Periodontal Regeneration in Humans Using Recombinant Human Platelet-Derived Growth Factor-BB (rhPDGF-BB) and Allogenic Bone

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Background: Purified recombinant human platelet-derived growth factor BB (rhPDGF-BB) is a potent wound healing growth factor and stimulator of the proliferation and recruitment of both periodontal ligament (PDL) and bone cells. The hypothesis tested in this study was that application of rhPDGF-BB incorporated in bone allograft would induce regeneration of a complete new attachment apparatus, including bone, periodontal ligament, and cementum in human interproximal intrabony defects and molar Class II furcation lesions.

Methods: Nine adult patients (15 sites) with advanced periodontitis exhibiting at least one tooth requiring extraction due to an extensive interproximal intrabony and/or molar Class II furcation defect were entered into the study. Eleven defects were randomly selected to receive rhPDGF-BB. Following full-thickness flap reflection and initial debridement, the tooth roots were notched at the apical extent of the calculus, the osseous defects were thoroughly debrided, and the tooth root(s) were planed/prepared. The osseous defects were then filled with demineralized freeze-dried bone allograft (DFDBA) saturated with one of three concentrations of rhPDGF-BB (0.5 mg/ml, 1.0 mg/ml, or 5.0 mg/ml). Concurrently, four interproximal defects were treated with a well accepted commercially available graft (anorganic bovine bone in collagen, ABB-C) and a bilayer collagen membrane. Radiographs, clinical probing depths, and attachment levels were obtained preoperatively (at baseline) and 9 months later. At 9 months postoperatively, the study tooth and surrounding tissues were removed en bloc. Clinical and radiographic data were analyzed for change from baseline by defect type and PDGF concentration. The histologic specimens were analyzed for the presence of regeneration of a complete new attachment apparatus coronal to the reference notch.

Results: The post-surgical wound rapidly healed and was characterized by firm, pink gingivae within 7 to 10 days of surgery. There were no unfavorable tissue reactions or other safety concerns associated with the treatments throughout the course of the study. In rhPDGF/allograft sites, the vertical probing depth (vPD) reduction for interproximal defects was 6.42 ± 1.69 mm (mean ± SD) and clinical attachment level (CAL) gain was 6.17 ± 1.94 mm (both P < 0.01). Radiographic fill was 2.14 ± 0.85 mm. Sites filled with ABB-C had a PD reduction and CAL gain of 5.75 ± 0.5 and 5.25 ± 1.71, respectively. Furcation defects treated with rhPDGF/allograft exhibited a mean horizontal and vertical PD reduction of 3.40 ± 0.55 mm (P < 0.001) and 4.00 ± 1.58 mm (P < 0.005), respectively. The CAL gain for furcation defects was 3.2 ± 2.17 mm (P < 0.030). Histologic evaluation revealed regeneration of a complete periodontal attachment apparatus, including new cementum, PDL, and bone coronal to the root notch in four of the six interproximal defects and all evaluable (four of four) furcation defects treated with PDGF. Two of the four interproximal intrabony defects treated with ABB-C and membrane exhibited regeneration.

Conclusions: Use of purified rhPDGF-BB mixed with bone allograft results in robust periodontal regeneration in both Class II furcations and interproximal intrabony defects. This is the first report of periodontal regeneration demonstrated histologically in human Class II furcation defects. J Periodontol 2003;74:1282-1292.

KEY WORDS
Bone regeneration; clinical trials; furcation/therapy; grafts, bone; growth factors, platelet-derived; periodontal regeneration; wound healing.

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A major goal of periodontal therapy continues to be regeneration of the attachment structures of teeth, including new bone, periodontal ligament (PDL), and cementum, which have been destroyed by periodontal diseases or trauma. Although a number of treatment modalities are currently available, clinicians continue to seek more predictable regenerative therapies that are less technique sensitive, lead to faster tissue regeneration, and are applicable to the broad array of periodontal and peri-implant defects encountered daily by clinicians.

Bone grafts and a limited number of bone substitutes are generally recognized to assist in the regeneration of lost bone in the orofacial region and, when used around teeth, may lead to long-term clinically satisfactory results and restoration of complete new attachment apparatus. Autogenous bone grafts appear to provide the best results, but the added operating time and increased pain and postoperative complications associated with their harvest, as well as the limited available volume of intraoral autogenous bone, reduce patient acceptance and the clinician’s desire to harvest autogenous bone for use in routine periodontal surgery.

