Emerging Regenerative Approaches for Periodontal Reconstruction: A Systematic Review From the AAP Regeneration Workshop

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More than 30 years have passed since the first successful application of regenerative therapy for treatment of periodontal diseases. Despite being feasible, periodontal regeneration still faces numerous challenges, and complete restoration of structure and function of the diseased periodontium is often considered an unpredictable task. This review highlights developing basic science and technologies for potential application to achieve reconstruction of the periodontium. A comprehensive search of the electronic bibliographic database PubMed was conducted to identify different emerging therapeutic approaches reported to influence either biologic pathways and/or tissues involved in periodontal regeneration. Each citation was assessed based on its abstract, and the full text of potentially eligible reports was retrieved. Based on the review of the full papers, their suitability for inclusion in this report was determined. In principle, only reports from scientifically well-designed studies that presented preclinical in vivo (animal studies) or clinical (human studies) evidence for successful periodontal regeneration were included. Hence, in vitro studies, namely those conducted in laboratories without any live animals, were excluded. In case of especially recent and relevant reviews with a narrow focus on specific regenerative approaches, they were identified as such, and thereby the option of referring to them to summarize the status of a specific approach, in addition to or instead of listing each separately, was preserved. Admittedly, the presence of subjectivity in the selection of studies to include in this overview cannot be excluded. However, it is believed that the contemporary approaches described in this review collectively represent the current efforts that have reported preclinical or clinical methods to successfully enhance regeneration of the periodontium. Today's challenges facing periodontal regenerative therapy continue to stimulate important research and clinical development, which, in turn, shapes the current concept of periodontal tissue engineering. Emerging technologies—such as stem cell therapy, bone anabolic agents, genetic approaches, and nanomaterials—also offer unique opportunities to enhance the predictability of current regenerative surgical approaches and inspire development of novel treatment strategies.
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KEY WORDS

Guided tissue regeneration; tissue engineering; wound healing.

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The periodontium is a functional unit that is composed of the alveolar bone, the periodontal ligament (PDL), and the cementum (tooth-supporting apparatus), as well as the free and attached gingiva. It is a highly specialized, adaptive, and dynamic tissue that is able to sustain a variety of microbiologic, inflammatory, and mechanical challenges through a number of complex molecular events.[1] Disturbances of this equilibrium in the form of different periodontal diseases affect a significant percentage of the adult population.[2,3]

Regeneration of the deteriorated periodontium has been the ideal goal in periodontal therapy for over three decades.[4] However, re-establishing the original structure, properties, and function of the diseased periodontium constitutes a significant challenge.[5] Different approaches have been proposed, but the quantity of regenerated tissue has often been limited and unpredictable. By definition, successful periodontal regeneration implies the simultaneous regeneration of cementum, PDL, and alveolar bone, because the periodontium functions as a unit (Fig. 1). This process requires a specific temporal sequence and spatial distribution, which is based on a number of essential factors.[6-9]

Although the exact cellular and molecular events are still not clear, specific cells must first migrate to the healing area and proliferate to provide the basis needed for the new tissue to grow and differentiate. This process is mediated and coordinated by soluble factors, many cell types, extracellular matrix (ECM), and matricellular interactions. Ideally, scaffolds will provide a three-dimensional (3D) template structure to support and facilitate these processes. Angiogenic signals and new vascular networks provide the nutritional base for tissue growth and homeostasis. Later, normal mechanical stimuli will increase and promote an organized ECM synthesis and organization, as well as cementum and bone formation and maturation. Once those structures are established, PDL fibers become organized and connect the tooth to the alveolar bone. Finally, because of the microbial load in the periodontal area, strategies to control infection and its subsequent host responses are required to optimize periodontal healing and regeneration.[8-10] Understanding the natural history of the initial development of the healthy periodontium may provide inspiration and cues to discover pathways for regeneration techniques.

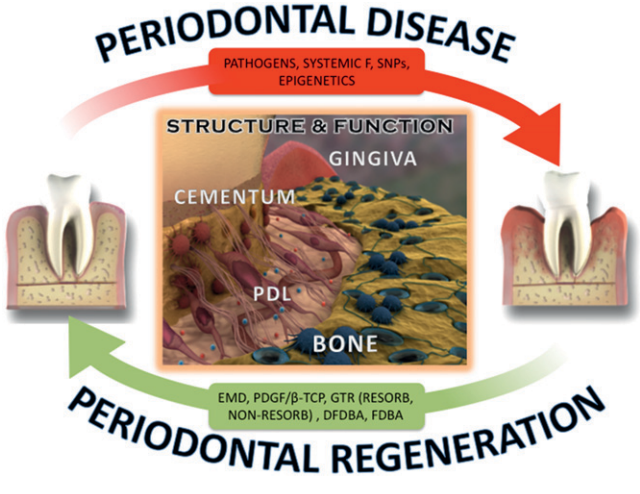

Figure 1.
Regeneration of the periodontium as a functional unit. Throughout life, the periodontal homeostasis is challenged by genetic and environmental factors. During periodontal disease, the structure and function of the tooth-supporting tissues are progressively compromised. Emerging biologic therapeutic wound-healing mediators provide a promising approach to assist clinicians in tailoring treatment to enhance periodontal regenerative outcomes. systemic F = systemic factors; SNPs = single nucleotide polymorphisms; EMD = enamel matrix derivative; PDGF = platelet-derived growth factor; β-TCP = beta-tricalcium phosphate; GTR = guided tissue regeneration; resorb = resorbable; non-resorb = non-resorbable; DFDBA = demineralized freeze-dried bone allograft; FDBA = freeze-dried bone allograft.

This study focuses on key clinical and preclinical evidence that illustrates promising therapeutic approaches to different aspects of tissue engineering of the periodontium. First described is therapy with proteins/peptides and systemic anabolic agents, followed by cell-based treatment, gene therapy, scaffolds, systemic anabolic agents, and laser therapy. Along the way, various cellular and molecular signaling events that guide these processes are explained briefly. Appropriate signals may be delivered directly by proteins/peptides or indirectly by genetic approaches. The goals are to highlight the next generation of techniques and strategies in periodontal regeneration, stimulate discussion, and provide guidance for future research needed to meet the challenges facing periodontal regenerative therapy. Supplementary Table 1 in online *Journal of Periodontology* provides an explanation of selected terms and abbreviations used throughout the review.

