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Regenerative Periodontal Therapy

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Abstract

Traditional treatment for loss of bone and attachment due to periodontal disease has focused around repairing the damage induced. However, over the past few decades, clinicians have begun to utilize regenerative techniques to rebuild bone, cementum and the periodontal ligament. Conventional procedures most often involve the use of barrier membranes with bone grafts that foster selective cell repopulation and regrowth of osseous structures. Since the predictability of these techniques may be limited to certain case types, pharmacologically based efforts are underway to investigate the possibility of harnessing osseous regrowth potential. Clinical research has found that proteins are potent biological mediators that promote many of the events in wound healing, and have been shown to promote bone formation in human clinical studies.

Conventional periodontal therapy, including surgical and non-surgical approaches, may result in tissue shrinkage after healing [1]. In advanced defects, bone contouring may be applied during the surgical procedures in order to create a maintainable environment. This bone contouring approach may also create esthetic concerns, especially in the anterior area. However, treatments without performing bone contouring may result in residual pockets and create an inaccessible environment for proper cleaning procedures [2]. In addition, the risks associated with the healing process of conventional periodontal treatment can result in the growth of a long junctional epithelium attachment; this secondary structure may be a disadvantage due to a tendency to form new pockets [3, 4].

At the micro-structural level, the epithelium and its surrounding connective tissue often matures into various non-functional types of scar tissue after conventional periodontal therapy; this result is considered as a 'repair' part of the healing process [5]. These adverse effects of conventional periodontal therapy can be avoided or reduced by applying regenerative procedures to restore the loss of periodontal tissue [6]. The regenerative treatment procedures are designed to restore lost parts of the tooth-supporting structures. Several studies have demonstrated that regenerative

periodontal procedures give more favorable results compared to conventional periodontal therapies [7–9].

In comparison to simple repair, periodontal regeneration is an ideal healing process that occurs through the reconstruction of new periodontal attachment apparatus, which involves the formation of new alveolar bone, a functionally aligned periodontal ligament (PDL) and new cementum [10]. Several studies, including meta-analysis results, have shown that periodontal regeneration procedures tend to show more clinical improvements than conventional procedures [11–15].

Regenerative periodontal procedures require biomaterials that lead to regeneration of the PDL, new attachments, new cementum and new alveolar bone. An ideal tissue engineering material should deliver the regenerative signals in an optimal manner to stimulate a cellular response. Initial efforts to achieve guided tissue regeneration (GTR) have aimed to regenerate attachment apparatus by isolating the defect with the barrier membranes and/or had grafting to maintain space so that regeneration could have enough time to take place. GTR is based on the exclusion of connective tissue and epithelium in favor of PDL regeneration, following the establishment of new attachments. Thus, GTR is the purposeful selection of cell types that repopulate at the wound with the intention of directing the healing tissue composition. Recently, the stimulation of periodontal regeneration with growth factors has become an effective and predictable technique. Based on the biological enhancement of wound healing, these molecules produce a true histological regeneration. Growth/regeneration factors and differentiation factors – such as enamel matrix proteins (amelogenin), growth factors (like platelet-derived growth factor; PDGF), as well as polypeptide mitogens (like bone morphogenic protein-2; BMP-2) – are being used to stimulate regeneration. These molecules augment and/or stimulate the natural healing response, and include stimulatory effects on angiogenesis, cellular differentiation, cellular proliferation and extracellular matrix biosynthesis, respectively. The stimulation by signaling molecules (growth factors) has improved predictability.

Periodontal Regenerative Techniques

Periodontal therapy has evolved from mainly resective concepts to regenerative treatments. The advantage of a regenerative approach not only includes a gain in clinical attachment, but the reduction or elimination of local factors. These include minimizing anatomical factors – such as root concavities, deep interproximal defects or furcations – where periodontal breakdown can easily progress. In addition, esthetic enhancements can be achieved, especially in the anterior regions.

Guided Tissue Regeneration

GTR includes multiple components, such as new attachment, reattachment, regeneration and repair. The term was first used when a new connective tissue attachment

was demonstrated with human histology utilizing an expanded polytetrafluoroethylene (ePTFE) membrane (non-resorbable) [16]. 'New attachment' is the union of connective tissue or epithelium with the root surface which has been deprived of its original attachment. 'Reattachment' is the reunion of epithelium or connective tissue with root surfaces and bone, such as occurs after incision or injury. 'Repair' is the healing of a wound with periodontium that has not been fully restored to its original form and function. Lastly, 'true regeneration' is the histological term that describes the reconstruction of bone, cementum and the PDL.

