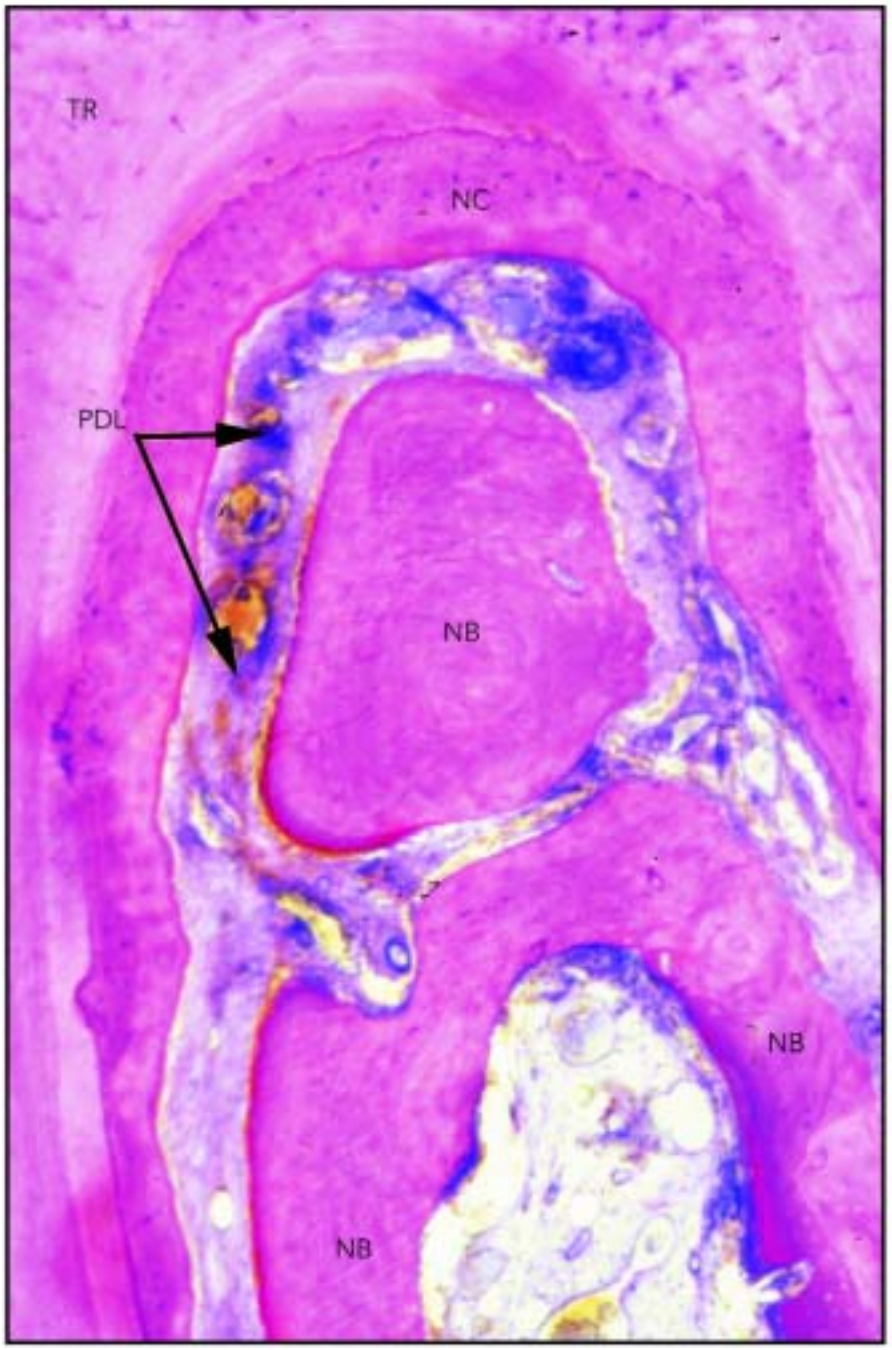


Periodontal Regeneration in Human Class II Furcations Using Purified Recombinant Human Platelet-Derived Growth Factor-BB (rhPDGF-BB) with Bone Allograft

Marcelo Camelo/Marc L. Nevins, DMD, MMSc**/*
*Robert K. Schenk, MD, Prof Dr Med***/Samuel E. Lynch, DMD, DMSc****/*
*Myron Nevins, DDS******

This human clinical trial evaluated the clinical and histologic response to recombinant human platelet-derived growth factor-BB (rhPDGF-BB) delivered in bone allograft for the treatment of advanced Class II furcation defects. Three mandibular and one maxillary molar furcation defects were treated: Two received 0.5 mg/mL and two received 1.0 mg/mL rhPDGF-BB, in all cases mixed with DFDBA. Clinical probing depths and attachment levels were obtained presurgically and 9 months postsurgical, after which the teeth and surrounding tissues were removed en bloc. Both concentrations of rhPDGF-BB resulted in substantially improved horizontal (mean 3.5 mm) and vertical (mean 4.25 mm) probing depths and attachment levels (mean 3.75 mm). Histologic evaluation revealed periodontal regeneration, including new bone, cementum, and periodontal ligament coronal to the reference notch. Regeneration was also present coronal to the original osseous crest. In one case where an enamel projection extended into the fornix of the furcation, new calcified tissue with new inserting connective tissue fibers was observed over the enamel. This study documented the favorable tissue response to rhPDGF-BB treatment at both the clinical and microscopic levels, provided the first human histologic evidence that new calcified tissue with inserting collagen fibers can occur over enamel projections within furcations, and demonstrated for the first time that complete periodontal regeneration can be achieved in advanced Class II furcation defects using a combination of purified recombinant growth factor and bone allograft. (Int J Periodontics Restorative Dent 2003;23:213–225.)

*Private Practice, Belo Horizonte, Brazil.
**Instructor, Harvard School of Dental Medicine; and Private Practice, Boston.
***Professor Emeritus, University of Bern, Switzerland.
****BioMimetic Pharmaceuticals, Franklin, Tennessee.
*****Director, Institute for Advanced Dental Studies, Swampscott, Massachusetts.

Reprint requests: Dr Myron Nevins, 90 Humphrey Street, Swampscott, Massachusetts 01907.