METHODS

This critical review is designed to introduce and describe the developing basic science and technologies for potential application to achieve reconstruction of the periodontium. A comprehensive search of the electronic bibliographic database PubMed was conducted to identify different emerging therapeutic approaches reported to influence either biologic pathways and/or tissues involved in periodontal regeneration. Only reports from scientifically well-designed studies or case reports that presented preclinical in vivo (animal studies) or clinical (human studies) evidence for successful periodontal regeneration were excluded. Hence, in vitro studies,

namely those conducted in laboratories without any live animals, were excluded. Based on the citation and abstract, each publication was assessed for possible inclusion, and the full text of those that were considered potentially eligible was subsequently retrieved. Using this thorough assessment of the full-text papers, their suitability for inclusion in this report was determined. The selected manuscripts were categorized by the type of method reported. The most promising contemporary techniques were described in more detail, and then novel approaches that are still in the early phases of development were summarized briefly. The major categories described in the following are as follows: 1) protein/peptide therapy; 2) cell-based therapy; 3) gene/RNA therapy; 4) scaffolds; and 5) lasers.

APPROACHES TO PERIODONTAL REGENERATION

Protein/Peptide Therapy

Over the past two decades, numerous studies have explored the potential of using biologic proteins and peptides in periodontal regeneration.[11,12] Currently, three products are commercially available: 1) enamel matrix derivative (EMD); 2) recombinant human platelet-derived growth factor-BB (rhPDGF-BB)/beta tricalcium phosphate (β-TCP); and 3) synthetic peptide binding protein P-15/anorganic bovine bone matrix.

Two other growth factors, namely recombinant human fibroblast growth factor-2 (rhFGF-2) and recombinant human growth and differentiation factor-5 (rhGDF-5), are undergoing testing in Phase II/III clinical trials, and several others are under active investigation in preclinical studies. Examples of such studies are displayed in Tables 1 and 2.[13-34] The addition of biologic proteins/peptides regulates the necessary cellular and biologic activities, thereby facilitating the regeneration process. The biologic functions of these proteins and peptides vary widely. However, they are selected as candidate targets for periodontal regeneration primarily because of their roles in the development of periodontium and wound healing, specifically, their effects on cell chemotaxis, proliferation, differentiation, matrix synthesis, and angiogenesis. Other proteins, such as parathyroid hormone (PTH) and sclerostin (SOST) antibody, mostly administered systemically, have also been shown to prevent periodontal disease progression and promote regeneration.[34,35]

EMD, a mixed peptide combination derived from immature enamel of 6-month-old piglets, is the first Food and Drug Administration (FDA)–approved biologic product for periodontal regeneration. More than 30 randomized clinical trials (RCTs) and several meta-analyses and systematic reviews have been published reporting the clinical outcomes after application of EMD in the treatment of intrabony defects.[36-39] Evidence indicates that use of EMD for treatment of periodontal intrabony defects, when compared with open flap debridement (OFD), EDTA, root conditioning or placebo, results in significant gain in clinical attachment level (CAL) (1.30 mm), reduction in periodontal probing depth (PD) (0.92 mm), and improvement in radiographic bone level (1.04 mm).[38] However, a recent network meta-analysis showed that, when comparing EMD plus grafting material or EMD plus barrier membrane with EMD only, the additional benefits were limited.[40] Furthermore, when compared with graft material or guided tissue regeneration, the clinical advantage of using EMD is still not clear.[37-39] Studies have been done to dissect the peptide elements of EMD and define their biologic effects both in vitro and in vivo.[41-43] For example, it has been found that five pools of EMD proteins showed a stronger angiogenic activity than the EMD parent.[41] Stout et al.[43] demonstrated that low-molecular-weight protein pools (7 to 17 kDa) within EMD have greater osteoinductive effects through increased bone morphogenetic protein (BMP) signaling and increased osterix (a transcription factor) and vascular endothelial growth factor (VEGF). Such studies provide the potential to better formulate EMD for optimum regenerative outcomes.

PDGF-BB is a growth factor that has broad wound-healing activities in both hard (bone) and soft (skin, gingiva) tissue, affecting cell proliferation, migration, and angiogenesis.[44]

In an FDA Phase III, multicenter RCT, PDGF-BB/β-TCP was tested in periodontal regeneration.[16] CAL gain, linear bone gain, and percentage defect fill were significantly greater at 3 months for the rhPDGF group (0.3 mg/mL) compared with vehicle controls. Interestingly, low-dose rhPDGF-BB (0.3 mg/mL) seemed to have a stronger effect than the high dose (1.0 mg/mL). The 24-month follow-up showed substantial radiographic changes in the appearance of the intrabony defect fill for both rhPDGF-BB treatment groups (i.e., 1.0- and 0.3-mg/mL dosage levels).[45] The more recently published 36-month follow-up results used a composite analysis for clinical and radiographic evidence of treatment success, defined as the percentage of cases with CAL ≥2.7 mm and linear bone growth ≥1.1 mm.[46] The authors reported that participants exceeding this composite outcome benchmark in the 0.3-mg/mL rhPDGF-BB dosage level group went from 62.2% at 12 months and 75.9% at 24 months to 87.0% at 36 months compared with 39.5%, 48.3%, and 53.8%, respectively, in the scaffold control group. The efficacy of rhPDGF-BB has also been demonstrated in another multicenter RCT,[17] with 4.3 ± 0.9 mm CAL

Table 1.

Representative RCTs for the Application of Biologic Proteins/Peptides in Periodontal Regeneration (adapted from Reynolds et al., 2012[13])

Study	Protein/ Peptide	Study Participants (n)		Baseline Defect Depth (mean ± SD mm)		CAL Gain (mean ± SD mm)		Defect Fill (mean ± SD mm)		Bone Fill (mean ± SD %)	
		Protein/ Peptide Group	Periodontal Surgery Group	Protein/ Peptide Group	Periodontal Surgery Group	Protein/ Peptide Group	Periodontal Surgery Group	Protein/ Peptide Group	Periodontal Surgery Group	Protein/ Peptide Group	Periodontal Surgery Group
Heiji et al.[14]*	EMD	31	31	7.1 ± 2.2	6.5 ± 2.3	2.3 ± 1.6	1.7 ± 1.2	2.2 ± 1.6	−0.2 ± 0.6	31	−4
Yukna et al.[15]	ABM/P-15	33	33	4.0 ± 0.8	4.3 ± 1.0	2.2 ± 2.0	2.1 ± 1.8	2.9 ± 1.4	2.2 ± 1.4	72.9 ± 23.3	50.6 ± 26.9
Nevins et al.[16]†	rhPDGF-BB/ β-TCP	60	59	6.0 ± 0.2	5.7 ± 0.2	3.8 ± 0.2	3.5 ± 0.2	2.6 ± 0.2	0.9 ± 0.1	57 ± 6	18 ± 6
Jayakumar et al.[17]	rhPDGF-BB/ β-TCP	27	27	6.3 ± 1.9	6.7 ± 1.9	3.7 ± 1.0	2.8 ± 0.9	3.7 ± 1.1	2.8 ± 1.2	65.6 ± 21.7	47.5 ± 19.8
Kitamura et al.[18]‡	rhFGF-2/ HPC	19	19	5.7 ± 2.6	4.7 ± 1.5	2.2 ± 1.3	2.6 ± 1.5	1.9 ± 1.8	1.0 ± 1.3	58.6 ± 46.7	23.9 ± 27.5
Kitamura et al.[19]‡	rhFGF-2/ HPC	57	61	4.8 ± 1.7	5.0 ± 1.8	2.3 ± 1.7	1.8 ± 1.5	NA	NA	50.6 ± 31.5	15.1 ± 21.9
Starvropoulos et al.[20]	rhGDF-5/ β-TCP	10	10	6.7 ± 2.8	6.4 ± 2.1	3.2 ± 1.7	1.7 ± 2.2	2.2 ± 1.6	0.8 ± 1.0	NA	NA