The occlusive-based regeneration of the periodontium is primarily dependent on epithelial exclusion. Exclusion of epithelium is essential in barrier membrane regeneration. Barrier materials include natural absorbable polymers, such as collagen (types I, II, III, IV), collagen and GAG copolymer, synthetic absorbable polymers (such as polylactic acid and polyglycolic acid), fibrin, non-resorbable polymer PTFE and titanium-reinforced ePTFE. Generally, cementum deposition and PDL formation is slower than the downgrowth migration of epithelium. This natural process limits or prohibits regeneration and results in repair or a long junctional epithelium. In a novel investigation, Nyman et al. [6] demonstrated that the placement of a Millipore filter excluded the epithelium from the healing site. This exclusion with the addition of space creation/maintenance is essential in effective regeneration. Once epithelium exclusion is accomplished, wound healing originates from the PDL [17]. The peak mitotic activity of the PDL occurs at approximately 3 weeks [18]. The importance of the PDL cell response in GTR is also important to cementum formation. With cementum formation, the PDL fibers are also able to insert and attach. Therefore, cementum formation can be viewed as the rate-limiting step in periodontal regeneration [19].

The clinical outcome of GTR regeneration is dependent on the periodontal defect morphology, remaining periodontium and practitioner skill. Membrane GTR is a challenging procedure. The success of the procedure often is related to the premature exposure of the membrane to the oral cavity. Soft tissue management generally includes concepts that maximize volume, such as intrasulcular and releasing incisions. Following flap reflection, the surgical site must be fully debrided and the root surfaces prepared. The next surgical step is trimming and adapting the membrane. Osseous architecture may require conservative osteoplasty to accommodate a passively fitting membrane. For closure of the site, suture material such as ePTFE or polyglycolic acid should be chosen since these retain the least amount of plaque. Lastly, a periodontal dressing may be used to secure the surgical area, especially from food impaction or trauma. Postoperative care is important and patients are typically prescribed an antibiotic, anti-inflammatory and antibacterial rinse during the initial healing period.

Developmental clinical studies have demonstrated that GTR is a predictable procedure. Predictability in these studies was related to the severity and position of the furcation. Class II furcations and maxillary buccal furcations are more predictable

[20–22]. When graft material is added for space maintenance, a GTR and demineralized freeze-dried bone allograft combination produced more defect filling, pocket depth reduction and greater levels of clinical attachment [23]. Depending on the number of bone walls in the defect, GTR showed 95% filling of 3-wall defects, 82% filling of 2-wall defects, and 39% of 1-wall defects [24]. In addition, sites with the most disease showed the greatest benefit [25]. Long-term stability of periodontal regeneration has been evaluated, and the results showed that if the patient is under a professional supportive periodontal maintenance program and maintains good oral hygiene, the newly regenerated tissue can be maintained in the long term [2, 7, 26–30].

Signaling Molecules in Periodontal Tissue Regeneration

There has been mounting evidence that the material utilized as graft material may play a significant role in altering wound healing, from repair to regeneration. Analyses of autografts and allografts have found that signaling molecules contained in the material may be able to stimulate regeneration. The molecular basis of these factors has been extensively identified and characterized. As a result, several signaling molecules are currently being investigated in preclinical and clinical studies.

Enamel Matrix Proteins

Clinical investigations support periodontal regeneration with enamel matrix proteins or their derivative, enamel matrix derivative (EMD). The main constituent of this product is the amelogenin protein. Commercially available EMD is isolated from porcine tooth buds [31]. These porcine-derived proteins are very similar to proteins expressed during human enamelogenesis [32, 33]. Amelogenin has been shown to have a positive effect on acellular cementum, the PDL and alveolar bone development. The biological response of PDL cells to amelogenin in vitro demonstrates that RNA expression is stimulated, cellular functions are improved and metabolism is increased [34, 35]. In addition, enamel proteins have been shown to facilitate new blood vessel formation. EMD also has an effect on osseous formation/resorption. Osteoclastic activity and resorption are limited by EMD treatment, whereas osteoblastic activity is upregulated. Moreover, important osteoblastic activity markers were also enhanced at 2 and 3 weeks as an indication of continued biological activity [36]. A preclinical mouse model demonstrated new cellular cementum-like tissue formed along enamel-matrix-protein-treated root slices [37]. EMD treatment of human gingival fibroblasts has found a reduction in cell death from TNF and produces a tissue inhibitor of MMP-2 [38, 39].