One of the greatest challenges in the field of periodontics continues to be the treatment of multirooted teeth demonstrating inter-radicular loss of the periodontium (furcation invasion). While added stability may be provided by extra root anchorage, furcated teeth and their surrounding tissues possess anatomic characteristics that make treatment difficult and results unpredictable with current therapies. Although there are reports of "clinical success" in the treatment of Class II furcations, there are no reports of periodontal regeneration in humans, as verified by the histologic documentation of new bone, periodontal ligament (PDL), and cementum coronal to a reference notch placed at the base of calculus at the time of surgery. Thus, while it is always preferable to regenerate lost periodontium, this goal has not been demonstrated in human furcations. To overcome the prevailing healing limitations in furcation defects, the principles of tissue engineering were applied using a purified growth factor together with an osteoconductive scaffold to stimulate the

patients' own cells toward a regenerative response.

Tissue engineering is the relatively new, highly promising field of reconstructive biology that draws on recent advances in medicine and surgery, molecular and cellular biology, polymer chemistry, and physiology. In its broadest sense, tissue engineering is considered to be any attempt to regenerate tissues of the body, whether accomplished in the laboratory or directly in the patient, by combining three key elements: scaffolds or matrices, signaling molecules, and cells. By combining these three elements, tissue (and organ) regeneration that was not previously possible can often be accomplished.

Bone autografts have been referred to as the "gold standard" in osseous grafting procedures because they are generally believed to provide the best results of available materials, presumably because of the presence of conductive bone trabeculae, cells, and signaling molecules (eg, growth factors). However, their use is often contraindicated because of insufficient availability of intraoral graft, frequency of postoperative pain at the donor site, and increased potential for postsurgical complications related to the graft harvest site. Equally important, even in autografts, the number of viable osteoprogenitor cells may be small and the amount of growth factors limited, especially in patients over the age of 50. Because of these limitations, periodontal therapies aimed at regenerating the periodontium have for many years used nonviable, osteoconductive matrices. These mostly inert, physical matrices exert just one key element of the tissue-engineering triad and therefore, although they are clinically effective in some cases (eg, deep intrabony defects), they are not predictable in the wide variety of bone defects encountered routinely by the practitioner.

Growth factors are signaling molecules that have received a great deal of attention in the periodontal and craniomaxillofacial fields as clinicians continue to seek an "off-the-shelf" material that could replace and/or enhance autografts and provide better, more consistent results than current bone scaffolds and matrices. Growth factors are mitogenic (proliferative), chemotactic (stimulate directed migration of cells), and angiogenic (stimulate new blood vessel formation). Therefore, they appear to be critical to the wound-healing process. The growth factor that has received the most attention in hard and soft tissue wound healing is platelet-derived growth factor (PDGF). PDGF is the natural wound-healing "hormone." It is naturally produced by the body at sites of soft tissue (gingiva and skin) and bone injury. Isoforms of PDGF are present in bone matrix and are produced locally at fracture sites.[1–4] Gene knockout studies (in which a specific gene is deleted) have shown that when a PDGF receptor gene is deleted, gross abnormalities occur in the embryologic development of the skeleton, suggesting the critical importance of PDGF in skeletal development.[5]

PDGF is the most thoroughly studied growth factor in periodontics. Since the original report[6] that demonstrated periodontal regeneration following in vivo application of PDGF in the late 1980s, nearly 100 studies on PDGF's effects on PDL and alveolar bone cells and regeneration of the periodontium in animals and humans have been published. An initial human clinical trial demonstrated that application of 150 μg/mL of recombinant human PDGF-BB (rhPDGF-BB) and recombinant human insulin-like growth factor (rhIGF)-1 results in a significant improvement in bone fill compared to open-flap debridement plus placebo.

The primary objective of this study was to determine if it is possible to achieve regeneration in advanced Class II furcation defects in humans following placement of a tissue-engineering product that combines purified rhPDGF-BB with allogeneic bone matrix. The secondary objectives of this study were to determine the safety and biocompatibility of the materials evaluated and the osteogenic potential of the product in advanced human furcation defects.

Method and materials

All patients screened for the study signed informed consent statements after discussing any questions they may have had with the study investigator(s). Four molars with advanced Class II furcation defects were selected for treatment after two clinicians not involved in the study judged these teeth to have a hopeless prognosis. Initial preparation included complete-mouth scaling and root planing (except for the study tooth), oral hygiene instruction, and selective occlusal grinding when indicated. A baseline examination was performed after initial preparation was completed, no more than 14 days before surgical treatment (or the day of surgery). The baseline examination included the following detailed measurements of the target tooth defect:

1. Horizontal probing pocket depth within the furcation (hPD; ≥ 5 mm)
2. Vertical probing pocket depth (vPD; ≥ 5 mm)
3. Clinical attachment level
4. Free gingival margin
5. Generalized probing measurements of all teeth
6. Radiographs of the target tooth
7. Oral hygiene measures, including complete-mouth plaque, gingival, and calculus assessments
8. Record of cigarette use
9. Record of selected medication use (nonsteroidal antiinflammatory drugs, steroids)

Following administration of local anesthesia, full-thickness mucoperiosteal flaps were elevated. Granulation tissue was carefully enucleated from the osseous defects, and the root surface was then notched using a small round bur at the apical extent of the calculus. Subgingival soft and hard deposits on the root surface were removed by scaling and root planing using hand and/or ultrasonic instruments to ensure thorough degranulation and root planing.

Direct measurements of the furcation area were performed using a calibrated probe, scoring the following dimensions:

1. Horizontal bone depth of the furcation defect at its deepest location, using a reference probe placed across the adjacent root surfaces
2. Vertical bone depth of the furcation defect at its deepest location, using the fornix of the furcation as the fixed reference
3. Width of the furcal orifice at its base, measured in a horizontal dimension at the level of the alveolar crest within the furcation

After completing scaling and root planing and obtaining all measurements, the root surface was conditioned with a tetracycline paste for 4 minutes for decontamination and removal of the smear layer. During this period, an amount of demineralized freeze-dried bone allograft (DFDBA) sufficient to fill the periodontal defect was saturated with a solution of rhPDGF-BB (0.5 or 1.0 mg/mL), and the rhPDGF-BB/allograft mixture was allowed to sit for about 10 minutes.

Following root conditioning, the wound was rinsed thoroughly with sterile saline. The root surfaces were then completely dried, and application of the rhPDGF-BB solution began at the coronal aspect of the exposed roots and proceeded apically as far as possible into the furcation and the base of the defect. The rhPDGF-BB/allograft mixture was then packed into the osseous defect. Throughout the procedure, care was taken to protect the root surface and defect from saliva. The gingival flaps were secured with interdental sutures to obtain coverage of the surgical site. The surgical site was protected with a periodontal dressing. The patient was instructed not to brush or floss the surgical site until the sutures were removed. Ten days after surgery, or when the flap had become stabilized by healing, the sutures were removed. Patients were instructed to rinse with chlorhexidine mouthrinse (0.12%) daily for 6 weeks. Analgesics were prescribed for management of postoperative discomfort. A record of analgesics taken (prescribed and over the counter) was kept.

