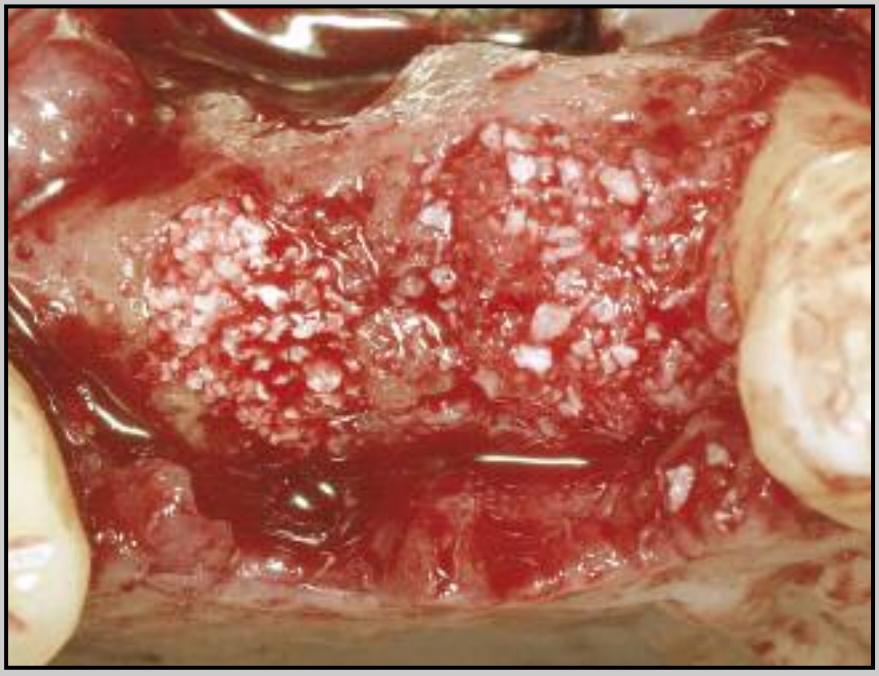
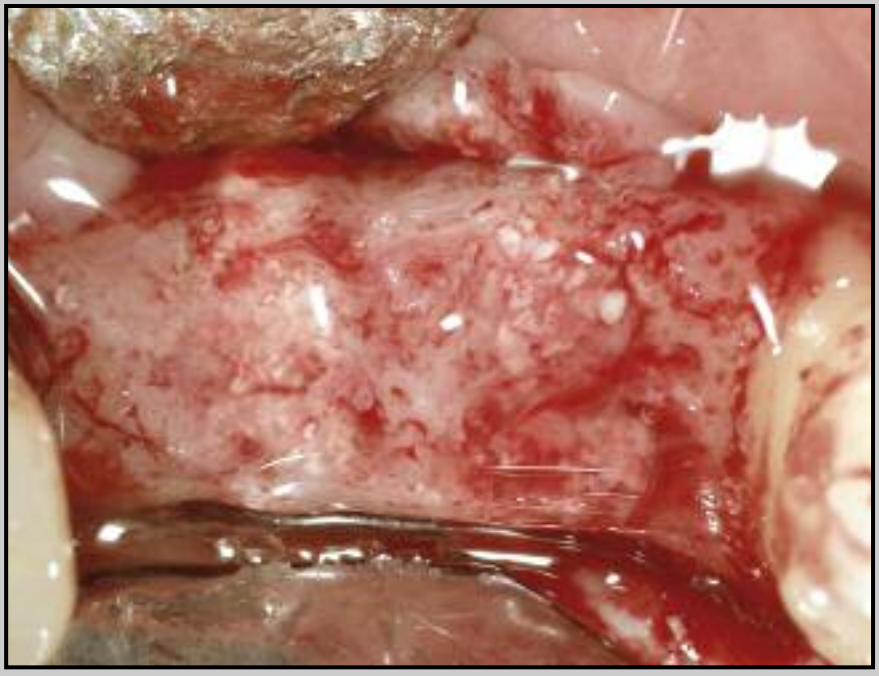




Histologic Evaluation of Recombinant Human Platelet-Derived Growth Factor-BB After Use in Extraction Socket Defects: A Case Series

*Bradley S. McAllister, DDS, PhD**
*Kamran Haghighat, BDS, MS***
*Hari S. Prasad, BS, MDT****
*Michael D. Rohrer, DDS, MS*****