As a substitute for autogenous bone grafts, bone allografts have been in treating bone defects associated with periodontal disease. Several reports in humans have shown decalcified freeze-dried bone allograft (DFDBA) to produce greater defect fill with new bone than debridement procedures alone. Histologically, DFDBA has been shown to result in regeneration of the periodontium. Likewise anorganic bovine bone (ABB) has been shown to induce periodontal and peri-implant regeneration, especially when used in conjunction with membranes and mixed with autograft.

**GROWTH FACTORS AND MORPHOGENS**

Bone, platelets, and a variety of other cells and tissues naturally contain potent bioactive proteins termed growth factors or morphogens. Growth factors and morphogens have received a great deal of attention in the periodontal, craniofacial, and orthopedic fields as clinicians continue to seek an “off-the-shelf” material that could replace the need for autografts and provide better, more predictable results than non-stimulatory bone substitutes. The two categories of molecules that have received the greatest attention are the growth factors, which are primarily mitogenic (cell proliferative) and chemotactic (cell recruitment) agents, and morphogens, that act mostly by osteoinduction, i.e., causing the differentiation of stem cells into bone-forming cells. Within the growth factor class, the protein that has been the most thoroughly studied and is being developed clinically is platelet-derived growth factor (PDGF). PDGF, a 25 to 30 kDa protein, is present in bone matrix, secreted by platelets during early fracture repair, and produced locally at fracture sites. It is both chemotactic and mitogenic for osteoblasts and stimulates osteoblast type I collagen synthesis, which is the primary extra-cellular component of bone. Cell surface receptors for PDGF are increased during fracture healing, further suggesting the role of these proteins in normal fracture healing. PDGF is critically important in the embryologic development of the skeleton, and localized injection of PDGF into the medullary cavity accelerates fracture healing in animals. PDGF has also been successfully used to treat osteoporosis in animal models, resulting in improved trabecular bone density and strength in both the flat bones and long bones throughout the skeleton.

**Materials and Methods**

**Patient Characteristics**

Nine adult patients 27 to 51 years of age (8 female, 1 male; all non-smokers) were enrolled in this study. Each patient presented with radiographic evidence (Figs. 1 and 2B) of at least one advanced periodontal defect (interproximal and/or severe Class II furcation defect) measuring 5 mm vertically and horizontally for furcation defects (Fig. 1A) and 7 mm vertically for interproximal defects (Fig. 2A). Teeth associated with the osseous defect sites had been assigned a hopeless prognosis by two independent dentists or required extraction for prosthetic or other reasons clearly specified in the dental treatment plan. Patients were systemically healthy and there were no contraindications to periodontal therapy. Informed consent was obtained following presentation and discussion of the study protocol.

**Presurgical Phase**

Initial periodontal therapy consisted of full-mouth scaling and root planing utilizing both hand and ultrasonic
instruments under local anesthesia. Oral hygiene instructions were given at each visit and were reinforced throughout the study period. Occlusal adjustments were performed by selective grinding when required. Following completion of initial therapy no more than 2 weeks prior to or on the day of surgery, a baseline examination was performed. The baseline examination assessed probing depth measurements, attachment level measurements, full-mouth plaque, calculus, and gingival indices, as well as additional radiographic...
films required beyond those obtained at the screening visit.

**Measurements**

All baseline clinical parameters were recorded no more than 14 days prior to or on the day of surgery. Measurements were made with a calibrated probe and recorded to the nearest millimeter at the mid-facial, mid-lingual, mesial, and distal line angles from the cemento-enamel junction (CEJ) to the free gingival margin (FGM) to evaluate recession. Additional measurements included those obtained from the FGM to the base of the pocket to evaluate probing depth (PD) changes and CEJ to the base of the pocket to evaluate attachment level (CAL) changes. Hard tissue measurements were obtained during surgery as follows: vertically from the alveolar crest to the base of the osseous defect (vertical bone depth), horizontally from the adjacent root prominence to the base of the