Postoperative examination and professional cleansing of the surgical site with chlorhexidine occurred at 1, 2, 4, 8, and 12 weeks and every 6 weeks thereafter until en bloc biopsies were obtained at 9 months. At 3 and 6 months postsurgical, periodontal maintenance was performed, and at 6 months, periodontal probing depth and vertical clinical attachment level measurements were obtained. Bleeding upon gentle probing was noted when ob-

Table 1 Clinical measurements (mm)

	Vertical probing depth			Horizontal probing depth			Clinical attachment level			Recession		
Case	Pre	Post	Change	Pre	Post	Change	Pre	Post	Change	Pre	Post	Change
1 (0.5 mg/mL rhPDGF)	8	2	6	7	3	4	8	2	6	0	0	0
2 (0.5 mg/mL rhPDGF)	8	3	5	7	4	3	8	4	4	0	1	1
3 (1.0 mg/mL rhPDGF)	6	2	4	5	2	3	9	5	4	3	3	0
4 (1.0 mg/mL rhPDGF)	5	3	2	6	2	4	7	6	1	2	3	1

served. Immediately prior to biopsy, at 9 months postsurgical, the clinical assessments were again performed, and a second periapical radiograph was obtained. Following administration of local anesthetic, the region of the original osseous defect and adjacent tooth structure was removed en bloc as previously described. The marginal gingiva and osseous tissue to the base of the original periodontal defect were included, with a minimum of extra tissue. After block extraction, the resultant defect was reconstructed with autogenous bone grafts, and postsurgical care was provided to achieve proper healing. No data collection occurred after biopsy. The reconstructed site was restored with dental implants and the appropriate prosthesis.

The biopsies were immersed in a solution of 4% formaldehyde, dehydrated in ethanol, and infiltrated and embedded in methyl methacrylate. Undecalcified sections approximately 300 μm in thickness were obtained using a low-speed diamond saw with coolant. The sections were glued onto opalescent acrylic glass, ground to a final thickness of approximately 80 μm, and stained with toluidine blue and basic fuchsin. Step serial sections were obtained in a mesiodistal plane. The following histologic parameters were evaluated:

1. Overall tissue health
2. Degree of inflammation associated with the graft site, as determined by the presence or absence of inflammatory cells, eg, neutrophils and macrophages
3. Presence or absence of a complete new attachment apparatus, including bone, PDL, and cementum, coronal to the root reference notch

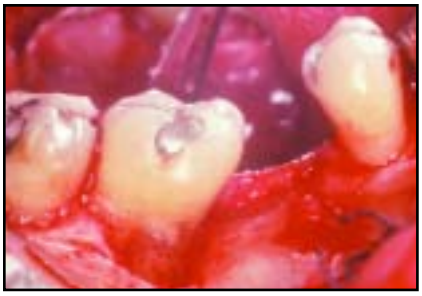
Analyses were performed to compare changes in clinical parameters from their baseline values. Categoric measurements were displayed as counts and percentages, and continuous variables were displayed as means, medians, standard deviations, and ranges.

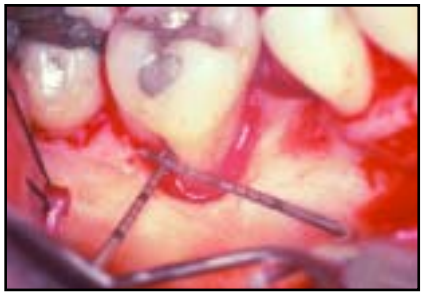
Results

All sites healed uneventfully. Clinically, wound healing appeared enhanced, with the gingiva appearing pink, firm, and completely closed 1 week postsurgical. Pre- and postsurgical probing pocket depths and clinical attachment levels for each patient are shown in Table 1. Complete new attachment apparatus regeneration was present in all four cases.

Case 1

The first patient was a 47-year-old woman with advanced periodontal disease (type IV) who was otherwise healthy. The patient presented with a vPD of 8 mm, a 7-mm hPD, and an 8-mm attachment loss on the buccal aspect of the mandibular right second molar. Following flap reflection, extensive deposits were observed on the roots, extending to within 1 to 2 mm of the base of the osseous defect. There was a 6-mm vertical, 5-mm horizontal, circumferential moat-type intrabony defect (Figs 1a and 1b). After thorough debridement and root preparation, the defect was treated with 0.5 mg/mL rhPDGF-BB in allograft (Fig 1c). Healing progressed well, with the tissue already





Figs 1a and 1b *Case 1 exhibits a 6-mm vertical, 5-mm horizontal, circumferential moat-type intrabony defect. Extensive calculus is apparent on the root surfaces to within 1 to 2 mm of the base of the defect. Roots are notched at the base of calculus and carefully planed, and defect is debrided to remove all granulation tissue.*

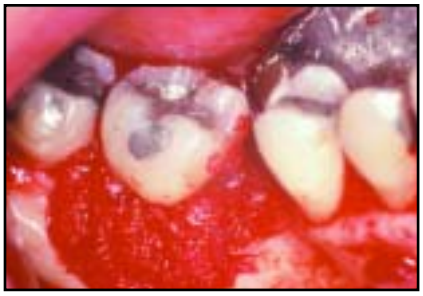


Fig 1c *rhPDGF-BB solution is applied directly to the root surfaces and also used to saturate sufficient DFDBA to completely fill the osseous defect.*

pink and firm by 1 week. No serious adverse events were reported for any of the cases. At 9 months post-surgery, there was a clinical attachment gain of 6 mm, no recession, and vPD and hPD of 2 mm and 3 mm, respectively. The tissues appeared healthy, with no signs of inflammation.

Histologic evaluation of the 9-month biopsy revealed that the 0.5 mg/mL rhPDGF-BB plus allograft was completely biocompatible as determined by the lack of any histologic markers of inflammation, eg, inflammatory cell infiltrate. Periodontal regeneration, including new bone, cementum, and PDL, was present coronal to the reference notch placed during surgery at the base of calculus (Fig 1d). The new bone was most dense at the apical aspect of the original defect. In this area, the new bone was continuous with the original bone and was of the same density. It was a composite of lamellar and woven bone, which appeared to be undergoing normal remodeling. Osteocytes were abundant, particularly in the woven bone. Haversian systems were forming normally.