Recent advancements in the arena of therapeutic molecular enhancement have shown favorable clinical findings for periodontics. However, further studies to optimize clinical outcomes using this technology are warranted. Twelve premolar extraction sockets were assigned randomly for treatment with 0.3 mg/mL recombinant human platelet-derived growth factor (rhPDGF-BB) combined with either a collagen containing anorganic deproteinized bovine bone (xenograft) or beta-tricalcium phosphate (β-TCP). Histologic evaluation of extraction socket healing was performed at 3 months. Histologic findings were similar with β-TCP and the xenograft, having 21% and 24% vital bone, respectively. The use of rhPDGF-BB with either β-TCP or a xenograft resulted in uneventful socket healing. At reentry, all implants were placed without the need for further grafting, and 100% implant success was recorded at the time of final evaluation (restoration completion). (Int J Periodontics Restorative Dent 2010;30:365–373.)

*Private Practice, Tigard, Oregon; Assistant Professor, Department of Periodontology, Oregon Health and Science University, Portland, Oregon.
**Private Practice, Tigard, Oregon.
***Senior Research Scientist, Hard Tissue Research Laboratory, University of Minnesota School of Dentistry, Minneapolis, Minnesota.
****Professor, Director of the Hard Tissue Research Laboratory and Oral Pathology Laboratories, University of Minnesota School of Dentistry, Minneapolis, Minnesota.

Correspondence to: Dr Bradley S. McAllister, 11525 SW Durham Rd, Suite D6, Tigard, OR 97224; fax: 503-968-5419; email: mcallister@portlandimplantdentistry.com.

Tooth removal secondary to periodontal disease, caries, or trauma is universally the most common surgical procedure. Evian et al[1] described the histologic sequence of events occurring within the human extraction socket following tooth removal. Many studies, mostly case series, document loss of alveolar bone height and width, especially in areas with thin facial or buccal bony cortices following tooth removal,[2–5] supporting the need for socket preservation through grafting and guided bone regeneration techniques.[5–12] Various approaches have been suggested for the preservation of ridge dimensions following tooth extraction.[6,8,13–15] Despite promoting hard tissue formation, grafting of sockets is shown to delay healing.[16–18] Araújo et al[19] reported recently that placement of a graft into extraction sockets failed to prevent remodeling of the socket walls but did promote de novo bone formation at the margins, which improved the ridge profile. Nevertheless, the current lack of randomized controlled trials, along with a lack of recognized standards of care in the management of extraction sockets, suggests the need for unambiguous

clinical guidelines based on firm scientific evidence.

Universally, however, it is accepted that rapid bone formation within extraction sockets is a desirable outcome when planning immediate or early placement of implants. In a recent canine study, Suba et al[20] suggested that the addition of growth factors within the extraction socket may accelerate bone formation at early time points. In this split-mouth study, beta-tricalcium phosphate (β-TCP) was combined with platelet-rich plasma and placed within premolar extraction sockets (test group). The contralateral extraction sites were grafted with β-TCP alone (control). Histomorphometric data revealed a statistically significantly higher percentage of bone fill at 6 weeks in the test group (45.9%) than in the control group (30.8%). However, by 24 weeks, the percent bone fill in both test and control groups was similar with no statistical differences. The investigators concluded that the addition of platelet-rich plasma to the ceramic osteoconductive matrix may accelerate bone formation within extraction sockets, but suggested that further investigation in humans was warranted. Since more rapid bone formation and volumetric stability within extraction sockets are desirable outcomes when planning immediate or early implant placement, and may prove beneficial for implant stability and subsequent osseointegration, a study examining the effects of growth factor in mediated bone regeneration within extraction sockets at an early time point is appropriate. Therefore, the primary purpose of this study was to examine the histologic and histomorphometric parameters of human bone regeneration within platelet-derived growth factor (PDGF)–grafted extraction sockets.

