

Minimally Invasive Alveolar Ridge Augmentation Procedure (Tunneling Technique) Using rhPDGF-BB in Combination with Three Matrices: A Case Series



Marc L. Nevins, DMD, MMSc¹/Marcelo Camelo, DDS²
 Myron Nevins, DDS³/Peter Schupbach, PhD⁴
 Bernard Friedland, BChD, MSc, JD⁵
 Joao Marcelo Borges Camelo, DDS⁶/David M. Kim, DDS, DMSc⁷

This study investigated a minimally invasive surgical procedure for alveolar ridge augmentation that combined recombinant human platelet-derived growth factor BB (rhPDGF-BB) and three different matrices. The minimally invasive tunneling ridge augmentation procedure was applied to 12 patients randomized into three groups: rhPDGF-BB (0.3 mg/mL) was combined with freeze-dried bone allograft (FDBA; group A), anorganic bovine bone graft (ABBG; group B), or anorganic bovine bone graft/mineralized collagen bone substitute (ABBG/MCBS; group C). Computed tomography (CT) scans were obtained presurgically and prior to 14-week re-entry surgery. Clinical reentry revealed adequate bone volume to place implants in all patients in groups A and B and two of four patients in group C. Trepine core biopsies were obtained and evaluated by microCT, backscatter scanning electron microscopy (BE-SEM), and light microscopy. New bone formation was consistently observed with BE-SEM and histologic analysis for group A and B specimens. Newly formed woven and lamellar bone were in close contact with graft particles. The ABBG/MCBS specimens (group C) had more variable results, with fibrous encapsulation of graft particles and limited histologic evidence of new bone formation. Within the limits of this study, the FDBA and ABBG carriers appear to be appropriate scaffolds to deliver rhPDGF-BB for ridge augmentation via minimally invasive surgical techniques. (Int J Periodontics Restorative Dent 2009;29:371–383.)

¹Assistant Clinical Professor, Division of Periodontology, Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, Massachusetts; Private Practice, Boston Periodontics and Dental Implants, Boston, Massachusetts.

²Director, Institute for Advanced Dental Studies, Belo Horizonte, Brazil.

³Associate Clinical Professor, Division of Periodontology, Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, Massachusetts.

⁴Adjunct Professor, Department of Periodontics, School of Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

⁵Assistant Professor, Division of Oral and Maxillofacial Radiology, Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, Massachusetts.

⁶Private Practice, Institute for Advanced Dental Studies, Belo Horizonte, Brazil.

⁷Assistant Professor, Division of Periodontology, Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, Massachusetts.

Correspondence to: Dr Marc Nevins, 175 Cambridge Street, Suite 310, Boston, MA 02114; fax: +617-720-0836; email: marc_nevins@hms.harvard.edu.

Traditional surgical techniques to correct alveolar ridge deficiencies to place dental implants include guided bone regeneration (GBR), onlay block grafting, ridge splitting, forced eruption, and distraction osteogenesis.^{1–8} These procedures have been used frequently after dental extractions.^{9–16}

Techniques of GBR, which utilize barrier membranes to exclude epithelium and connective tissue, are predictable procedures to provide ridge augmentation.^{3,5,6,17–19} However, this surgical approach may be associated with wound dehiscences, postsurgical swelling, and edema, and often requires a secondary surgical site to harvest the autogenous bone graft. Thus, current surgical trends lean toward minimally invasive surgical technologies that result in minimal surgical trauma, postoperative discomfort, and morbidity. These techniques have been reported for periodontal regeneration and alveolar ridge augmentation procedures.^{20–25}

Lateral ridge augmentation with subperiosteal tunneling is performed with a small incision and minimal tissue dissection and flap reflection to gain access to a defect site without disrupting the soft tissue profile.^{26–31}

This surgical approach appears to reduce postoperative discomfort and swelling. A number of variations on the subperiosteal tunneling technique have been attempted and reported, with particulate hydroxyapatite and particulate human mineralized bone allograft used to enhance thin alveolar ridges.^{26,28,29} Decortication of the recipient site and the use of a resorbable collagen membrane in conjunction with anorganic bovine bone graft have been reported.²⁹