The width of the new PDL adjacent to the notch was remarkably similar to the width of the PDL in the preexisting periodontium (Fig 1e). In this area, the new PDL was well-organized, with primarily horizontal and tangential fibers and a clearly visible vascular network. In fact, the regenerated PDL was indistinguishable in all aspects from the preexisting PDL apical to the original osseous defect. New cementum was continuously present on the root surfaces from the apical extent of the root planing, completely around the fornix of the furcation, to the apical extent of root planing on the adjacent root (Figs 1d to 1f). The new cementum was cellular in nature. The apical extent of the root planing and new cementum corresponded to the original base of the osseous defect and was 1 to 2 mm apical to the base of the root notch.

In the coronal aspect of the original defect area, the new bone was less dense. The presence of osseous trabeculae gave the appearance of "islands" of new bone, but these were most likely interconnected in planes other than the plane of the section. The osteoid nature of the new bone in the coronal aspect of the furcation and the presence of osteoblasts lining the osteoid suggest that bone formation was continuing to occur at the time the biopsy was obtained. Small remnants of bone allograft were present. These islands of allograft were often, although not always, partially or completely incorporated in new bone (Fig 1f). Interestingly, no epithelium was present within the furcation, even though no membrane was used.

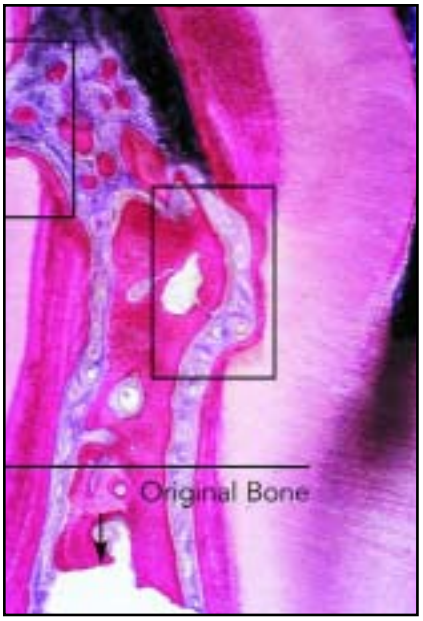


Fig 1d *Nine-month biopsy shows regeneration coronal to the root notch (toluidine blue–basic fuchsin stain; original magnification × 6.3).*

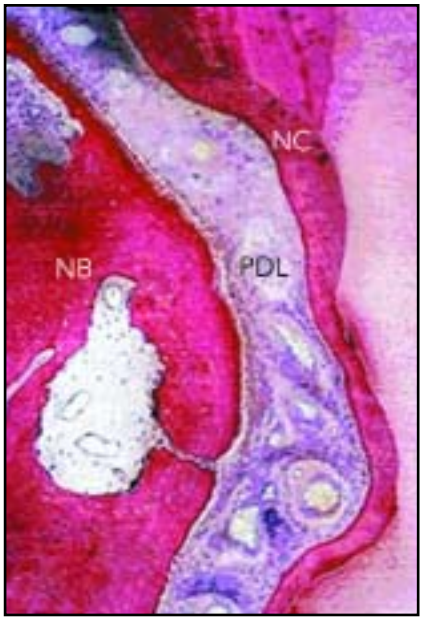


Fig 1e *Right box from Fig 1d: New cementum* (NC) *is present along the entire length of the root surfaces within original defect. New bone* (NB) *adjacent to the notch has similar density as preexisting alveolar bone. New periodontal ligament* (PDL) *is indistinguishable from that apical to original defect. There is no long junctional epithelium, although no membranes were placed (toluidine blue–basic fuchsin stain; original magnification × 25).*

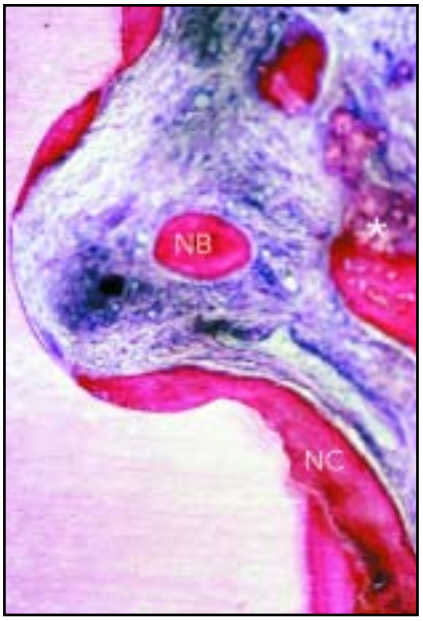


Fig 1f *Left box from Fig 1d: Remnants of DFDBA* (*) *are still visible (toluidine blue–basic fuchsin stain; original magnification × 25).* NB = *new bone;* NC = *new cementum.*

Case 2

The next treated defect, in a 27-year-old woman on the lingual surface of a mandibular right second molar, exhibited an 8-mm vertical attachment loss and a vPD and hPD of 8 mm and 7 mm, respectively. Upon flap reflection, the osseous defect was found to be a 6-mm vertical, 4-mm horizontal, one- to three-walled intrabony defect. The regenerative treatment regimen used 0.5 mg/mL rhPDGF-BB in allograft. As in all cases, the postoperative healing progressed uneventfully and appeared to be more rapid than is customarily observed following full-thickness flap reflection. Nine months following treatment, there was a 4-mm gain in clinical attachment and 1 mm of recession. The vPD was 3 mm, and the hPD was 4 mm. All gingiva in the treated area exhibited minimal edema or erythema.

Histologic analysis of the 9-month biopsy revealed that the treated site was healthy, with no signs of inflammation, and had a histologic appearance similar to that observed in case 1. A highly interesting finding in this case was the presence of an enamel projection/pearl covered with a calcified tissue with inserting collagen fibers (Fig 2). The enamel projection was present at the fornix of the furcation. In some sections, the cementum-like calcified matrix was continuous with the new cementum that lined the entire length of the planed root surfaces and exhibited approximately the same thickness and histologic appearance (Fig 2a).