β-TCP is a purified, multicrystalline, porous form of calcium phosphate with a $Ca:PO_4$ ratio similar to natural bone mineral. Extensive animal and human clinical studies over the past 25 years have demonstrated the biocompatibility of β-TCP, with no published reports presenting any adverse reactions.[21–27] β-TCP fills bone defects physically, preventing the collapse of soft tissue into the bone defect, and provides a matrix or scaffolding for new bone formation, which subsequently undergoes remodeling and finally is replaced by host bone. This has been demonstrated in numerous animal and human clinical studies.[21,23,27–38]

PDGF is the most widely studied growth factor in periodontics. Since the initial observations of PDGF-induced periodontal regeneration (bone, cementum, and periodontal ligament) in the late 1980s,[39] nearly 100 studies have since been published, demonstrating its effects on periodontal ligament and alveolar bone cells as well as regeneration of the periodontium in both animals and humans. These studies have clearly illustrated the action mechanism of PDGF, highlighting the presence of cell surface receptors for PDGF on periodontal ligament and alveolar bone cells, and elucidated PDGF's stimulatory effect on the proliferation and chemotaxis of these cells.[40,41] The use of recombinant human PDGF-BB (rhPDGF-BB) has been shown to promote the regeneration of periodontal tissues, including bone, cementum, and periodontal ligament, in numerous animal studies.[39,42–48] Additionally, a recent clinical trial involving 180 patients demonstrated the safety and effectiveness of the combination of rhPDGF-BB and β-TCP in periodontal defects.[49]

Method and materials

Patient selection

Patients (n = 11) between the ages of 25 and 75 who required maxillary premolar extractions were screened, consented to treatment, and accepted for this study if none of the exclusion criteria outlined in Table 1 were present and they desired an implant-supported crown for replacement of their extracted tooth.

Surgical procedures

Extraction sockets were assigned randomly to treatment with either rhPDGF-BB and β-TCP (Gem21, Osteohealth) or rhPDGF-BB and a xenograft (BioOss Collagen, Osteohealth). Following administration of a local anesthetic, tooth extraction was performed using minimally traumatic techniques followed by socket debridement, and all inter-radicular bone was removed prior to bone graft placement (Fig 1). The graft material was supplied in "kit" form—a cup of β-TCP and a separate syringe containing a solution of rhPDGF-BB (0.3 mg/mL). At the time of surgery, the clinician fully saturated either the β-TCP particles or the substitute

Table 1 **Exclusion criteria for patient selection**

Buccal dehiscence defects > 4 mm from the alveolar crest
Buccal fenestration defects > 4 mm
Periapical pathology, as represented by periapical radiolucencies > 3 mm beyond the root apex
Pregnant women or women intending to become pregnant during the study period
History of cancer or human immunodeficiency virus (HIV)
Allergic to yeast-derived products
Untreated periodontal disease, especially adjacent to a tooth requiring removal
Presence of any medical condition or therapeutic regimen that alters soft or hard tissue healing (eg, osteoporosis, hyperparathyroidism, autoimmune diseases, chemotherapeutic or immunosuppressive agents, steroids, Actonel, Fosamax, or other similar drugs)

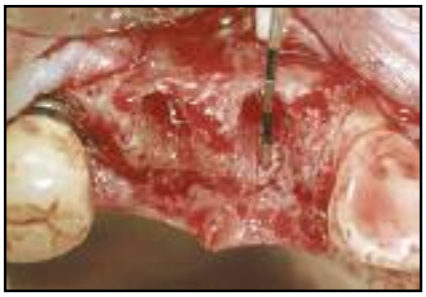


Fig 1 *Teeth were extracted using atraumatic techniques to preserve the alveolar housing.*

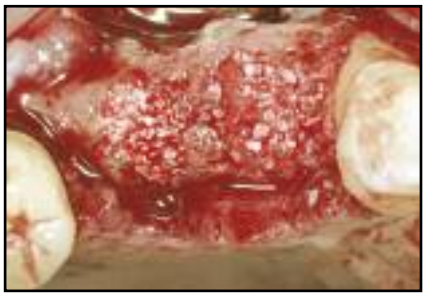


Fig 2 *rhPDGF-saturated bone grafts were placed loosely within the sockets. β-TCP was used at the second premolar site and xenograft collagen at the first premolar site.*

Fig 3 *Three-month postoperative view of the grafted sockets prior to sample harvesting.*

xenograft with the rhPDGF-BB solution. One 0.5-mL rhPDGF syringe was added to completely saturate the graft material (either 0.5 mL of β-TCP, as packaged by the manufacturer, or a portion of the 0.5-mL package of collagen containing the xenograft). There was a sufficient amount of rhPDGF in a single syringe (0.5 mL) to treat each defect so no additional solution was required. After allowing for adequate hydration of the graft and bonding of the rhPDGF-BB (15 minutes), the graft material was packed loosely into the sockets (Fig 2).