**Figure 2.**

A) Intraoperative photo following full-thickness flap reflection and root debridement. Six-millimeter, one-wall intrabony defects are present on the mesial and distal aspects of tooth #6. B) Presurgical radiographic appearance showing extensive bone loss. C) Post-surgical radiograph taken 9 months following treatment of the site with rhPDGF-BB in bone allograft. The notches placed at the time of surgery at the apical extent of the calculus are evident (arrows). Bone formation coronal to the notches is clearly visible. D) Histologic section of tooth #6 taken 9 months following treatment with rhPDGF-BB mixed with allograft showing regeneration of a CNAA. Note new bone (NB) coronal to the level of the original bone (OB) and root notches. The solid line demarcates the original bone from the new bone. A physiologic PDL is also clearly seen coronal to the notches (arrows) and adjacent NB. (Original magnification x25.) E) Higher power view of the box seen in 2D. Regeneration of the attachment structures including a thin layer of new cementum (NC) with adjacent new PDL and new bone (NB) is seen. New blood vessel formation (BV) is also apparent. The new bone is a dense construct of lamellar and woven bone and appears to be undergoing normal remodeling. The new PDL is well organized with bundles of collagen fibers coursing perpendicularly from the new bone to the root surface. (Original magnification x25.)
osseous defect (horizontal bone depth for furcation defects), and fornix of the furcation to the base of the osseous defect (vertical bone depth for furcation defects). Only those defects with an intrabony component of at least 2 mm vertically and 4 mm horizontally for furcation defects, and 4 mm vertically for interproximal defects were included in the study.

Radiographic bone height was determined in the following manner: total tooth length was determined by measuring from the root apex to the cusp tip. A correction factor was obtained by dividing the presurgical total tooth length value by the 9-month post-surgical total tooth length value. Radiographic bone height measurements were obtained for both time points by measuring from the root apex to the radiographic osseous crest at the defect site. The measurement obtained from the presurgical radiograph was recorded and the measurement obtained from the 9-month post-surgical radiograph was multiplied by the correction factor and this value was then recorded.

**Surgical Procedures**

The proposed surgical area was anesthetized using local anesthetic. Following intracrevicular incisions, buccal and lingual full-thickness (mucoperiosteal) flaps were elevated extending at least one tooth mesial and distal to the treated tooth defect. Care was taken to preserve as much of the gingivae as possible. Vertical releasing incisions were performed to facilitate coronal displacement of the flap.

Following reflection of the mucoperiosteal flap, all granulation tissue associated with the osseous defect was removed. The root surface was then notched with a small round bur at the apical extent of the calculus and subgingival soft and hard deposits on the root surface were removed utilizing both hand and ultrasonic instrumentation to assure thorough degranulation and root planing. The surgical site was rinsed thoroughly with sterile saline in order to allow the investigator to evaluate the site for pathology or irregularities which would exclude the patient from the study.

Final eligibility for inclusion in the study was determined at this point by recording defect depth measurements utilizing a calibrated probe (vertical and horizontal intrabony measurements for furcation defects and vertical intrabony measurements for interproximal defects). The furcation defect was carefully examined to confirm that it was a Class II furcation defect.

Upon completion of the scaling and root planing and obtaining all measurements, the root surfaces were conditioned with a tetracycline paste for 3 minutes. The paste was prepared by mixing the contents of one tetracycline HCl 250 mg capsule with a small amount of sterile saline or sterile water to a paste consistency. The paste was carefully applied to the tooth root surface and care was taken to avoid excessive overflow of the paste onto adjacent bony sites. During this period, an amount of allograft, sufficient to fill the periodontal defect, was combined with one of the three concentrations of rhPDGF-BB solution (0.5, 1.0, or 5.0 mg/ml) for a minimum of 10 minutes. The exact volume of solution adsorbed depended on the amount of allograft to be applied, which, in turn, was determined by the size of the periodontal defect.