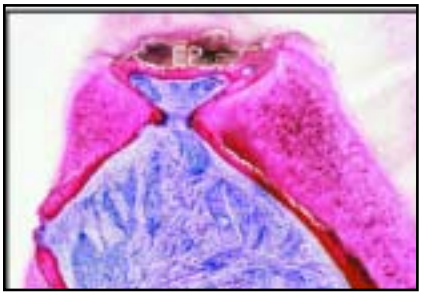


Fig 2a *Furcation area from case 2 shows an enamel projection/pearl* (EP) *in the fornix of the furcation. The enamel is covered with a layer of cementum-like calcified tissue continuous with the new cementum lining the root surfaces (toluidine blue–basic fuchsin stain; original magnification × 25).*

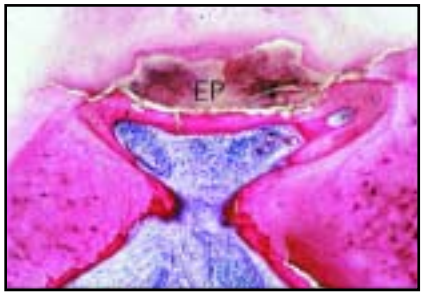


Fig 2b *New collagen fibers insert into the cementum-like material. Separation of new cementum-like material from enamel is likely an artifact. Mild inflammation is present. The morphology of the calcified tissue in the upper right corner gives the appearance of a secondary osteon or new cementum bridging the narrow gap between new cementum on the root surface and that covering the enamel projection* (EP) *(toluidine blue–basic fuchsin stain; original magnification × 25).*

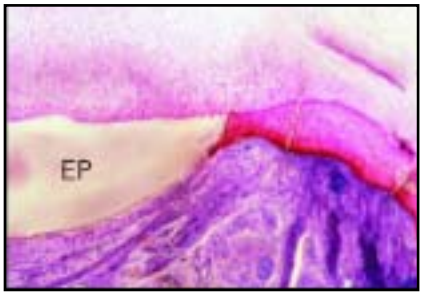


Fig 2c *In some sections, the new calcified material completely covered the enamel, while in others it partially covered the enamel, with the remainder of the enamel covered with dense collagen fibers oriented longitudinally (toluidine blue–basic fuchsin stain; original magnification × 25).* EP = *enamel projection/pearl.*

New collagen fibers inserted into the cementum-like substance, suggesting that a new attachment had formed over the enamel projection (Fig 2b). In other sections, the edges of the enamel projection were partially covered with the new cementum-like calcified tissue, while the remainder of the enamel was in contact with dense connective tissue fibers coursing primarily longitudinally from the new cementum-like layer covering one root to the new cementum-like layer covering the adjacent root (Fig 2c).

While the continuity of the calcified substance with the new cementum covering the remainder of the root surfaces in the defect area suggested that this substance was indeed cellular cementum, this could also have been a thin layer of new bone. Suggestive of the possible bony nature of this substance is the formation of a structure resembling a secondary osteon at one side of the enamel projection (Fig 2b). Although it is difficult to determine with certainty the exact nature and origin of this calcified layer, clearly visible collagen fibers inserting into it formed a new attachment to the tooth root.

Case 3

The third case, which was in a 40-year-old woman, evidenced an uncontained zero-walled horizontal osseous defect on the mesiopalatal aspect of a maxillary first molar. The defect measured 6 mm vertically from the fornix and 5 mm horizontally. The attachment loss measured 9 mm on the palatal aspect. The site was treated with 1.0 mg/mL rhPDGF-BB in allograft. Postoperative healing was uneventful and appeared to be enhanced. Nine months later, there was a gain of 4 mm in clinical attachment, no additional recession, a vPD of 2 mm, and an hPD of 2 mm. The gingival tissues surrounding the defect were pink and firm.

Histologically, the defect site appeared healthy, without the presence of inflammatory cells. Periodontal regeneration was present, with new bone and adjacent new PDL and new cementum filling most of the original defect. The new cementum was present continuously from one root surface to the other, including throughout the fornix of the furcation.

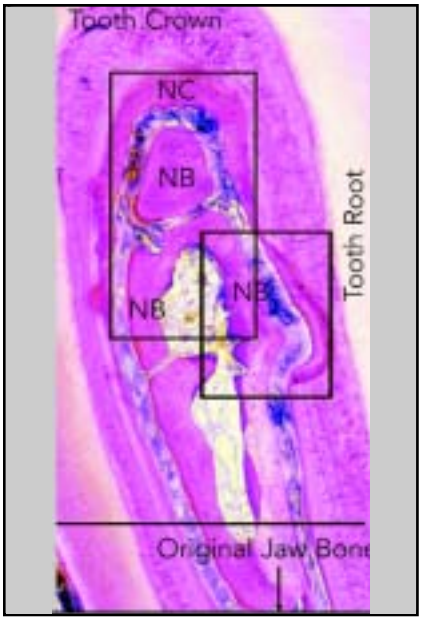
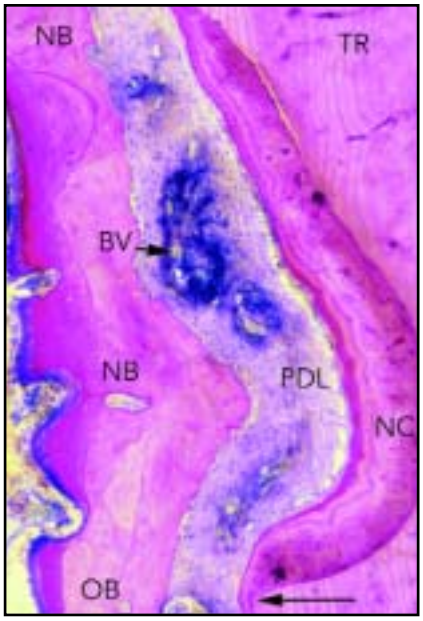
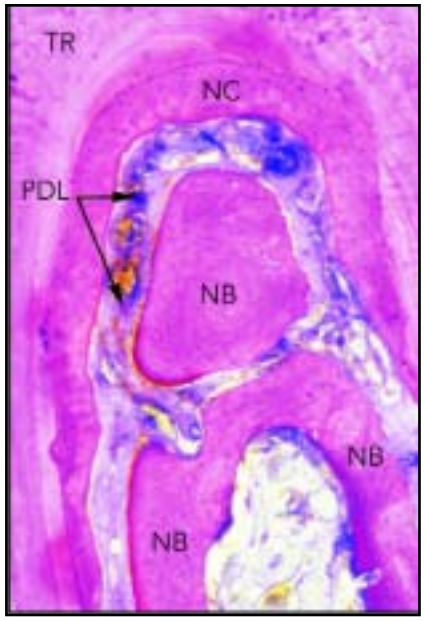