CAL = clinical attachment level; ABM = anorganic bone matrix; HPC = hydroxypropyl cellulose; NA = not available.
* Data at 16 months.
† Data at 24 weeks. The test group was 0.3 mg/mL rhPDGF-BB.
‡ Data at 36 weeks. The test group was 0.3% rhFGF-2.

gain in the test group compared with 3.2 ± 1.6 mm in vehicle control group and 3.7 ± 1.1 mm linear bone growth versus 2.8 ± 1.2 mm in the control group (values are mean ± SD throughout). Human histologic observations of periodontal bony defects treated with rhPDGF-BB plus bone allograft showed regeneration characterized by new bone, cementum, and functionally oriented PDL fibers.[24-26] In contrast, β-TCP treatment alone healed only by fibrous connective tissue (CT) repair and a long junctional epithelial attachment.[47] It should also be mentioned that optimal clinical outcomes have been observed when biologic peptides/proteins (EMD and rhPDGF-BB) are used for root coverage[25,38] as alternatives for the traditional CT graft procedure.

P-15 is a polypeptide consisting of 15 amino acids that mimics the cell-binding domains of Type I collagen, which has been shown to increase the rate and extent of cell attachment and migration to root surfaces.[48] The commercial product combines P-15 with bovine-derived hydroxyapatite (HA) (anorganic bone matrix [ABM]). An early clinical trial showed that ABM/P-15 used in the treatment of periodontal osseous defects demonstrated significantly greater mean defect fill when compared with ABM alone.[15] Longer observation suggested that the treatment outcomes may be stable up to 3 years.[49,50] A case report based on human histology after treatment of periodontal defects with ABM/P-15 also showed evidence of regeneration.[51] One may be concerned that the xenograft carrier ABM is the main contributor to the regenerative effect of ABM/P-15, because a study has shown periodontal regeneration after grafting with a bovine-derived xenograft alone.[52] It is important to know that the chemical extraction process used for the

Table 2.

Candidate Growth Factors/Peptides for Periodontal Regeneration

Growth Factors/ Peptides	Development Stage	Biologic Function	Histologic Evidence for Periodontal Regeneration
EMD	FDA approved	Enhances cell adhesion, stimulates cell proliferation, angiogenesis, osteogenesis, cementogenesis, and ECM synthesis	Human[21-23]
P-15	FDA approved	Enhances cell adhesion	Human[51]
rhPDGF-BB	FDA approved	Increases chemotaxis of inflammatory cells and MSC progenitors, stimulates cell proliferation, enhances angiogenesis	Human[24-26]
rhFGF-2	Phase II/III clinical trial	Stimulates fibroblast proliferation and ECM synthesis, increases chemotaxis, proliferation, and differentiation of endothelial cells	Non-human primate[27]
rhGDF-5	Phase II clinical	Promotes cell proliferation, increases chemotaxis of osteoblast progenitors, and enhances osteoblast differentiation	Human[20]
BMP-2	Preclinical	Stimulates osteogenic differentiation of MSCs	Non-human primate;[28] beagle dog[29]
OP-1 (BMP-7)	Preclinical	Increases mitogenesis and differentiation of osteoblasts	Beagle dog[30]
BMP-6	Preclinical	Enhances osteogenesis	Beagle dog[31]
BMP-12	Preclinical	Induces expression of tendon- and ligament-specific genes, limited effect on osteogenesis	Beagle dog[32]
BDNF	Preclinical	Stimulates osteogenesis and angiogenesis	Beagle dog[33]
PTH	Clinical	Bone anabolic effect	N/A
SOST antibodies	Preclinical	Bone anabolic effect and antiresorption	Rat[34]

BMP = bone morphogenetic protein; OP = osteogenic protein; BDNF = brain-derived neurotrophic factor; PTH = parathyroid hormone; SOST = sclerostin; MSC = mesenchymal stem cell; N/A = not applicable.

xenograft carrier in this study was implemented at a temperature of 300°C in contrast to the 1,100°C used on the xenograft found on ABM/P-15. The low temperature preserves the exact trabecular architecture and porosity of the original bone. However, to the best of the authors' knowledge, so far, there is no published RCT evaluating the clinical influence of treatment with P-15 alone.

FGF-2 belongs to a large family of growth factors that binds heparin and possesses broad mitogenic and angiogenic capabilities.[53] It has been implicated in diverse biologic processes, such as embryonic development, wound healing, and angiogenesis.[53] In a randomized, placebo-controlled trial, FGF-2 was radiographically shown to improve bone union.[54] With respect to periodontal regeneration, an exploratory FDA Phase IIa study showed that FGF-2 significantly improved the percentage of bone fill compared with vehicle alone at 36 weeks after treatment.[18] A follow-up multicenter RCT confirmed the superior effect of rhFGF-2 on the percentage of bone fill, with the best outcome obtained with a concentration of 0.3% rhFGF-2. However, no significant differences among treatment groups were noted for CAL gain.[19] Therefore, future studies appear necessary to demonstrate the clinical influence of rhFGF-2.

GDF-5, a member of the BMP family, plays important roles in joint development.[55] It has been shown that rhGDF-5 promotes the healing of ligaments and tendons,[56,57] as well as bone formation in pure bone defects.[58-61] GDF-5 also promotes the proliferation of cells derived from periodontal tissue, including cementoblasts, PDL fibroblasts, and osteoblasts.[62,63] It also has a chemo-attractive effect for osteoblast progenitor cells and enhances osteoblast differentiation.[64] Preclinical studies in dogs and non-human primates have shown that treatment using rhGDF-5 resulted in alveolar bone, cementum, and PDL formation.[65,66] In a recent FDA Phase IIa RCT, rhGDF-5 delivered in a β-TCP carrier resulted in greater PD reduction and CAL gain.[20] Human histologic analysis confirmed periodontal regeneration without root resorption in the treatment group. However, the small number of study participants (n = 10) did not allow for the calculation of statistical significance in differences in clinical parameters between the test and control groups.[20]

Studies with large sample sizes should be conducted to help clarify the clinical outcomes and relevance.

