimensional polarity and patterning within the defect geometry to guide and establish cell tissue integration and directionality.



**Figure 3.**

The hybrid scaffold concept has proved effective at establishing an adequate periodontal tissue interface that integrates the newly formed cementum, bone, and properly oriented PDL fibers. **A)** The main two compartments of the scaffold are depicted as PDL region (red) and bone region (blue). **B)** A rapid prototyping technique is used to generate the three-dimensional geometry. **C)** Wax molds for PDL and bone architectures are manufactured and fabricated to cast polymeric materials, poly-glycolide (PGA) for PDL and poly- $\epsilon$ -caprolactone (PCL) for bone of the hybrid scaffold. **D)** Microcomputed tomography ( $\mu$ -CT) showed that the designed hybrid scaffold guides multiple tissue formation with the specific dimension in PDL interface (red dash-lined box) and bone region (blue dash-lined box). The hematoxylin and eosin staining was used to analyze mineralized tissue formation and fibrous

connective tissue formation (scale bar = 50  $\mu\text{m}$  original magnification  $\times 10$ ). In PDL interface, fibrous tissue orientation along the PDL topography (yellow dash-lined border) was found with blood vessel formations (red triangles) and limited cementum-like layer formation on the tooth dentin surface (yellow triangles).<sup>86</sup>

**Table 1**

## Preclinical Applications of Cell Therapies for Periodontal Tissue Engineering

Vector	Type	Advantages	Disadvantages
Retrovirus	Viral	Non-immunogenic Constitutive transgene expression	Infects only dividing cells Insertional mutagenesis
Lentivirus	Viral	Infects dividing and non-dividing cells Infects wide range of cell types Low immune response	Insertional mutagenesis Potential pathogenicity Complex large-scale preparation
Adenovirus	Viral	Infects dividing and non-dividing cells	Potential immunogenicity
Adeno-associated virus	Viral	Does not integrate into target cell genome Infects dividing and non-dividing cells	Transient expression Difficult to produce at high titers
Plasmid	Non-viral	Low immunogenicity Non-pathogenic in human Non-immunogenic Non-pathogenic	Small transgenes Low transduction efficiency
DNA polymer complexes	Non-viral	Infects dividing and non-dividing cells Cell-specific targeting	Low transduction efficiency

**Table 2****Viral and Non-Viral Gene Therapy Vectors Used in Tissue Engineering**

<b>Cell Type</b>	<b>Autograft/Allograft</b>	<b>Defect Type</b>	<b>Reference(s)</b>
Bone marrow stromal cells	Auto	Class III defects	8,9
	Auto	Periodontal fenestration	10
	Auto	Osteotomy	11-13
Adipose stromal cells	Auto	Periodontal palatal defects	14
Periodontal ligament cells	Auto	Class II defects	15
	Auto	Periodontal fenestration	16
	Allo/Xeno	Periodontal fenestration	17
Periodontal ligament stem cells	Allo	Ectopic	18
	Allo	Periodontal fenestration	15,19
	Auto	Periodontal defects	20
Cementoblasts	Allo	Ectopic	21
	Allo	Periodontal fenestration	22
Dental follicle cells	Allo	Ectopic	21,22
	Allo	Periodontal fenestration	22

Xeno = xenograft; Allo = allograft; Auto = autograft.

**Table 3**  
Scaffolding Matrices for Delivery of Cells and Genes for Periodontal Engineering

Scaffold Origin	Biomaterial	Components	Application
Naturally Derived	Allografts	Calcified freeze-dried bone, decalcified freeze-dried bone	Non-viral vector (PDGF-BB) <sup>89</sup>
	Xenografts	Bovine mineral matrix, bovine-derived HA, bovine inorganic bone mineral	Non-viral vector (TGF- $\beta_1$ ) <sup>90</sup>
	Collagen (bovine/porcine xenograft)	Sponge	Oral/craniofacial MSCs (PDL cells) <sup>91</sup>
		Membrane	Non-viral vector (b-FGF) <sup>92</sup>
		Gel/Gelatin	Oral/craniofacial MSCs (PDL cells) <sup>17</sup>
			Extraoral MSCs (bone marrow MSC) <sup>8</sup>
			Adenovirus vector (PDGF-B) <sup>44</sup>
Synthetic/Alloplasts	Polymers	PLLA	Oral MSCs (PDL fibroblasts) <sup>93</sup>
		PGA	Retroviral vector (BMP7); periosteal cells <sup>94</sup>
		PLGA (copolymer of PLLA and PGA)	Oral/craniofacial MSCs (cementoblasts) <sup>29</sup>
	Ca-P-based ceramics	$\beta$ -TCP, Ca-P cement	Retroviral vector (BMP2); W-20 cell line <sup>95</sup>
	Hydroxyapatite-based scaffolds	Hydroxyapatite dense HA, porous HA, resorbable HA, non-porous non-resorbable granular HA	Non-viral vector (TGF- $\beta_1$ ) <sup>90</sup>
	Hydrogels	HA ester	Oral/craniofacial MSCs (PDL cells) <sup>32</sup>
		Methylcellulose	Oral/craniofacial MSCs (PDL cells) <sup>6</sup>
	CaCO <sub>3</sub>	Coralline calcium carbonate ester	Non-viral vector (PDGF-BB; IGF-I) <sup>96</sup>
			Non-viral vector (TGF- $\beta_1$ ) <sup>97</sup>

PGA = polyglycolic acid; PLLA = polylactic acid; PLGA = poly(lactic-co-glycolic acid); TCP = tricalcium phosphate; TGF = transforming growth factor; FGF = fibroblast growth factor; IGF = insulin-like growth factor; Ca-P = calcium phosphate; bFGF = basic fibroblast growth factor.