Fig 3a *Nine-month biopsy from case 4 shows complete periodontal regeneration within furcation. New bone* (NB) *formed in continuity with original bone, and new PDL is indistinguishable from original PDL apical to original defect. PDL fibers are perpendicular, traversing between new cementum* (NC) *and bone* (NB) *(toluidine blue–basic fuchsin stain; original magnification × 6.3).*





Figs 3b and 3c *Higher magnifications of boxes from Fig 3a show that tooth roots (TR) are continuously lined with new cementum* (NC). *There is no epithelium within the furcation. Robust angiogenesis is present, as evidenced by numerous new blood vessels* (BV) *present within the new periodontal ligament* (PDL) *and bone* (NB) *(toluidine blue–basic fuchsin stain; original magnifications × 25 [Fig 3b] and 16 [Fig 3c]).* OB = *original bone;* arrow = *base of calculus notch (Fig 3b).*

Case 4

The final case in this series was a 34-year-old woman who presented with a vPD of 5 mm, hPD of 6 mm, and attachment loss of 7 mm on the lingual surface of a mandibular second molar. Flap reflection revealed a 5-mm vertical, 5-mm horizontal, uncontained osseous defect. Following debridement and root preparation, the site was treated with 1.0 mg/mL rhPDGF-BB in allograft. Healing was uneventful and rapid. Nine months after treatment, the vPD and hPD were 3 mm and 2 mm, respectively. There was 1 mm of recession. The gingiva was pink and firm, with no clinical signs of inflammation.

Histologically, complete periodontal regeneration was observed, including new bone, cementum, and PDL coronal to the root notches (Fig 3). New cementum formation was continuous and progressed from just apical to the notches in both roots completely across the fornix, without discontinuity (Figs 3a and 3c). The adjacent PDL was well-organized and mature, with collagen fibers traversing primarily horizontally from the bone and inserting into the new cementum (Fig 3b). The bone was formed in such a manner that the normal PDL space was maintained, even in the fornix of the furcation (Fig 3c). There was no ankylosis or root resorption. The new PDL could not be distinguished from the original PDL present apical to the original osseous defect.

There was also extensive new bone formation that was the same density as the preexisting alveolar bone. The bone was mostly lamellar, with small areas where the woven bone was still remodeling. Revascularization (angiogenesis) was present to the same degree as in the original alveolar bone and ligament. There was no long junctional epithelium, even though a barrier was not used. The new periodontium appeared completely regenerated and healthy in all respects analyzed.

Discussion

Effective treatment of advanced furcation defects continues to be one of the most challenging, and least predictable, areas of periodontal therapy. Current periodontal regenerative materials, including bone allograft, bone substitutes, barrier membranes, root conditioning agents, enamel matrix derivatives, and various combinations thereof, have not been shown to lead to regeneration in human furcations.[7]

Clinically successful therapy, as assessed by clinical measurements, has certainly been reported. Expanded polytetrafluoroethylene (e-PTFE) barrier membrane–treated Class II furcation defects show a significant gain in clinical attachment and decreased probing depth compared to debrided controls.[8–11] An enhanced clinical effect is demonstrated using e-PTFE membranes in combination with root conditioning and composite osseous grafting (autogenous bone mixed with either DFDBA or TCP) in furcations.[12] A 5-year follow-up of that study indicated that grafted sites retained clinical attachment, while nongrafted sites lost previously gained attachment.[13] A subsequent study confirmed these findings, showing greater regeneration with the combined therapy of e-PTFE and DFDBA compared to e-PTFE alone.[14] Resorbable barrier membranes alone and in combination with various graft materials have also been reported to improve clinical outcomes.[15–23]

Although clinical studies may demonstrate significant improvement in clinical parameters, to establish true periodontal regeneration, which is considered the ideal goal of regenerative therapy, there must be histologic evidence of new alveolar bone, PDL, and cementum formation on the root surface coronal to the apical extent of calculus at the time of surgery.[24] Materials meeting these criteria when used for treatment of interproximal, intrabony defects include autogenous bone,[25–27] DFDBA,[28,29] DFDBA with osteogenin,[30] certain barrier membranes (which demonstrated the formation of new cementum with inserting collagen fibers but limited amounts of new bone),[31,32] citric acid root conditioning,[33] bovine-derived xenograft,[34,35] and enamel matrix derivative.[36–38] However, these results are limited to interproximal intrabony defects, and no prior materials have demonstrated regeneration in furcation-type defects.

PDGF, among other growth factors thought to be important to tissue repair, is released by activated platelets, among the first cells to reach an injured site. PDGF is a 25- to 30-KDa heat- and acid-stable dimeric protein. It is present in relatively high concentrations during early fracture repair, where it acts to stimulate osteoblast cell proliferation (mitogenesis) and recruitment (chemotaxis). In addition, it stimulates osteoblast type I collagen synthesis, the predominant component of bone matrix. In addition to platelets, PDGF is made by endothelial cells, mesenchymal stem cells,

osteoblasts, chondrocytes, and osteoclasts present in the later stages of fracture repair. Recent data demonstrate that rhPDGF-BB causes osteoblasts to produce vascular endothelial growth factor (VEGF), one of the most potent mediators of new blood vessel formation, ie, angiogenesis.[39] In vivo, localized application of rhPDGF-BB to fractures in animals results in quicker healing of the fracture site.[40] Systemic application of rhPDGF-BB in an osteoporotic animal model results in increased bone formation, trabecular bone density, and strength in both long bones, eg, tibia and femur, and flat bones such as the vertebrae.[41]

In the periodontal environment, PDGF stimulates proliferation and chemotaxis of PDL cells and has led to robust periodontal regeneration in numerous studies in dogs, nonhuman primates, and other models.[42–45] A plethora of cell culture studies have shown that PDGF is probably the most potent of the recombinant growth factors for stimulating PDL cells, and that it enhances wound healing in vitro.[44,46–49] Likewise, many animal studies have shown that rhPDGF-BB, alone and in combination with IGF-1, enhances periodontal regeneration.[45,46,50–52] An earlier phase I/II human clinical trial also indicated that rhPDGF-BB and rhIGF-I, delivered in a methyl cellulose gel, improve bone fill in intrabony periodontal defects.[53]

While rhPDGF-BB appears to hold great promise for improving wound healing and clinical outcomes in periodontics and other fields, results can likely be improved by combining this potent growth factor with osteoconductive graft materials and bone substitutes. An osteoconductive bone void filler physically fills bone defects, providing a matrix or scaffolding for bone formation. By filling the defect, it also prevents collapse of the soft tissues into the bone defect and, if appropriately porous, facilitates stabilization of the blood clot and ingrowth of new blood vessels. If the product is similar to natural bone in macroscopic structure and chemical and physical properties, it can integrate into the newly formed bone and improve its volume and strength. The addition of rhPDGF-BB to an osteoconductive graft material would be expected to improve the beneficial effect of these materials by accelerating cellular ingrowth and revascularization of the wound site.