Other growth factors are currently studied in animal models by multiple teams throughout the world. For instance, BMP-2 has been shown to stimulate the regeneration of periodontal tissue, especially alveolar bone, in murine,[67] canine,[29,68] and non-human primates.[28,69] However, root resorption and ankylosis were observed frequently in teeth receiving BMP-2 treatment. Giannobile et al.[30] used BMP-7, also known as osteogenic protein-1 (OP-1), in a canine model using surgically created, critical-size Class III molar furcation defects. The authors reported pronounced stimulation of osteogenesis, regeneration of cementum, and new attachment but limited root ankylosis. Similar observations were seen by Ripamonti et al.[28] Interestingly, in the same study, the researchers found that combined applications of OP-1 and BMP-2 did not enhance alveolar bone regeneration or new attachment formation over and above the single applications of the morphogens individually.[28]

Chiu et al.[31] applied BMP-6 polypeptide to a rat periodontal fenestration defect model and significantly enhanced new bone, cementum, and functionally oriented PDL formation were noted, with minimal root resorption and no ankylosis. BMP-12 has been shown to be involved in tendon development and healing. Application of rhBMP-12 exhibited a functionally oriented PDL bridging the gap between newly formed bone and cementum.[32] Brain-derived neurotrophic factor (BDNF) is important for the survival and differentiation of peripheral neurons, as well as various types of non-neural cells, including PDL cells. Interestingly, BDNF appears able to promote angiogenesis and stimulate formation of periodontal supporting structures.[33,70]

Proteins and peptides have emerged to play important roles in the future of regenerative therapy because of their profound biologic effects. Nevertheless, there are two concerns that must be addressed before routine therapeutic application can be realized: 1) experimental clinical dosages are far above physiologic levels, which may increase systemic side effects; and 2) the high cost of production. Such concerns will become significant issues when considering regeneration in a large-sized defect or at multiple sites.[71,72] Part of the reason for the supraphysiologic dose is that many of the growth factors are delivered by a burst-release system, in which most of the products are released in the first 24 hours after application. In the future, it would be highly desirable to develop controlled delivery systems with significantly lowered doses of growth factors that still achieve the intended therapeutic effect. These systems should also meet the temporal expression pattern of growth factors during healing, which will help reduce the required dosage level.

The approach of systemic administration of anabolic agents has also been studied in periodontal regeneration. The response of alveolar bone to PTH has been evaluated by several investigators.[73-75] In a study by Miller et al.,[74] PTH significantly increased crestal bone levels in the mandibles of ovariectomized rats. A recent preclinical investigation demonstrated the ability of teriparatide (a recombinant form of PTH) to promote dental implant osseointegration.[76] In addition, evidence suggests a promising potential of teriparatide to promote CAL gain and alveolar bone regeneration when combined with periodontal surgical procedures.[35]

A new emerging bone anabolic agent is monoclonal antibody against SOST, an osteocyte-specific protein encoded by the SOST gene. Mutations of this gene can cause two rare bone disorders characterized by high bone mass: 1) van Buchem disease and 2) sclerosteosis.[77,78] These findings highlight the role of SOST in the homeostasis of bone mass and provide the basis for targeting SOST with monoclonal antibodies to enhance bone formation. In a preclinical postmenopausal osteoporosis study, treatment with SOST antibody actually did increase bone mass at all skeletal sites and completely prevented bone loss associated with estrogen deficiency.[79] In a Phase 1 study, a single dose of SOST antibody was well tolerated and increased bone formation markers.[80] More recently, the delivery of monoclonal antibodies inhibiting SOST has shown the potential to inhibit alveolar bone loss in a preclinical model of periodontal disease.[34] The administration of SOST antibodies was able to both prevent and treat experimental periodontitis in a rodent model system. This approach suggests that bone anabolics, such as SOST inhibitors, have potential in increasing alveolar bone density in the context of periodontal diseases.[34]

Cell-Based Therapy

Cells are obviously central to 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. Cell delivery approaches are used to accelerate periodontal regeneration through two primary mechanisms: 1) the use of cells as carriers to deliver regenerative signals, including endogenous cytokines/growth factors/chemokines secreted by the delivered cells or specific factors that are ectopically overexpressed, and 2) the provision of stem cells that are able to differentiate toward multiple cell types to promote regeneration.[9]

Cell transplantation has been an important therapy for hematopoietic diseases and saved thousands of lives in the past 50 years.[81] With the blooming of stem cell research in the past few years, especially in adult pluripotent stem cells and embryonic stem (ES)

cells, cell therapy has also been studied extensively as a valuable therapeutic option in regenerative medicine.[82,83] In the context of periodontal regeneration, the cells seeded into periodontal defects should be easy to harvest, non-immunogenic, and highly proliferative and should have the ability to differentiate into the various types of cells comprising the periodontal tissues.[84] Different types of cells, from both extraoral and intraoral origins, have been proposed for periodontal regeneration (Table 3).

Mesenchymal stem cells (MSCs) are adult pluripotent stem cells that are self-renewable and can differentiate into multiple cell types, such as osteoblasts, chondrocytes, adipocytes, and neurons, and secrete growth factors that favor the regeneration process and an array of cytokines with immunoregulatory effects.[85-87] MSCs have tremendous potential in regenerative medicine, and, by September 2013, a total of 354 human clinical trials associated with MSCs were registered for treatment of a variety of diseases. Bone marrow stromal cells (BMSCs) are the most widely investigated MSCs partially because they are easily accessible. Many authors have shown that BMSC transplantation induces periodontal regeneration characterized by new cementum and bone and PDL ligament formation in experimental periodontal defects in rats, rabbits, mini-pigs, and dogs.[9,88] By cell-labeling techniques, it is shown that BMSCs can differentiate into cementoblasts, PDL fibroblasts, and alveolar bone osteoblasts in vivo.[89-91]