The bone graft was covered with a pediculated palatal connective tissue graft for graft containment and primary closure.[50] Suturing was performed using Vicryl 4-0 sutures. Routine postoperative instructions and medications were given and care was taken that no prosthesis came into contact with the area during the first 2 weeks of healing. No patient showed any postoperative complications. Retrieval of biopsy specimens was planned at the time of implant placement, following a 12-week healing phase (Fig 3).

Fig 4a *Specimen treated using a xenograft with rhPDGF-BB. The entire core, harvested at 3 months, is shown (×25 magnification).*

Fig 4b *New bone* (red) *can be seen forming directly on the xenograft particles* (tan) *(×150 magnification).*

Core biopsies

At the 3-month implant placement appointment, a biopsy core was taken with a 2.7-mm internal diameter (3.5-mm external diameter) trephine. Biopsies were left within the trephine and placed in 10% neutral buffered formalin for fixation. Implants were placed in the osteotomy sites created following the trephine core biopsies.

Histologic preparation

Specimens were retrieved and placed in 10% neutral buffered formalin. Upon receipt at the Hard Tissue Research Laboratory, University of Minnesota, specimens were dehydrated with a graded series of alcohols for 9 days. Following dehydration, specimens were infiltrated with a light-curing embedding resin (Technovit 7200 VLC, Kulzer). After 20 days of infiltration with constant shaking at normal atmospheric pressure, specimens were embedded and polymerized by a 450-nm light, with the temperature of the specimens never exceeding 40°C. Specimens were then prepared by the cutting/grinding method of Donath and Breuner.[51,52] Specimens were cut to a thickness of 150 µm on an EXAKT cutting/grinding system (EXAKT Technologies). Following this, cores were polished to a thickness of 45 to 65 µm using a series of 800- to 2,400-grit polishing sandpaper disks using an EXAKT microgrinding system followed by a final polish with 0.3-µm alumina polishing paste. The slides were stained with Stevenel's blue and Van Gieson's picro fuchsin for histologic analysis by means of bright field and polarized microscopic evaluation.

Histomorphometry

Following nondecalcified histologic preparation, the cores were evaluated morphometrically. All cores were digitized at the same magnification using a Zeiss Axiolab microscope and a Nikon Coolpix 4500 digital camera. Histomorphometric measurements were completed using a combination of Adobe PhotoShop (Adobe Systems) and the public domain NIH Image program.[53] At least two slides of each core were evaluated. Parameters evaluated included the total area of the core, the percentage of new bone formation, and the percentage of residual graft material.

Results

Histology

For most specimens, the qualitative core evaluation revealed more new bone in the coronal region of the xenograft specimens (Figs 4a and 4b)

Fig 5a *Specimen treated using β-TCP with rhPDGF-BB. The entire core, harvested at 3 months, is shown (×25 magnification).*

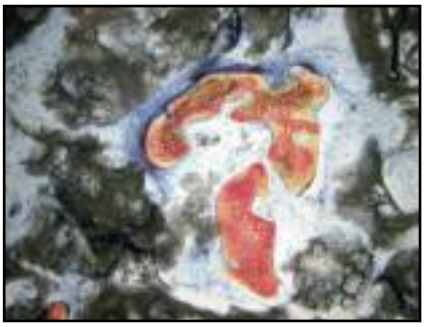
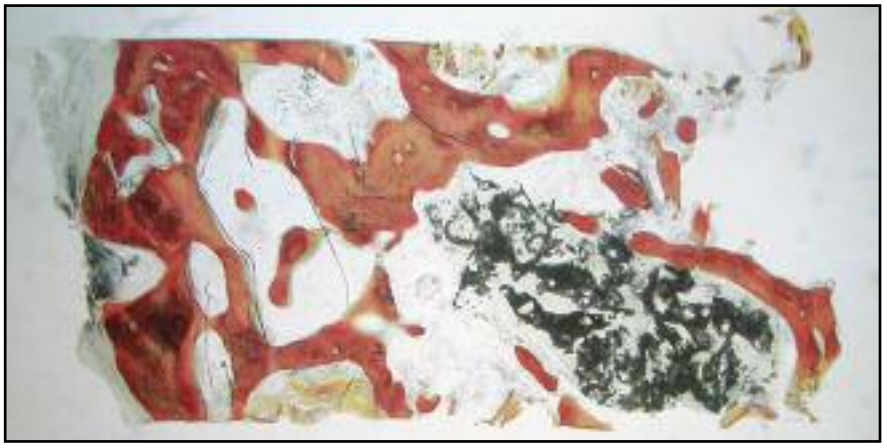