Recombinant human platelet-derived growth factor BB (rhPDGF-BB) is a natural biologic molecule that mediates and regulates key cellular events such as cell proliferation, chemotaxis, and matrix synthesis by binding to specific cell-surface receptors.³²⁻³⁵ The wound-healing response is activated when PDGF is released locally during clotting by platelets at the site of soft and hard tissue injury.³⁶ Once released from the platelets, PDGF binds to cell surface receptors to promote rapid cellular migration (chemotaxis) and proliferation (mitogenesis).^{36,37} It is proangiogenic in that it acts in synergy with endogenous vascular epithelial growth factor to stimulate neovascularization at the defect site.³⁸⁻⁴⁰

Preclinical and clinical studies, including a large-scale randomized controlled trial, have demonstrated the clinical safety and efficacy of rhPDGF-BB-mediated therapy in both periodontal regeneration and implant site development.⁴¹⁻⁵⁸ These studies of ridge augmentation have provided evidence of the periosteal influence on rhPDGF-BB-mediated osteogenesis.⁵⁵⁻⁵⁸

The present study evaluated a minimally invasive ridge augmentation procedure (tunneling technique) that used rhPDGF-BB in combination with three particulate scaffolds, including freeze-dried bone allograft (FDBA), anorganic bovine bone graft (ABBG), and mineralized collagen bone substitute (MCBS).

Method and materials

This investigation was designed and implemented as a single-center, prospective, open-label clinical study. Subjects were selected for enrollment from the population of patients requesting dental implant placement in the maxillary anterior region and presenting with a ridge that was inadequate in width to place 4-mm-diameter dental implants. To be eligible, prospective subjects had to be between the ages of 18 and 70 years and willing and able to follow the study protocols. Subjects signed informed consent documents according to the Declaration of Helsinki. In addition, subjects were excluded from entry into the study if (1) they presented with systematic disorders that would prevent them from undergoing surgery or (2) were current smokers (within 6 months of entry into the study). Pregnant women were also excluded from the study.

At the screening visit, the eligibility criteria were assessed with reviews of dental and medical histories, along with extraoral and intraoral examinations. Once the eligibility criteria were met, the patient was scheduled for clinical photographs, periapical

radiographs, dental computed tomography (CT) scan, and dental prophylaxis, and was scheduled for the surgery.

Alveolar ridge augmentation was performed in the maxillary anterior region with local anesthesia (2% lidocaine with 1:100,000 epinephrine). A minimally invasive tunneling ridge augmentation procedure using two short vertical releasing incisions adjacent to the site of augmentation was performed. The buccal tissue was carefully dissected and lifted from the alveolar crest with a periosteal elevator to form a subperiosteal pouch. Decortication of the buccal plate was performed manually with a custom-fabricated decorticator to stimulate bleeding into the defect and allow for access to progenitor cells and angiogenesis.

Prior to grafting, the 12 subjects were randomized into three experimental groups (Table 1).

- Group A: FDBA (The University of Miami Tissue Bank) hydrated with rhPDGF-BB (Gem21S, Osteohealth)
- Group B: ABBG (Bio-Oss, Osteohealth) hydrated with rhPDGF-BB
- Group C: ABBG/MCBS (Bio-Oss Collagen, Osteohealth) hydrated with rhPDGF-BB

In groups A and B, 1 g of collagen (Hemostop, Technew) was mixed with graft material to enhance its handling characteristics.