Following root conditioning, the wound was rinsed thoroughly with sterile saline. The root surfaces were then completely dried and the rhPDGF-BB solution was applied to the tooth root surfaces using the saturated allograft (similar to a sponge). 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/or the base of the defect. The allograft saturated with rhPDGF-BB solution was then packed into the osseous defect. Concurrently, four interproximal intrabony defects in the same patients received ABB-C alone or in combination with bilayer collagen membrane. Care was taken to isolate the root surface and defect from saliva during the grafting procedures. The gingival flaps were secured with interdental sutures to achieve complete coverage of the surgical site. Periodontal dressing was applied. Patients received penicillin VK (1 g per day for 7 days) and were instructed to rinse with chlorhexidine digluconate twice daily for 8 weeks. Analgesics were prescribed for management of postoperative discomfort and a record was kept of analgesics taken (prescribed and over the counter). In addition, the patient’s subjective evaluation of the relative discomfort of the operative site was recorded as well as any adverse events. Sutures were removed approximately 10 days following surgery when the flap had become stabilized by healing.

**Post-Surgical Phase**

Postoperative examination occurred at 1, 2, 4, 8, and 12 weeks, and every 6 weeks thereafter until en bloc biopsies were obtained at 9 months. At each visit the condition of the soft tissues was examined, supragingival prophylaxis was carefully performed, oral hygiene instructions were reviewed, any abnormalities were noted, and the treatment tooth and the presence and extent of graft exposure (in millimeters) was recorded. At 3 and 6 months post-surgery, periodontal maintenance was performed, and at 6 months probing depth and vertical clinical attachment level measurements were obtained and bleeding upon gentle probing was noted. Immediately prior to biopsy, at 9 months postsurgery, the clinical assessments were again performed and a second periapical radiograph was obtained. This

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† Bio-Oss collagen, Geistlich AG, Lucerne, Switzerland.
†† Coe Pak, GC America, Alsip, IL.
** Gore-Tex suture CV-5, W.L. Gore & Associates, Inc., Flagstaff, AZ.
# Bio-Gide, Geistlich AG.
radiograph was compared to the pretreatment radiograph for changes in bone height and density as well as any signs of abnormal healing such as ankylosis or root resorption.

After completing these assessments the study tooth and a small amount of surrounding soft and hard tissue were removed en bloc as described previously. Any remaining tooth structure was extracted and the site was reconstructed with autogenous bone grafts and an ePTFE or collagen augmentation membrane. Appropriate post-surgical care was given to achieve proper healing after biopsy. No data collection occurred after biopsy. The reconstructed biopsy site was restored with dental implants and the appropriate prosthetic. The biopsies were immersed in a solution of 4% formaldehyde, dehydrated in ethanol, and infiltrated and embedded in methylmethacrylate. Undecalcified sections of 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 toludine blue and basic fuchsin. Step serial sections were obtained in a mesiodistal plane.

**Statistical Analysis**

Analyses were performed to compare changes in clinical parameters from their baseline values. Categorical measurements were displayed as counts and percents, and continuous variables were displayed as means, medians, standard deviations, and ranges. Comparisons between changes from baseline were made using McNemar’s test for binary outcomes and paired t tests for continuous variables. To assess dose response, models were fit using analysis of variance (ANOVA) techniques for continuous outcomes or multiple logistic regression for categorical outcomes. Since there were only one or two observations at each dose level for each type of defect, these analyses were more descriptive than inferential. In addition, if certain patients provided more than one defect for analysis, analyses were performed that account for this correlation within patient.

**RESULTS**

All 9 patients completed treatment and experienced no adverse reactions related to treatment. Post-surgical healing was uneventful in the 15 sites involved in the study. Clinically, wound healing appeared enhanced in the PDGF/allograft sites with the gingivae appearing pink, firm, and completely closed 1 week post-operatively.

**Furcation Defects**

The clinical results in furcation defects are shown in Table 1. For this patient group, horizontal PD decreased from a mean of 6.2 ± 0.84 mm to 2.8 ± 0.84 (P < 0.001). Vertical PD (vPD) decreased from a mean of 6.8 ± 1.30 mm to 2.8 ± 0.84 (P = 0.005). CAL decreased from a mean of 7.8 ± 0.84 mm to 4.6 ± 1.67 (P = 0.030). The increase in FGM from a mean of 1.0 ± 1.41 mm to 1.8 ± 1.30 was not statistically significant.