A few clinical studies were conducted to test the safety and effectiveness of BMSC transplantation in craniofacial and periodontal regeneration (Table 4).[92-94] Yamada et al.[95] developed a cell transplantation strategy by using expanded, autogenous BMSCs mixed with platelet-rich plasma (PRP) gel. This technique was applied in a large clinical study with 104 participants, 17 of whom were treated to obtain periodontal regeneration and the rest for alveolar bone augmentation.[92] Such BMSC therapy appeared to be safe for all study participants. In the periodontal regeneration group, the average reduction in PD, gain in CAL, and radiographic bone gain was 5.12 ± 2.45, 4.29 ± 1.32, and 3.12 ± 1.23 mm, respectively. Significantly improved bone regeneration with no side effects in 87 other cases, including guided bone regeneration (GBR), sinus floor elevation, and socket preservation, was reported as well.[92] Kaigler et al.[96] demonstrated that cells harvested from bone marrow and expanded via a single-pass perfusion process have strong angiogenic and osteogenic potential and were able to promote bone regeneration in tooth extraction sockets and sinus floor augmentation procedures. In the subsequent Phase I/II feasibility RCT, they showed that BMSC for treatment of alveolar bone defects appeared safe. By clinical, radiographic, tomographic, and histologic measures, stem cell therapy seemed to accelerate alveolar bone regeneration compared with traditional GBR treatment.[97] McAllister et al.[98] reported that stem cells could be preserved in allograft material by a cryopreservation technique and therefore could be used for sinus lift procedures[99] and periodontal regeneration.[100] In the future, RCTs are needed to demonstrate whether this stem cell-containing graft matrix has additional regenerative effects when compared with traditional allograft.

The PDL tissue contains a population of MSCs that is essential for osteogenesis and cementogenesis during development and remodeling of periodontium, as well as for the healing response to injury. A large body of literature has demonstrated that PDL progenitors can differentiate into osteoblasts, adipocytes, chondrocytes, and other cell types.[9,88] New cementum, PDL, and alveolar bone are seen after PDL progenitor cells are implanted into periodontal defects in small and large animals.[9,88,94] PDL cells differentiate into cementoblasts and osteoblasts after transplantation, as demonstrated by green fluorescent protein (GFP) and other labeling techniques.[101-104] Published results from only one clinical study in which PDL cells were applied for periodontal regeneration were identified.[93] All three of the patients receiving PDL cell therapy reported no adverse effects.

It is worth noting that several research groups have developed cell sheet techniques for periodontal tissue engineering.[105-107] PDL cells are cultured on temperature-responsive polymer dishes and hyaluronic acid carriers. When transferred into a low-temperature environment (<32°C), the polymer becomes hydrated, and cells start to detach from culture dishes. This facilitates the harvest and delivery of cell sheets for clinical applications. In several studies in small and large animals, significant cementum formation and anchoring PDL fibers were observed together with new alveolar bone formation after PDL cell sheets were delivered into periodontal osseous defects.[105,108-110] The safety and efficiency of autologous PDL cell sheets in periodontal tissue regeneration is undergoing testing in humans.[94]

PDL progenitor cells have been incorporated in different scaffolds for periodontal tissue-engineering purposes. For example, Sonoyama et al.[111] generated a bioengineered tooth root (bioroot) structure encircled with PDL tissue by loading PDL progenitors with apical papilla stem cells from extracted teeth in a root-shaped HA/β-TCP scaffold. This "bioroot" was further used to support an artificial crown restoration.[112] In another study, Gault et al.[113] harvested and expanded autologous PDL progenitor cells from extracted teeth and then delivered them onto titanium implants in a cell transplantation approach. After this feasibility demonstration in dogs, the researchers tested the concept in humans. Ligamentous

Table 3.
Cells Able to Differentiate Into Periodontal Tissues

Cell Type	Origin	Advantages	Disadvantages	Investigative Status
BMSC (autogenous)	Bone marrow	Relatively easy accessibility, multipotency, no immune rejection, no carcinogenesis	Invasive technique to harvest cells, slow proliferation rate, limited cell source	Clinical trial
PDL progenitor cell	PDL	Multipotency, no immune rejection, no carcinogenesis	Relatively low accessibility, slow proliferation rate, limited cell resource, depends on cell banking and cannot "harvest when needed"	Clinical trial
BMSC (allogenous)	Allogenous bone graft	Relatively easy accessibility, multipotency, no carcinogenesis	Small cell number, immune response, risk of contamination of pathogens from donors, amount and quality of stem cell may vary between donors	Clinical study
Adipose-derived stem cell	Adipose tissue	Easy accessibility, multipotency, no immune rejection, no carcinogenesis	Slow proliferation rate, limited cell source, less potential to osteogenic differentiation	Preclinical (small animal)
iPS cell–derived MSC	Differentiate from iPS cell	Multipotency, no immune rejection, abundant cell source	Possible carcinogenesis, difficulty in cell purification	Preclinical (small animal)
ES cell	Inner cell mass of the blastocyst	Pluripotency, potential resource from abandoned in vitro fertilization embryos, immortal and fast proliferation rate	Immune rejection, potential carcinogenesis, differentiation into unwanted cell types after implantation, relatively rare cell source, migration to distant organs	Preclinical (small animal)
iPS cell	Induced from somatic cells by ectopic gene expression or small molecules	induced from any somatic cells, easy accessibility, pluripotency, abundant cell source, immortal, fast proliferation rate, lack of immune rejection	Differentiation into unwanted cell types after implantation, migration to distant organs, potential carcinogenesis	Preclinical (small animal)

BMSC = bone marrow stromal cell; iPS = induced pluripotent stem.

attachments anchoring bone-to-implant surfaces (consistent with PDL) were formed, and these "ligaplants" were able to support functional loading for 4 to 60 months.[113] Hence, the combination of cell therapy and advanced implantable biomaterials offers another potential avenue for oral tissue-engineering strategies.

ES cells are pluripotent stem cells derived from the inner cell mass of a blastocyst. ES cells have great promise in regenerative medicine because of their capacity to divide for long periods of time and differentiate to all cell types within the organism. Yang et al.[114] studied the effect of ES cell transplantation in the treatment of periodontal furcation defects in a porcine model. Three months after the delivery of GFP-labeled ES cells, significantly better clinical parameters were seen compared with control sites without cell therapy, and no obvious evidence of rejection or teratoma was found. However, GFP-expressing cells were detected in the repaired cementum at the control site, as well as in remote organs, including lung, urinary bladder, colon, and liver, suggesting the migration of ES cells to remote tissues through blood vessels, especially to tissues with a high turnover rate.