The clinical application of EMD was compared to traditional GTR in lower molar class II buccal furcation defects. Results indicated an equivalent reduction in horizontal probing at 14 months after surgery [40]. Similar improvements were found in a comparison of contralateral randomized infrabony defects using EMD versus

GTR with a resorbable membrane. These improvements in periodontal parameters were maintained for up to 8 years [41]. Case reports have demonstrated significant periodontal regeneration with EMD [42]. One such report describes the treatment of a localized bone defect associated with a palatal groove [43]. In a 1-year follow up, the site maintained an 8-mm clinical attachment gain and a 2-mm residual probing depth. Esposito et al. [44] reported in a Cochrane systematic review of intrabony defects that EMD significantly improved attachment levels and reduced probing pocket depths when compared to placebo or controls. The authors also indicated that there was great heterogeneity in the trails, suggesting that the significant difference found should be interpreted with caution.

Platelet-Derived Growth Factor

PDGF, formed by the combination of two amino acid chains, are dimeric structural protein ligands of about 30,000 kDa in molecular weight. The nomenclature for their characterization uses an alphabetical suffix (A–D), based on the amino acid homology. The 3D structure of the protein leads to the formation of either homo- or heterodimers (PDGF-AA, AB, BB, CC, DD) [45]. These dimer molecules have two receptor-binding epitopes that function as active sites to induce the proliferation of preosteoblastic cells, chemotaxis and matrix apposition [46]. The A and B chains of PDGF, which have been more extensively studied, are both synthesized as precursor molecules that undergo proteolytic processing. These attach only to cells that have receptors to accommodate them. The PDGF receptor, based on the most recent literature reviews, has two general types: a and b receptors [47]. PDGF was originally isolated because of its ability to promote proliferation of mesenchymal cells [48]. It has since been recognized as initiating additional cellular responses, such as cell migration and survival [49].

Periodontal Utility of Platelet-Derived Growth Factor

The potential of recombinant human PDGF (rhPDGF) in tissue engineering has probably made it one of the most thoroughly studied growth factors in periodontics [50]. Originally PDGF and insulin-like growth factor I (IGF-I) were found in combination to synergistically upregulate DNA and protein synthesis in osteoblasts, and have demonstrated mitogenic and chemotactic effects on the PDL and alveolar bone cells [51–56]. Histological results have shown that PDGF/IGF-1 treated sites showed the formation of new attachment apparatus, while the control carrier gel group indicated the presence of long junctional epithelium without any clear regeneration [57]. In another study, rhPDGF-BB was used alone (without IGF), with bone allograft as the carrier, in patient teeth with interproximal intrabony defects and molar class II furcation defects and poor-to-hopeless prognosis requiring extraction. A notch was placed at the base of the defect on the root surfaces, osseous bony defects were then

filled with demineralized freeze-dried bone allograft with 1 of 3 concentrations of rhPDGF-BB (0.5, 1.0 or 5.0 mg/ml). The hopeless teeth and surrounding tissue were taken en block after 9 months. Histomorphometric analyses were carried out in reference to the notch placed on the root surface. In the rhPDGF/allograft-treated defects, the vertical probing depth reductions as well as clinical attachment level gain for interproximal defects were approximately 6 mm, while radiographic bone fill was approximately 2 mm. Furcation defects treated with the rhPDGF/allograft demonstrated horizontal and vertical pocket depth reductions with a gain in clinical attachment levels of approximately 3 mm. The histological evaluation also indicated the regeneration of a complete new periodontal attachment apparatus. This study demonstrated that rhPDGF-BB alone can stimulate periodontal regeneration in both class II furcations and interproximal intrabony defects [51].

A prospective randomized clinical study to determine the safety and effectiveness of rhPDGF-BB was completed. rhPDGF-BB and β-tricalcium phosphate (β-TCP) were utilized for the treatment of advanced periodontal osseous defects and compared to the control. This multicenter study examined patients who required surgical treatment for a ≥4-mm intrabony periodontal defect; they were evaluated after 6 months. Experimental groups received β-TCP and either 0.3 or 1.0 mg/ml rhPDGF-BB, while the control group was given β-TCP alone. The clinical parameters used to assess PDGF-BB-induced effects included clinical attachment levels, gingival recession, radiographic linear bone growth and percent bone fill. Clinical attachment level gain was 3.8 mm for 0.3 mg/ml rhPDGF and significantly higher compared to β-TCP alone at 3 months following the surgical procedures, but at 6 months this difference was not statistically significant. The 6-month radiographic analysis showed linear bone gain of 2.6 mm and percent defect fill of 57% in the rhPDGF-BB-treated group (0.3 mg/ml), compared to 0.9 mm and 18%, respectively, in the control group [58]. The rhPDGF-BB-treated patients also demonstrated increased radiographic defect fill at 18–24 months after surgery compared to 6 months after surgery [59]. In summary, this multicenter study demonstrated the safety of rhPDGF-BB in the clinical treatment of periodontal defects.