Histologic evaluation was performed for four of the five furcation defects as one specimen was not evaluable due to difficulties encountered during processing. All four remaining defects exhibited periodontal regeneration (complete new attachment apparatus, CNAA: new bone, cementum, and PDL) coronal to the reference notch placed in the base of calculus. Representative photomicrographs are shown in Figures 1C, D, and E. New cementum formation was continuous and progressed from just apical to the notch in one tooth root completely across the fornix without discontinuity. The adjacent PDL was well organized (Fig. 1D) and mature with collagen fibers transversing primarily horizontally from the bone and inserting into the new cementum. The bone was formed in such a manner that the normal PDL space was maintained. The maintenance of the PDL was true even in the fornix of the furcation. 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 the

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**Table 1. Clinical Evaluation: Furcation Defects Treated with rhPDGF-BB Mixed with DFDBA**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (N = 5)</th>
<th>9-Month (N = 5)</th>
<th>Change from Baseline (N = 5)</th>
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<tbody>
<tr>
<td>hPD (mm)</td>
<td>Mean 6.20</td>
<td>2.80</td>
<td>−3.40</td>
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<td></td>
<td>SD 0.84</td>
<td>0.84</td>
<td>0.55</td>
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<td></td>
<td>P value*</td>
<td>&lt;0.001</td>
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<tr>
<td>vPD (mm)</td>
<td>Mean 6.80</td>
<td>2.80</td>
<td>−4.00</td>
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<tr>
<td></td>
<td>SD 1.30</td>
<td>0.84</td>
<td>1.58</td>
</tr>
<tr>
<td></td>
<td>P value*</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>Mean 7.80</td>
<td>4.60</td>
<td>−3.20</td>
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<tr>
<td></td>
<td>SD 0.84</td>
<td>1.67</td>
<td>2.17</td>
</tr>
<tr>
<td></td>
<td>P value*</td>
<td>0.030</td>
<td></td>
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<tr>
<td>FGM (mm)</td>
<td>Mean 1.00</td>
<td>1.80</td>
<td>0.80</td>
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<tr>
<td></td>
<td>SD 1.41</td>
<td>1.30</td>
<td>0.84</td>
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<tr>
<td></td>
<td>P value*</td>
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*P values for change from baseline from paired t tests.
original alveolar bone and ligament. There was no long junctional epithelium, even though a barrier was not used. In summary, the new periodontium was regenerated and physiologic in all respects analyzed.

**Interproximal Defects**

The clinical and radiographic results in interproximal defects are shown in Table 2. For this patient group, PD decreased from a mean of 9.67 ± 1.63 mm to 3.25 ± 1.08 (P < 0.001). Sites receiving ABB-C alone or in combination with a collagen membrane decreased from a mean PD of 8.5 ± 1.29 mm to 2.75 ± 1.26 mm. CAL increased from a mean of 11.08 ± 1.69 mm to 4.92 ± 2.25 mm (P < 0.001) in the PDGF sites compared to ABB-C sites that increased from a mean of 10.5 ± 1.29 mm to 5.25 ± 0.96 mm. Bone height measured radiographically increased from a mean of 7.38 ± 2.48 mm to 9.52 ± 2.16 (P = 0.002) as measured from the root apex in the PDGF sites (Fig. 2B and C, Table 3). Bone height was not assessed radiographically in the ABB-C sites due to the radiopaque nature of this material. There was no significant change in FGM in any group.

Four interproximal defects treated with rhPDGF-BB/allograft exhibited regeneration coronal to the reference notch. Representative photomicrographs are shown in Figures 2D and E and Figures 3A through F. Histologically, the PDGF/allograft sites were characterized by dense new supracrestal bone, a well organized, highly vascularized new PDL, and extensive new cementum coronal to the reference notch. A long junctional epithe

<table>
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<th>Table 2. Clinical and Radiographic Evaluation: Intrabony Defects Treated with rhPDGF-BB/DFDBA</th>
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<tr>
<td><strong>Baseline</strong> (N = 6)</td>
</tr>
<tr>
<td>vPD (mm)</td>
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<tr>
<td>SD</td>
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<td>P value*</td>
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<td>CAL (mm)</td>
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<td>P value*</td>
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<td>FGM (mm)</td>
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<td>P value*</td>
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<tr>
<td>Bone height (mm)</td>
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<td>SD</td>
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<td>P value*</td>
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*P values for change from baseline from paired t tests.