Induced pluripotent stem (iPS) cells are a population of stem cells generated from somatic cells through the forced expression of specific genes. These cells are highly similar to ES cells in many aspects, including their proliferation and differentiation capability, which suggests that iPS cells could be a more easily accessible source of pluripotent stem cells for clinical application. Duan et al.[115] reported that implantation of iPS cells combined with EMD in

Table 4.
Clinical Studies Using Cell Therapy for Periodontal Regeneration as of April 18, 2014 (all with unknown defect types)

Study	n	Study Type	Status	Cell Source	Cell Expansion in Laboratory	Delivery Carrier	Outcome (improvement; mean ± SD)
Yamada et al.[92]	17	Case report	Completed	Autologous BMSC from iliac crest	Yes	PRP, thrombin/10% calcium chloride	5.12 ± 2.45 mm PD loss; 4.29 ± 1.32 mm CAL gain; 3.12 ± 1.23 mm bone gain (radiographic)
Feng et al.[93]	3	Case report	Completed	Autologous PDL cells from third molars	Yes	Synthetic bone grafting material	No adverse effects during 32 to 72 months of follow-up; possible therapeutic benefit for periodontal defects but data from only one patient
Chen et al.;[94] clinicaltrials.gov identifier NCT01357785	35	RCT	Recruiting	Autologous PDL cells from third molars	Yes: cell sheet technique	N/A	N/A

N/A = not applicable.

a mouse fenestration model promoted periodontal regeneration. To overcome the safety issue and other disadvantages of ES and iPS cells, Hynes et al.[116] recently induced iPS cells into MSCs and then delivered them into mouse periodontal fenestration defects. The magnitude of regeneration and newly formed mineralized tissue increased significantly.

Advances have also been made in the application of cell therapy to periodontal soft tissue problems. A bilayer tissue-engineered cell sheet (allogeneic cultured keratinocytes and foreskin fibroblasts) has been developed to serve as a reservoir for regenerative molecules, including cytokines and growth factors, to stimulate wound healing.[117] During early wound-healing events, expression of angiogenic-related biomarkers, such as angiostatin, PDGF-BB, VEGF, FGF-2, and interleukin-8, is upregulated in sites treated with tissue-engineering cell sheets.[118] In a multicenter and within-patient RCT, this living cellular construct resulted in a gain of >2 mm keratinized gingiva in 95.3% of patients, with a mean of 3.2 ± 1.1 mm.[119] Gingiva regenerated with cell therapy matched the color and texture of the adjacent gingiva.[119,120] Cell therapy has also been tested for papillae augmentation. After a long-term clinical study (mean 55.3 ± 17.7 months), Yamada et al.[121] reported that injectable MSCs delivered in a hyaluronic acid scaffold mixed with PRP resulted in a mean improved interproximal "black triangle" value of 2.55 ± 0.89 mm. Thus, it can be argued that cell therapy also may provide alternative options to periodontal soft tissue regeneration.

Based on information from preclinical studies and exploratory clinical trials that examine feasibility, cell therapy holds great promise in periodontal regeneration. Nevertheless, several key questions will need to be addressed. First of all, safety is a critical concern, especially for cells with carcinogenic potential, such as ES cells. Autogenic cell therapy based on adult MSCs appears to have a better safety profile; however, issues related to culture systems, such as the use of xenogenic serum proteins, should be addressed. Second, the cell delivery system has to be improved, because current delivery methods usually lead to low cell viability or dispersal of cells away from target sites and therefore have limited clinical utility.[122] Third, criteria are needed to assess and predict the outcome of cell therapy.[123] For example, how many cells are needed for certain defects? What is the relationship between the clinical outcome and the quantity of growth factors/cytokines the delivered cells produce? How do cell purity and heterogeneity affect the clinical outcome? Fourth, it has been shown that MSCs from different sites, such as the mandible and the tibia, are not identical,[124] and PDL cells and dental follicle cells behave differently in periodontal

wound healing.[125,126] Challenges remain in searching for the most suitable cell resource for periodontal regeneration.

In conclusion, cell-based therapies have great potential in periodontal regeneration. Nonetheless, more studies are necessary to evaluate the regenerative capacity of cells from different tissues and to demonstrate sufficient product safety for human application.

Gene/RNA Therapy

Gene therapy is defined as transferring genetic materials to patients' own cells to produce therapeutic agents for disease treatment.[127,128] The first successful treatment of human disease by gene therapy was reported in 2000 in patients with X-linked severe combined immunodeficiency.[129,130] Since then, gene therapy has emerged as a realistic therapeutic technology with >1,800 gene therapy clinical trials worldwide currently being completed, ongoing, or approved for initiation.[131]

In regenerative medicine, gene therapy has several advantages over other treatments, including greater sustainability, relatively low cost, and overcoming the manufacturing difficulties of protein expression, modification, and purification. Moreover, a broader array of candidate target genes exist, including secreted growth factors, intracellular transcription factors and regulators, and regulatory RNAs. Techniques developed to carry candidate genes into cells fall into one of two general categories: 1) viral vectors and 2) non-viral vectors.[9] Examples of viral vectors are adenovirus (Ad), adeno-associated virus, retrovirus/lentivirus, and herpesvirus.[132] Viral vectors that carry candidate genes will eventually insert into the intracellular fluid (cytosol). First, they attach to receptors on the cell membrane, then pass through the nuclear membrane, and eventually release DNA. The exogenous DNA is transcripted into messenger RNA (mRNA) in the cell nucleus and subsequently delivered into the cytosol for protein production. Gene delivery by viral vectors usually results in longer gene expression, ranging from days to weeks, and even years. Larger exogenous transgenes can also be delivered by viral vectors, although they are usually associated with stronger host immune reactions.[133] Non-viral vectors, such as lipid-based particles/nanoparticles, calcium phosphate nanoparticles, and ultrasound, have been used to transfer plasmid, modified mRNA, and small interfering RNA into cells.[128] A major hurdle for the clinical use of non-viral vectors in gene therapy is a low transduction efficiency.[127]

Gene therapy can be a viable treatment for periodontal regeneration, too. Initial studies show that Ad–PDGF can efficiently transduce cells derived from the periodontium—osteoblasts, PDL fibroblasts, gingival fibroblasts, and cementoblasts—and prolong PDGF signaling and enhance mitogenesis.[134,135] Using in vivo optical imaging, Chang et al.[136] reported sustained and localized gene expression in periodontal lesions for as long as 21 to 35 days after direct gene delivery by an adenoviral vector. It was also shown that Ad–PDGF-B treatment stimulated tissue regeneration in large periodontal defects, with a four-fold increase in bridging bone and six-fold increase in cementum repair.[137,138] Additionally, regenerative effects of Ad–PDGF-B treatment were seen in peri-implant alveolar bone defects.[139] It has also been reported that gene delivery of PDGF-B stimulated potent increases in cell repopulation and defect fill in an ex vivo gingival repair model.[140] Therefore, gene transfer would appear to have potential applications for periodontal soft tissue engineering as well.