The grafts were delivered to the deficient area in a syringe (3 mL, Embraplast) (Fig 1). Following its insertion, the graft was packed and condensed until stable. Primary wound coaptation was achieved with simple

Table 1 Breakdown of subject enrollment and the experimental groups

Group/ Subject	Age	Gender	Site(s)*	Graft (g)
Group A				
301	45	F	12,11,21,22	2 g FDBA
305	51	F	11,21,22	3.5 g FDBA
308	29	F	12,11,21,22	3 g FDBA
310	56	F	12,11,21,22	3.5 g FDBA
Group B				
302	37	M	12,11,21,22	3 g ABB
303	26	M	12,11,21,22	3 g ABB
307	45	M	12,11,21,22	3 g ABB
309	40	F	12,11,21,22	3 g ABB
Group C				
304	31	F	12,11	2 g ABB/1 g MCBS
306	39	M	14-11	2 g ABB/1 g MCBS
311	47	F	22	2 g ABB/0.25 g MCBS
312	39	F	12,11,21,22	2 g ABB/0.25 g MCBS

Two milliliters of rhPDGF-BB were used in each patient in combination with the selected graft material.

*FDI tooth-numbering system used.

Fig 1 Delivery of the composite graft via syringe.



interrupted sutures (nylon sutures, Ethicon). Subjects were instructed to take oral antibiotics (amoxicillin 500 mg three times daily for 7 days) and ibuprofen (800 mg four times daily for 3 days), use a mouth rinse (0.12% chlorhexidine two times a day), and avoid brushing of the treated site for 2 weeks. They were also provided instructions on when to return for follow-up visits, as indicated in the

protocol. Oral hygiene instruction was reviewed at each visit. The 3-month visit included periapical radiographs, a CT scan, and dental prophylaxis.

Surgical reentry of the treatment site was performed 3.5 months (14 weeks) after augmentation surgery under local anesthesia (2% lidocaine with 1:100,000 epinephrine) with the elevation of a full-thickness mucoperiosteal flap. Trephine drills were used to

obtain core biopsies (minimum 2 mm wide by 7 mm deep) from the center of the treated area as well as the lateral surface (horizontal core). These non-decalcified bone cores were scanned, and the data were quantified using three-dimensional (3D) microCT (μ CT 40, Scanco Medical). Dental implants were placed in the augmented sites when primary stability could be achieved.

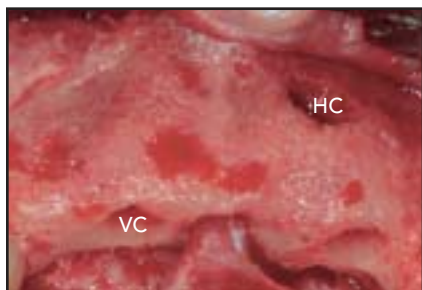


Fig 2 (left) Well-incorporated bone particulate was noted in group A sites. HC = horizontal core; VC = vertical core.

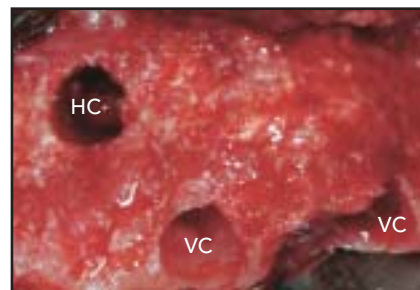


Fig 3 (right) Visual identification of the graft particles in group B sites. HC = horizontal core; VC = vertical core.

The primary outcome variable was bone quality and quantity assessed by light microscopy, histomorphometry, and microCT and backscatter SEM. The secondary outcome variables included bone volume, bone quality, soft tissue healing, and incidence of unanticipated adverse healing events.

Results

Twelve patients (four men with a mean age of 37.35 years and eight women with a mean age of 42.25 years) were enrolled based on the inclusion criteria and treated with the tunneling technique for ridge augmentation. There were no serious adverse events during the course of the study.

Clinical Results

Early wound healing demonstrated typical postsurgical sequelae, such as localized facial edema and gingival/mucosal erythema. Two patients in

group B and one patient in group C showed fenestration of soft tissues at the 7-day postoperative visit. All of the sites subsequently healed, with complete wound closure by the 28-day postoperative visit. The fenestrations occurred at the buccal aspect of the grafted sites, where the continuity of the mucosa had been compromised by instrumentation during elevation or condensing of the graft particulate.