**Combining all rhPDGF/Allograft Defects – Change from Baseline**

Table 3 summarizes the measurements at baseline and at the end of the study (9 months) for all growth factor/allograft-treated defects in the study, and summarizes the results of the paired t tests. For this patient group, hPD (n = 5) decreased from a mean of 6.2 ± 0.84 mm to 2.8 ± 0.84 (P < 0.001). vPD (n = 11) decreased from a mean of 8.36 ± 2.06 mm to 3.05 ± 0.96 (P < 0.001). CAL (n = 11) decreased from a mean
of 9.59 ± 2.15 mm to 4.77 ± 1.92 (P<0.001). Bone height (n = 6) measured radiographically increased from a mean of 7.38 ± 2.48 mm to 9.52 ± 2.16 (P = 0.002). Among all growth factor-treated sites there was an increase in the height of the FGM (n = 11) from a mean of 1.23 ± 1.60 mm to 1.73 ± 1.79 (P = 0.049).

For the outcome of bleeding on probing (BOP), there was no change for the furcation defects (all remained the same) and among the intrabony defects, three out of six improved while the remaining three stayed the same (there was no worsening).

Eight of the 10 evaluable defects that received rhPDGF-BB/DFDBA exhibited regeneration coronal to the reference notch histologically, while two of the four sites that received ABB-C demonstrated regeneration.

**Dose Response**

For the clinical and radiographic measurements, analyses were performed to explore whether there were differences in outcomes based on different concentrations of rhPDGF-BB. These analyses were only exploratory in nature since these were only one or two defects per dose per defect type. General linear models (analysis of variance) were fit using the change from baseline measurement as the outcome. Results of the among group comparisons showed no significant differences between groups (data not shown). All sites healed well and there were no adverse reactions even at the high dose of 5 mg/ml.

**DISCUSSION**

Biomimetics, or tissue engineering, is most effective when conductive matrices and the appropriate tissue growth factors are combined. The hypothesis tested in this study was that combining a thoroughly studied, FDA-approved growth factor, e.g., rhPDGF-BB, with a well known and widely used osteoconductive grafting material, i.e., DFDBA, would lead to favorable clinical and histological outcomes in clinical practice. The rationale was that rhPDGF-BB is a potent mitogen (stimulator of cell proliferation) and chemotactic (causes directed cell migration) protein for PDL fibroblasts and alveolar bone cells and improves angiogenesis (new blood vessel formation) while a bone allograft offers a
biological matrix conducive to cell growth and may contribute osteoinductive bone matrix proteins.

The highest standards of clinical evidence for a periodontal regenerative material are controlled multicenter prospective, randomized masked clinical trials, supplemented with a small number of human biopsies to demonstrate the type of healing that occurs using a particular product. The present study satisfies the latter requirement, which histologically demonstrates periodontal regeneration (CNAA) above a reference notch in the base of the calculus in humans. Regeneration was present in four of six severe interproximal intrabony cases and all four furcation cases treated with the rhPDGF-allograft mixture. All four cases (two interproximal and two furcation) treated with 1.0 mg/ml rhPDGF-BB exhibited remarkable regeneration.

This is the first study to demonstrate periodontal regeneration, including bone, PDL, and cementum documented in human histological specimens of severe class II furcation defects.

The basis for this study was derived from the results of many years of research that clearly document the osteobiologic action of PDGF. PDGF has been shown by numerous investigators to stimulate both the proliferation and recruitment of alveolar bone and PDL cells.12,13,20 In animals, it has been shown that, when PDGF was used with guided tissue regeneration (GTR) in dogs, 100% regeneration was observed within 8 weeks as compared to only 19.2% regeneration using GTR alone.24 Further, an initial human clinical trial demonstrated that application of 0.15 mg/ml of rhPDGF-BB and recombinant human insulin-like growth factor I (rhIGF-I) resulted in a significant improvement in bone fill compared to conventional surgery plus a placebo.25 This initial human clinical trial utilized a methylcellulose gel carrier for the rhPDGF-BB/rhIGF-I combination. While the results were promising, this therapy provided no osteoconductive scaffold to facilitate bone migration, stabilize the clot, and prevent the collapse of the soft tissue.

A clinically attractive feature of PDGF is that long-term administration is not required. In fact, the ideal administration of rhPDGF-BB appears to be either a short burst (much as occurs naturally during blood clot formation), or a series of pulsed applications. In vitro experiments in which cells are continuously exposed to PDGF for long periods of time, osteoblast differentiation is inhibited and proliferation is enhanced.