Other gene candidates have been investigated in periodontal regeneration. Direct or ex vivo gene delivery of BMPs regenerates not only significant quantities of bone[141] but also cementum, complete with Sharpey fiber insertion, and hence re-establishes the normal elements of the periodontal apparatus.[142,143] The so-called *Wnt* signaling pathways play an important role in skeletal development, homeostasis, and tooth morphogenesis.[144] Using an ex vivo approach, Chang et al.[145] demonstrated that *Wnt-4* gene transduction promotes alveolar bone wound healing in a rat model. LIM domain mineralization protein (LMP) is an intracellular protein that is highly upregulated in the early stages of osteoblast differentiation.[146] Recently, Lin et al.[147] reported that LMP is a positive regulator of PDL cells in osteogenesis. Overexpression of *LMP-3* in PDL cells by adenoviral vector significantly induced osteolineage differentiation in vitro.[148] Furthermore, combinatory gene delivery of *LMP-3* and *BMP-7* synergistically promoted ectopic bone formation in vivo.[148] Nevertheless, more studies are required to demonstrate the value of LMP gene delivery for periodontal regeneration.

An acceptable safety profile has been reported after localized gene delivery via a collagen matrix in a rodent periodontal fenestration defect model.[136] The Ad–PDGF-B transgene was well contained in a localized defect area without viremia or distant organ involvement. Although minor alterations in specific hematologic and blood chemistry were seen, most measures were within normal limits. In the future, studies in large animals are needed to further evaluate the safety and efficacy of gene therapy for periodontal regeneration.

The use of non-viral vectors in periodontal regeneration has been explored, and attempts have been made to increase their transduction efficiency. Elangovan et al.[149] used nano-sized calcium

phosphate particles to deliver PDGF plasmid into fibroblasts with a high level of biocompatibility. Sugano et al.[150] developed "bubble liposomes" as a useful carrier for gene delivery, and its efficiency in vitro and in vivo was shown to increase with simultaneous application of high-frequency ultrasound.[151] Recently, many researchers have concentrated their efforts on RNA-based gene delivery, which only needs to reach the cytoplasm to function and, consequently, should have a better safety profile because of the lack of ability to integrate into the host genome. It has been demonstrated that gene delivery of modified VEGF mRNA regulates heart progenitor cell differentiation and induces vascular regeneration after myocardial infarction in a mouse model.[152,153] This is promising for future use of RNA-based therapy in regenerative medicine and periodontal regeneration for transient expression of therapeutic molecules.

Gene therapy provides great opportunities to deliver a wide range of candidate genes to enhance periodontal regeneration, which is a complex biologic process in which many genes are involved. Challenges in translating this technique into clinical application lie in developing appropriate gene delivery vectors that can achieve controlled expression patterns with reasonable expression levels. At the same time, a sufficiently reliable safety profile, such as reduced immune responses and minimal insertional oncogenesis, should be provided. Although RNA-based therapy has great potential, extending the lifespan of the delivered RNA to achieve longer-lasting therapeutic effects is a challenge for the future.

Scaffolds

In general, scaffolds are used to provide and maintain the space necessary for the cells to grow and physically support the healing process. In the past two decades, scaffold matrices have been investigated extensively in periodontal regeneration as grafting materials. Advances in science and technology have propelled important innovative research, focused on the optimization of physicochemical and mechanical properties of novel scaffolds, to overcome common structural and biologic limitations that have hindered the predictability of periodontal regenerative therapy. Several fundamental properties for a successful scaffold have been proposed: 1) providing a 3D architecture that supports the desired volume, shape, and mechanical strength; 2) proper physical characteristics, such as hydrophilicity and porosity, which facilitate tissue infiltration; 3) biocompatibility; and 4) controlled degradation rate in a pattern that matches tissue regrowth. Well-designed scaffolds can also serve as delivery platforms to enhance the regenerative potential of the host.[154,155]

Extensive studies were conducted to apply scaffolds as infrastructures for tissue engineering. Scaffolds can be combined with cell- or gene-based approaches to serve as supportive carriers that conduct a sustained release of bioactive factors, thereby inducing stimuli for tissue formation.[156] Bioactive molecules, such as growth factors, may also be encapsulated into nanoparticles and microparticles to aid in their sustained release from scaffolds.[157] 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.[9,158] Moreover, the feasibility to establish a 3D polarity in scaffolding design constitutes an important advance to create biomimetic scaffold surfaces that can be applied for gene- and cell-therapy strategies.[159] Several other scaffold fabrication technologies have been used, including conventional prefabricated scaffolds, such as particulate, solid-form, and injectable scaffolds. Whatever the form of the scaffold, its purpose is to influence the environment in which it is implanted to promote a better outcome.[160,161]

Conventional scaffolds are usually prefabricated from both natural and synthetic polymeric materials. Naturally derived scaffolds include autografts, allografts, and xenografts. Other naturally derived scaffolds are ceramics, most commonly used in bone regeneration and implant therapy.[162] Alloplasts and other polymers are synthetically engineered materials consisting of bioactive molecules serving a purpose similar to that of natural scaffolds.

Biphasic calcium phosphate (BCP) has emerged as a promising graft material in periodontal regeneration because its degradation rate can be tuned by adjusting the ratio of fully synthetic HA and β-TCP. Studies showed that BCP is an effective bone replacement substitute in sinus augmentation and alveolar bone defect reconstruction. It has also been shown that BCP combined with EMD leads to clinical improvements in periodontal bony defects.[163]

Most of the biomaterials of natural origin in current use are based on the cross-linking or self-assembly properties. These materials have an innate ability to interact with and mediate degradation by cells[9] and can form hydrophilic polymers with >90% water. In this category, there are materials such as collagen, chitosan, dextran, alginate, aloe vera, or fibrin. Recently, some interesting studies have been published in the field of periodontal engineering using these materials. A novel porcine acellular dermal matrix maintaining the 3D collagen framework was tested both in vitro and in vivo. Together with HA, the construct showed an appropriate biodegradation pattern and favorable tissue compatibility.[164] Similarly, another new collagen-based 3D scaffold made of collagen

hydrogel, cross-linked to the ascorbate–copper ion system and injected into a collagen sponge, had good biocompatibility and biodegradability after 2 weeks of implantation in Class II furcation defects (of 5-mm depth and 3-mm width) created in beagle dogs. Reconstruction of alveolar bone, cementum, and PDL was observed frequently only 4 weeks after surgery.[165]

Biodegradable synthetic polymers have a long history in medicine starting in 1969 with FDA approval of polyglycolic acid sutures.[166] Compared with naturally derived biopolymers, synthetic polymers have drawn much attention because they can be fabricated in a variety of microstructures and provide greater freedom in the ability to control degradation time. Many products for regenerative purposes were developed in the past, including a polylactic–coglycolic acid-based bone filler and polyethylene glycol-based cartilage repair material.[167] Modification of nanoscale biopolymers has been shown to affect cell behavior as well. Synthetic nanofibers that mimic the fibrillar structure of collagen exhibit properties similar to natural collagen fibers and enhance osteoblast differentiation compared with scaffold with solid-walled architectures.[168] Recently, nanofibrous hollow microspheres, integrating the ECM-mimicking architecture in a highly porous injectable form, were designed as cell carriers for cartilage regeneration and exhibited superior outcome versus cell therapy alone.[156,169] Therefore, biomimetic scaffolds with 3D macrostructures and nanostructures will provide a suitable environment for cellular activity and tissue regeneration. These systems can also be adapted well for periodontal regenerative therapy.