A combination of rhPDGF-BB and an osteoconductive graft has its basis in the principles of tissue engineering.[54] Tissue engineering is based on using the combination of growth factors and modulators, scaffolds, and cells to regenerate tissues and organs either in vivo or ex vivo. By combining signaling molecules such as PDGF with conductive scaffolds and cells, tissue regeneration may be possible to achieve in situations in which it was not previously possible.

In the current study, purified rhPDGF-BB was combined with DFDBA to increase the level of growth factors in this matrix and thereby improve upon the stimula-

tory effect of DFDBA. This tissue engineering–based therapy was used in an attempt to achieve regeneration in an anatomic site where it has not been previously reported, ie, human Class II furcation defects. The study demonstrated that: *(1)* at both the clinical and microscopic levels, there was a favorable tissue response to the rhPDGF-BB–enhanced allograft; *(2)* new calcified tissue with inserting collagen fibers can occur over enamel projections within furcations; and *(3)* for the first time, complete periodontal regeneration, documented histologically, was achieved in advanced Class II furcation defects.

Acknowledgments

This study was supported by BioMimetic Pharmaceuticals. Samuel E. Lynch is a full-time employee of BioMimetic Pharmaceuticals.

References

1. Mohan S, Baylink DJ. Thereapeutic potential of TGF-β BMP, and FGF in the treatment of bone loss. In: Principles of Bone Biology. New York: Academic Press, 1996:111–123.
2. Lane JM, Tomin E, Bostrom MP. Biosynthetic bone grafting. Clin Orthop 1999;367: S107–S117.
3. Andrew JG, Hoyland JA, Freemont AJ, Marsh DR. Platelet-derived growth factor expression in normally healing human fractures. Bone 1995;16:455–460.
4. Bostrom MP, Lane JM, Berberian WS, et al. Immunolocalization and expression of bone morphogenetic proteins 2 and 4 in fracture healing. J Orthop Res 1995;13: 357–367.
5. Schatterman GC, Morrison-Graham K, Van Koppen A, Weston JA, Bowen-Pope DF. Regulation and role of PDGF receptor alpha subunit expression during embryogenesis. Development 1992;115:123–131.
6. Lynch SE, Williams RC, Polson AM, et al. A combination of platelet-derived growth factor and insulin-like growth factors enhances periodontal regeneration. J Clin Periodontol 1989;16:545–548.
7. Kalkwarf K, Kadahl WP, Patel KD. Evaluation of furcation region response to periodontal therapy. J Periodontol 1988;59: 794–804.
8. Pontoreiro R, Lindhe J, Nyman S, Karring T, Rosenberg E, Sanavi F. Guided tissue regeneration in degree II furcation-involved mandibular molars. J Clin Periodontol 1988;15:247–254.
9. Becker W, Becker BE, Mellonig J, et al. A prospective multi-center study evaluating periodontal regeneration for Class II furcation invasions and intrabony defects after treatment with a bioabsorbable barrier membranes: 1-year results. J Periodontol 1996;67:641–649.
10. Pontoriero R, Lindhe J, Nyman S, Karring T, Rosenberg E, Sanavi F. Guided tissue regeneration of furcation defects in mandibular molars. J Clin Periodontol 1989;16:170–174.
11. Lekovic V, Kenney EB, Kovacevic K, Carranza FA Jr. Evaluation of guided tissue regeneration in Class II furcation defects. A clinical reentry study. J Periodontol 1989;60:694–698.
12. Schallhorn RG, McClain PK. Combined osseous composite grafting, root conditioning, and guided tissue regeneration. Int J Periodontics Restrorative Dent 1998; 8(4):9–31.
13. McClain PK, Schallhorn RG. Long-term assessment of combined osseous composite grafting, root conditioning, and guided tissue regeneration. Int J Periodontics Restorative Dent 1993;13: 9–27.
14. Anderegg CR, Martin SJ, Gray JL, Mellonig JT, Gher ME. Clinical evaluation of the use of decalcified freeze-dried bone allograft with guided tissue regeneration in the treatment of molar furcation invasions. J Periodontol 1991;62:264–268.
15. Caffesse RG, Mota LF, Quiñones CR, Morrison EC. Clinical comparison of absorbable and non-absorbable barriers for guided periodontal tissue regeneration. J Clin Periodontol 1997;24:747–752.
16. Black BS, Gher ME, Sandifer JB, Fucini SE, Richardson AC. Comparative study of collagen and expanded polytetrafluoroethylene membranes in the treatment of human Class II furcation defects. J Periodontol 1994;65:598–604.
17. Caton J, Greenstein G, Sappa U. Synthetic bioabsorbable barrier for regeneration in human periodontal defects. J Periodontol 1994;65:1037–1045.