**Figure 3 (continued).**

D) Higher power view of bottom box (notch area) seen in Figure 3C. Arrow at bottom of photo marks the base of the notch. The bone is undergoing a normal remodeling process from woven bone to the more mature lamellar bone. TR: tooth root; BV: blood vessels; OB: original bone; NB: new bone; NC: new cementum; PDL: new periodontal ligament. (Original magnification ×25.)

E) Higher power view of the middle box seen in Figure 3C. Regeneration of the attachment structures, including bone, periodontal ligament, and cementum, is present. The PDL and bone are both well organized and dense. No inflammation, root resorption, or ankylosis was seen in any of the specimens. TR: tooth root; NB: new bone; NC: new cementum; PDL: new periodontal ligament. (Original magnification ×16.)

F) Higher power view of the top box in Figure 3C. Collagen fibers have physiologic orientation perpendicular and inserting into the root surface. Dense new bone is observed all the way to the crest. The new cementum ends adjacent to the crest of the NB. The junctional epithelium ends (JE arrow) coronal to the new cementum and NB. JE: apical extent of junctional epithelium; CT: gingival connective tissue; NB: new bone. (Original magnification ×25.)
leading to an abundant number of cells, but little mineralized matrix;\textsuperscript{26} in contrast, in in vivo experiments, cells are exposed to PDGF for a short period of time due to the rapid clearance (1 to 4 hours) of exogenous rhPDGF-BB,\textsuperscript{27} so that differentiation is inhibited only transiently and an increase in the number of functional osteoblasts (and PDL cells around teeth) is the predominant result. The resultant increase in osteoblasts and PDL cells, and their migration into the defect as a result of PDGF’s chemotactic properties, leads to improved bone fill and periodontal regeneration.

Allografts are also thoroughly studied and have a long history of clinical use. Bowers and co-workers showed the first large scale human histologic evidence of regeneration using DFDBA. Others have modified DFDBA by adding substances to aid in holding the allograft particles in place. For example, in a study by Francis et al.\textsuperscript{28} paired osseous defects ranging in depth from 3 to 12 mm, received either Grafton\textsuperscript{‡‡} (uses glycerin as the binding agent) or DFDBA alone. Results of both the radiographic analysis and clinical measurements showed that Grafton and DFDBA performed similarly demonstrating a mean defect fill of 69% and 77%, respectively, and reducing probing depths by 4.0 and 4.6 mm, respectively. Both graft types resulted in attachment level gains of 4 mm.

In the present study, both furcations and interproximal angular defects were treated. In all treated defects there was a significant improvement in hPD, vPD, and CAL as well as bone fill as judged radiographically. Changes from the baseline measurements in vPD and CAL for the interproximal intrabony defects may be compared to those observed in a meta-analysis reported by Laurell et al.\textsuperscript{29} by using paired $t$ tests. For the first analysis comparing the results of the current study to the results of the meta-analysis on intrabony defects treated with open flap debridement (OFD) alone, Laurell reported a probing depth reduction from 7.0 mm to 4.0 mm and a CAL gain of 1.5 mm. There was significantly greater reduction in PD ($P = 0.002$) and CAL gain ($P = 0.002$) in the present study compared to the changes reported by Laurell et al.

In a second analysis comparing the results of the current study to the results of the meta-analysis on intrabony defects treated with OFD plus bone grafts, Laurell et al. reported probing depth reduction from 6.9 mm to 4.4 mm and a CAL gain of 2.1 mm. There was significantly greater reduction in PD ($P = 0.002$) and CAL gain ($P = 0.004$) in the current study compared to the changes reported by Laurell et al. While one has to be careful not to overinterpret these comparisons, they do nonetheless provide encouraging evidence of the clinical benefits that may be achieved using a growth factor/allograft tissue engineered product.

In conclusion, this study proved the hypothesis that filling of periodontal defects with a mixture of rhPDGF-BB and DFDBA is safe and most frequently (eight of 10 cases) induces a robust regenerative response in severe human interproximal intrabony and Class II furcation defects. This finding occurred in the absence of the use of GTR membranes. This is the first time that regeneration of a CNAA has been documented histologically in human Class II furcation defects.

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