Imaging-based, computer-aided design is a more recent development in scaffold fabrication techniques, providing a personalized solution for tissue engineering.[170] In this technique, the 3D anatomic geometry of a defect can be acquired by high-resolution computed tomography or magnetic resonance imaging data, which can function as a template for a scaffold on a global anatomic level. The scaffold is fabricated with desired biomaterials by 3D printing that, in turn, will precisely match the spatial dimensions of the defect area. Because of the complexity of the periodontal apparatus, application of this technique requires a heterogeneous internal structure design in the scaffold to create region-specific variations in porous microstructure and scaffold surface topography. This, in turn, helps regulate the fate of ingrowing cells in a spatial-specific manner. Park et al.[171] manufactured biomimetic fiber-guiding scaffolds that custom fit complex periodontal osseous defects to guide functionally oriented ligamentous fibers in vivo. Predictably, oriented fiber architecture, greater control of tissue infiltration, and better organization of ligament interface were seen in scaffolds with guidance channels compared with random scaffold architectures. These findings demonstrate that high-resolution imaging, computer-aided design, biomaterial 3D printing, and fiber-guiding channel design together can facilitate the creation of customized implantable devices for regeneration of the tooth-supporting structures in the periodontium.

Additionally, there are a few commercially available biodegradable dermal allograft materials that mimic the ECM and function as scaffolds that are used for gingival regeneration aimed at root coverage and keratinized tissue augmentation.[172-175]

Undoubtedly, tremendously exciting advances in the development of scaffolds for periodontal regeneration were seen in the past decades. In the future, scaffolds that provide improved, controllable biodegradable profile and biomechanical parameters will be developed. These scaffolds should also satisfy the needs for minimally invasive surgery and individualized periodontal regenerative approaches.

Lasers

The term "laser" stems from the acronym LASER that stands for "light amplification by stimulated emission of radiation" but is now a commonly used noun. Laser therapy has received considerable attention for more than two decades because of its purported advantages, such as ease of soft tissue ablation, bactericidal effect, and increased hemostasis.[176] At the cellular level, it has been reported that low-power laser irradiation stimulates cell proliferation, migration, and differentiation.[177-180] However, there is great heterogeneity among studies in their designs and results reported in the existing literature, regardless of whether a laser is used as a monotherapy or as an adjunct to scaling and root planing (SRP). Consequently, several recent reviews have concluded that there is insufficient evidence to support the commonly held belief that lasers offer an enhanced clinical outcome when compared with SRP alone for up to 24 months after treatment.[181-184] Even when comparing laser-mediated surgery with traditional surgery, such as OFD and other debridement procedures, lasers appear to offer no additional benefits.[176]

Two relatively recent proof-of-principle human histologic studies[185,186] using the neodymium: yttrium-aluminum-garnet (Nd:YAG) laser, a short-wavelength laser, in a specific minimally invasive protocol,§ reported a potential regenerative effect of laser therapy. In this protocol, a free-running pulsed Nd:YAG laser is used to remove the pocket epithelium. After debridement, periodontal pockets are lased a second time, which purportedly seals the pocket as a result of blood clot stabilization. Yukna

§ Laser-Assisted New Attachment Procedure (LANAP), Millennium Dental Technologies, Cerritos, CA.

et al.[185] reported that new cementum, functional CT attachment, and bone formation were seen 3 months after laser treatment of intrabony pockets. In contrast, control defects treated only by SRP exhibited periodontal repair with long junctional epithelium. Nevins et al.[186] reported results of laser therapy on 10 teeth from eight patients. Histologic evidence of varying degrees of periodontal regeneration was noted in five of the teeth, i.e., formation of new cementum, PDL, and alveolar bone. Because of the limited number of participants in the two studies,[185,186] additional evaluation of the potential of laser therapy in the area of periodontal regeneration must include well-designed, masked, multicenter, RCTs.

CONCLUSIONS

Several different approaches and biologic agents for regenerating the compromised periodontium are in development and under study with varying degrees of clinical applications. The major challenge that remains is to establish control of the exact sequence of events required for cell recruitment, differentiation, and maturation to effectively promote healing and regeneration without compromising normal cell function. Therefore, new materials and signaling molecules delivered by gene therapy are of great interest. More evidence and practice standardization are needed to successfully obtain the required regulatory requirements to apply these technologies to the clinical scenario. Differences between chronic periodontal pathology and other defects, such as implant sites and extraction sockets, must be taken into consideration because their regenerative processes are different. Therefore, the application of periodontal engineering also requires a detailed understanding of the homeostasis and pathogenesis of these defects.

Identification of genetic susceptibility variants and their role in disease onset and progression is fundamental to identify novel determinants of periodontal stability. Currently, periodontal diagnosis is based on the clinical presentation of the disease. The current classification guides identification of "different" forms of the disease that manifest themselves with a common clinical presentation and clusters them within groups (i.e., chronic, aggressive, necrotizing, etc.). Therefore, it is a responsibility to acknowledge the complexity and heterogeneity of this group of conditions. The lack of a biology-based classification system prevents the establishment of more homogeneous diagnostic categories and more predictable treatment outcomes.[187] A molecularly based model for periodontal disease pathogenesis would provide an important insight that could assist in tailoring treatment to enhance regenerative outcomes while providing more predictable and individualized patient care.

Today, periodontal regeneration based on tissue-engineering approaches has a solid evidence base for clinical application in human periodontal defects. Although the cell-based, scaffold, and gene therapies interface and complement each other, some are still at the preclinical level. In the near future, the outcomes of periodontal regeneration will undoubtedly be enhanced by the ability to correctly identify clinical situations in which these techniques can be successfully applied with predictable results.

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