Fig 5b *New bone* (red) *can be seen forming independent of the β-TCP particles* (black) *(×150 magnification).*

Fig 6 *Specimen treated using β-TCP with rhPDGF-BB. The entire core, harvested at 11 months, is shown. New bone* (red) *can be seen forming independent of the* β-*TCP particles* (black). *The β-TCP particles have been replaced largely with bone (×25 magnification).*



compared to β-TCP specimens (Figs 5a and 5b). Most of the vital bone was found in the apical portion for both groups. The bone tended to form through apposition on xenograft particles, whereas with β-TCP, the new bone appeared to be more of a replacement of the graft material. One of the β-TCP sites was unable to initiate the core harvest and implant placement procedure at 3 months. This site's data was not included as part of the histomorphometry results. β-TCP replacement was especially evident in one long-term specimen (11 months). This specimen showed 39% new bone and 10% β-TCP (Fig 6).

Histomorphometry

Treatment with rhPDGF-BB and β-TCP resulted in an average of 21% (range, 15% to 31%) vital bone formation and an average of 24% residual β-TCP particulate graft material (five patients). Treatment with rhPDGF-BB and a xenograft resulted in an average of 24% (range, 10% to 51%) vital bone formation and an average of 17% residual xenograft particulate graft material (six patients).

Discussion

The combination of xenografts and collagen has been used in many oral bone grafting applications.[19,54–56] The presence of collagen dispersed in the mineralized bovine–derived bone grafting material is theorized to enhance the rhPDGF-BB binding capacity and slow its subsequent release, compared to the β-TCP currently marketed as a carrier for rhPDGF-BB. Clearly, further binding and release studies are needed to evaluate potential carriers for this particular bioactive molecule.

While rhPDGF-BB has been shown clearly to offer an effective therapeutic approach for periodontal regeneration applications,[49] the evidence for bone regeneration is limited.[57,58] Simion and colleagues demonstrated, in a small dog study and subsequently in two human studies, that rhPDGF-BB in combination with blocks of deproteinized cancellous bovine bone could result in significant vertical bone regeneration.[57,58] Others, however, have suggested that PDGF in animal models may have either a dose-dependent negative effect on bone formation by negating the actions of growth factors such as osteogenins and reducing graft resorption, or be of insignificant added value in promoting bone regeneration and osseointegration.[59–61] The varied responses reported show the importance of controlled studies to evaluate carriers and the optimal concentration for bone formation.

The qualitative core evaluation in this study revealed more new bone formation in the coronal region of xenograft specimens when compared to β-TCP specimens. Evaluation of the entire core revealed that the percentage of vital bone found was similar for the two groups. However, more vital bone was found in the apical portion of both groups. This is the end result of two contributing factors. First, new bone forms from the apical and lateral aspects of the socket, which is why the central coronal region was the final area to mineralize. This has been demonstrated in animal models.[2,62] Second, the core harvesting technique will not always be in the central region of the socket, nor does it include its most apical extent. The cores are taken from the most desirable implant position based on a prosthetic plan. This means that core harvests taken from healing sockets that previously contained teeth with curved roots will not include sites of new bone formation exclusively. Using maxillary premolars minimized this effect in this study and had the advantage of being able to take cores from the most central region of the healing socket. In contrast, using canines or incisors has the

disadvantage of prosthetic demands, requiring the core be taken from a position slightly palatal to the central region of the socket. While a more challenging defect, such as the presence of a buccal dehiscence, is more appealing, these types of defects are not as uniform and tend to occur most commonly in patients with a previous history of inflammatory disease or therapy, such as orthodontics. With conservative extraction techniques that aim at preservation of the buccal plate, the number of patients exhibiting significant bony defects become limited in number, thus making the premolar socket a realistic model. It is also acknowledged that such extraction socket defects heal uneventfully with normal bony infill in the absence of a bone graft.

Conclusion

Within the limits of this study, PDGF, when combined with either β-TCP or a xenograft and placed into premolar extraction socket defects, resulted in a similar volume of bone and adequate ridge preservation that allowed for successful implant placement and subsequent restorations. Clearly, further studies with a larger sample size and controls are warranted to assess the impact of various growth factors, including PDGF, on bone growth within extraction sockets and other alveolar defects frequently encountered during implant reconstruction.

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