18. Christgau M, Schmalz G, Reich E, Wenzel A. Clinical and radiographical split-mouth-study on absorbable versus non-resorbable GTR-membranes. J Clin Periodontol 1995;22:306–315.
19. Eickholz P, Kim TS, Holle R. Guided tissue regeneration with non-resorbable and biodegradable barriers: 6 months results. J Clin Periodontol 1997;24:92–101.
20. Garrett S, Polson AM, Stoller NH, et al. Comparison of a bioabsorbable GTR barrier to a non-absorbable barrier in treating human Class II furcation defects. A multi-center parallel design randomized single-blind trial. J Periodontol 1997;68: 667–675.
21. Yukna RA, Evans GH, Aichelmann-Reidy MB, Hayer ET. Clinical comparison of bioactive glass bone replacement graft material in expanded polytetrafluoroethylene barrier membrane in treating human mandibular molar Class II furcations. J Periodontol 2001;72:125–133.
22. Paolantonio M. Combined periodontal regenerative technique in human intrabony defects by collagen membranes and anorganic bovine bone. A controlled clinical study. J Periodontol 2002;73:158–166.
23. Leonardis D, Garg AK, Pedrazzoli V, Pecora GE. Clinical evaluation of the treatment of Class II furcation involvements with bioabsorbable barriers along or associated with demineralized freeze-dried bone allografts. J Periodontol 1999; 70:8–12.
24. Lynch SE. Methods for the evaluation of regenerative procedures. J Periodontol 1992;63:1085–1092.
25. Stahl S, Froum S, Kushner J. Healing responses of human intraosseous lesions following the use of debridement, grafting and citric acid root treatment. II. Clinical and histologic observations: One year post surgery. J Periodontol 1983;54: 325–338.
26. Drago MR, Sullivan HC. A clinical and histological evaluation of autogenous iliac bone grafts in humans. Part II. External root resorption. J Periodontol 1973;44: 614–625.
27. Hiatt WR, Schallhorn R, Aaronian A. The induction of new bone and cementum formation: IV. Microscopic examination of the periodontium following human bone and marrow allograft, autograft and non-graft periodontal regenerative procedures. J Periodontol 1978;49:495–512.
28. Bowers GM, Chadroff B, Carnevale R, et al. Histologic evaluation of new attachment apparatus formation in humans. Part III. J Periodontol 1989;60:683–693.
29. Bowers GM, Chadroff B, Carnevale R, et al. Histologic evaluation of new attachment apparatus formation in humans. Part II. J Periodontol 1989;60:675–682.
30. Bowers G, Felton F, Middleton C, et al. Histologic comparison of regeneration in human intrabony defects when osteogenin is combined with demineralized freeze-dried bone allograft and with purified bovine collagen. J Periodontol 1991; 62:690–702.
31. Gottlow J, Nyman S, Lindhe J, Karring T, Wennström J. New attachment formation in the human periodontium by guided tissue regeneration. Case reports. J Clin Periodontol 1986;13:604–616.
32. Stahl S, Froum S, Tarnow D. Human histologic response to guided tissue regeneration techniques in intrabony lesions. J Clin Periodontol 1990;17:191–198.
33. Cole RT, Crigger M, Bogle G, Egelberg J, Selvig KA. Connective tissue regeneration to periodontally diseased teeth. J Periodontal Res 1980;15:1–9.
34. Camelo M, Nevins ML, Schenk RK, Rasperini G, Lynch SE, Nevins M. Clinical, radiographic and histologic evaluation of human periodontal defects treated with Bio-Oss and Bio-Gide. Int J Periodontics Restorative Dent 1998;18:321–331.
35. Mellonig JT. Human histologic evaluation of a bovine-derived bone xenograft in the treatment of periodontal osseous defects. Int J Periodontics Restorative Dent 2000;20:19–29.
36. Sculean A, Reich E, Chiantella G, Brecx M. Clinical and histological evaluation of human intrabony defects treated with an enamel matrix protein derivative (Emdogain). Int J Periodontics Restorative Dent 1999;19:157–163.

37. Mellonig JT. Enamel matrix derivative for periodontal reconstructive surgery: Technique and clinical and histologic case report. Int J Periodontics Restorative Dent 1999;19:9–19.

38. Yukna RA, Mellonig JT. Histologic evaluation of periodontal healing in humans following regenerative therapy with enamel matrix derivative. A 10-case series. J Periodontol 2000;71:752–759.

39. Boultreau PJ, Warren SM, Spector JA, Steinbrech DS, Mehrara DJ, Longaker MT. Factors in the fracture microenvironment induce primary osteoblast angiogenic cytokine production. Plast Reconstr Surg 2002;110:139–148.

40. Nash TJ, Howlett CR, Martin C, Steele I, Johnson KA, Hicklin DJ. Effect of platelet-derived growth factor on tibial osteotomies in rabbits. Bone 1994;15:203–208.

41. Mitlak BH, Finkelman RD, Hill EL, et al. The effect of systemically administered PDGF-BB on the rodent skeleton. J Bone Miner Res 1996;11:238–247.

42. Matsuda N, Lin WL, Kumar NM, Cho MI, Genco RJ. Mitogenic, chemotactic, and synthetic responses of rat periodontal ligament fibroblastic cells to polypeptide growth factors in vitro. J Periodontol 1992;63:515–525.

43. Oates TW, Rouse CA, Cochran DL. Mitogenic effects of growth factors on human periodontal ligament cells in vitro. J Periodontol 1993;64:142–148.

44. Lynch SE, de Castilla GR, Williams RC, et al. The effects of short-term application of a combination of platelet-derived and insulin-like growth factors on periodontal wound healing. J Periodontol 1991;62: 458–467.

45. Rutherford RB, Niekrash CE, Kennedy JE, Charette MF. Platelet-derived and insulin-derived growth factors stimulate regeneration of periodontal attachment in monkeys. J Periodontal Res 1992;27:285–290.

46. Dennison D, Vallone D, Pinero G, Rittman B, Caffesse RG. Differential effect of TGF-beta1 and PDGF on proliferation of periodontal ligament cells and gingival fibroblasts. J Periodontol 1994;65:641–648.

47. McAllister BS, Leeb-Lundberg F, Mellonig JT, Olson MS. The functional interaction of EFG and PDGF with bradykinin in the proliferation of human gingival fibroblasts. J Periodontol 1995;66:429–437.

48. Piche JE, Graves DT. Study of growth factor requirements of human bonederived cells. A comparison with human fibroblasts. Bone 1989;10:131–138.

49. Lacker KP, Oates TW, Hoang AM, Takacs V, Cochran DL. Development of an in vitro wound healing model for periodontal cells. J Periodontol 2000;71:226–237.

50. Giannobile WV, Hernandez RA, Finkelman RD, et al. Comparative effects of platelet-derived growth factor-BB and insulin-like growth factor-I, individually and in combination, on periodontal regeneration in Macaca fascicularis. J Periodontal Res 1996;31:301–312.

51. Park JB, Matsuura M, Han KY, et al. Periodontal regeneration in Class III furcation defects of beagle dogs using guided tissue regenerative therapy with platelet-derived growth factor. J Periodontol 1995;66:462–477.

52. Cho MI, Lin WL, Genco RJ. Platelet-derived growth factor modulated guided tissue regeneration therapy. J Periodontol 1995;66:522–530.

53. Howell TH, Fiorellini JP, Paquette DW, Offenbacher S, Giannobile WV, Lynch SE. A phase I/II clinical trial to evaluate a combination of recombinant human platelet-derived growth factor-BB and recombinant human insulin-I like growth factor-I in patients with periodontal disease. J Periodontol 1997;68:1186–1193.

54. Lynch SE, Genco RJ, Marx R. Tissue Engineering Applications in Maxillofacial Surgery and Periodontics. Chicago: Quintessence, 1999.