The pharmacokinetics for growth factors used in periodontal regeneration have been optimized. The total load dose is dependent on protein stability, half-life and carrier properties. Gene therapy evaluation of PDGF has indicated that sustained release may amplify the intensity and duration of regeneration. In a small animal model, several different gene combinations for PDGF-A and B were utilized in critical size defects. Histomorphometric measurement found that PDGF-B stimulated cell proliferation compared to PDGF-A or control. Bone regeneration was greater for PDGF-B specimens compared to the other test and control groups. Gene expression was active in the periodontal defect for up to 3 weeks [60, 61]. PDGF-A was found to have a minimal effect on periodontal regeneration. Using similar gene therapy methods, cementoblasts were altered using PDGF-A gene application. These cells exhibited proliferation similar to continuous rhPDGF-AA protein application on native

cementoblasts [62]. Gingival fibroblasts in 3D collagen lattices were evaluated for the effects of PDGF-A and PDGF-B gene transfer. Findings of this study indicated that cell repopulation and defect fill was stimulated by more than four times for the PDGF-B-gene-applied gingival fibroblasts, while PDGF-A and controls exhibited similarly diminished cellular activity [63].

Bone Morphogenetic Protein

Molecular regenerative therapy has also included the application of recombinant human BMP-2 (rhBMP-2). BMPs have been shown to be mediators of several processes in embryogenesis. The majority of BMPs are members of the transforming growth factor family. BMPs can function singly or together with other BMPs or cytokines. BMP-2 and 7 have been demonstrated to produce bone and periodontal regeneration. rhBMP-2 has become a standard in clinical practice for the regeneration of bone. Extensive preclinical investigations have provided a basis for this human indication. Goldhaber [64] found that a diffusible substance induced bone formation across a membrane. Urist et al. [65] implanted this substance in the soft tissue of a rodent and bone was formed in a non-osseous site. Later, BMP/TCP carrier was surgically implanted in critical size defects in dogs. rhBMP-2 stimulated sites demonstrated more than 90% new bone; the control group demonstrated new bone formation below 10% [65]. More recently, the human clinical application of rhBMP-2 has focused on extraction socket preservation and sinus augmentations. A randomized masked placebo-controlled multicenter clinical study demonstrated that the novel combination of rhBMP-2 and a commonly utilized absorbable collagen sponge (ACS) had a striking effect on de novo osseous formation for the placement of dental implants [66]. In addition, the efficacy of different rhBMP-2 concentrations in the anterior maxilla for bone regeneration was assessed with radiographic evaluation. CT evaluations found a significant difference in bone formation between subjects treated with a concentration of 1.5 mg/ml rhBMP-2 and the other groups [67]. In the lateral sinus augmentation procedure, 1.50 mg/ml rhBMP-2/ACS was compared to a standard autograft. Sinuses with an alveolar ridge height <6 mm were included in the study. A total of 160 patients were enrolled in the study. The results indicated that the mean changes in bone height were similar; however, the rhBMP-2/ACS-induced bone was significantly denser on histological evaluation [68].

The rhBMP-2 regeneration of the periodontal apparatus has been extensively investigated in preclinical models; however, no human investigations have been published. rhBMP-2 periodontal regeneration in rodents was evaluated for early wound healing at 10 and 38 days. New bone formation was significantly increased 10 days after rhBMP-2 application on experimental periodontal wounds, as confirmed with histological sections. These exhibited new bone, cementum and collagen fiber formation. Importantly, more cementum growth coronally was detected with

rhBMP-2 application. Complete healing without any evidence of ankylosis occurred in all 38-day-treated sites [69]. Wikesjo et al. [70] found that in a canine model 0.02 mg/ml rhBMP-2 fostered periodontal regeneration. Bone height was significantly increased with the treatment as compared to the control (3.8 vs. 0.7 mm). However, there was limited cementum regeneration and ankylosis was noted in the rhBMP-2-treated sites. In a recent study, rhBMP-2/ACS was utilized in the treatment of 3-wall intrabony defects in the dog model. Surgical implantation of the rhBMP-2/ACS resulted in enhanced bone formation, but no improvement in cementum regeneration. As a result, there was minimal functional periodontal formation. The application of BMP gene vectors for bone and periodontal regeneration has been documented. Betz et al. [71] documented that a dose-dependent rhBMP-2 vector (adenovirus) for critical-size defects was on the femora of rats. The evaluations with radiographs and histology indicated that all of the defects treated with a high dose were bridged with bone, whereas in the medium and low dose groups, this occurred significantly less.

In conclusion, the goal of these various treatment modalities is to regenerate periodontal tissues lost secondary to the disease. Current regenerative periodontal therapies utilize the surgical placement of biomaterials and cytokines. The transitions from barrier membranes to growth-factor-mediated regeneration enhance predictably, are easy to use, and reduce complications.

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