Group A

Clinical examination of the group A patients at the 14-week postoperative visit revealed enhancement of ridge width in all four patients, as confirmed by the 12-week CT scan. The newly grafted bone was more radiopaque than the native bone. However, it was obvious that the ridge shape lacked uniformity, with significant irregularity of the surface contour.

Surgical reentry demonstrated well-incorporated bone particulate presenting increased ridge volume with minor surface irregularities and partial undercuts in the apical third of the

ridge (Fig 2). The regenerated bone appeared to be firm on the surface, but variable bone density was noticed at the time of implant site preparation. All planned implants were placed.

Group B

Clinical examination of the group B patients at the 14-week postoperative visit revealed significant enhancement of the ridge width in all four patients. The CT images evidenced well-incorporated graft material. Flap elevation and removal of soft tissues revealed enhanced edentulous ridge form, with clinical quality consistent with new bone (Fig 3). The augmented sites allowed for implant placement in all patients.

Group C

Clinical examination of the group C patients at the 14-week postoperative visit revealed moderate enhancement of the ridge width in all four patients. The graft particulate was embedded in the soft tissue flap and the lack of incorporation noted on the CT scan (Fig 4).

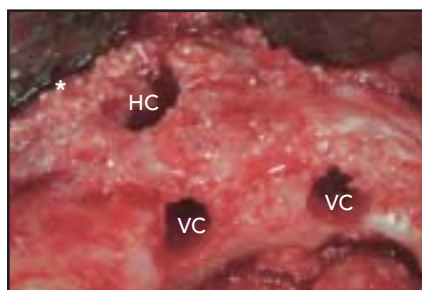
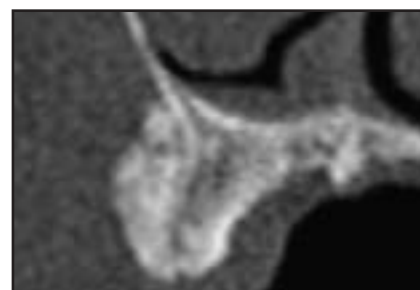


Fig 4 Lack of incorporation (asterisk) of the graft material to the host bone in group C sites. HC = horizontal core; VC = vertical core.



Figs 5a and 5b Group A patient (#310): CT scans obtained (left) preoperative and (right) at 12 weeks postoperative.

Two of the four patients showed no increase in ridge width, prohibiting implant placement.

Quantitative CT scan evaluation

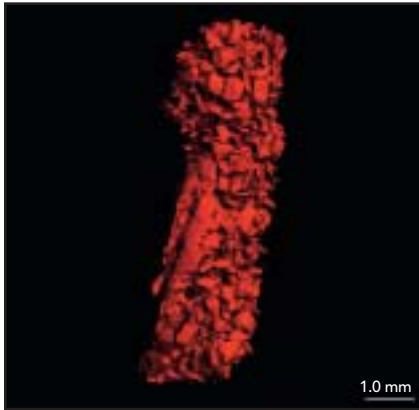
Postsurgical CT scans indicated that there was a volumetric increase at the defect sites in all three groups (Fig 5). Measurements were performed by an independent (blinded) radiologist to compare presurgical to postsurgical defect dimensions, evaluating for a width that would be sufficient for implant placement. The height of the ridge extending to a 6-mm width was evaluated (Table 2). Eleven of the 12 CT scans, including 20 of the 23 potential implant sites, were available for evaluation. The other three readings were discarded owing to poor CT scan technique.

Table 2 Preoperative and postoperative defect dimensions, as derived from CT scans

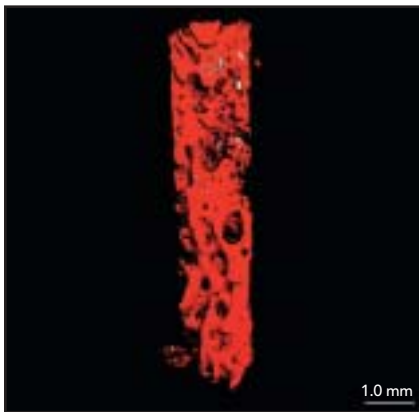
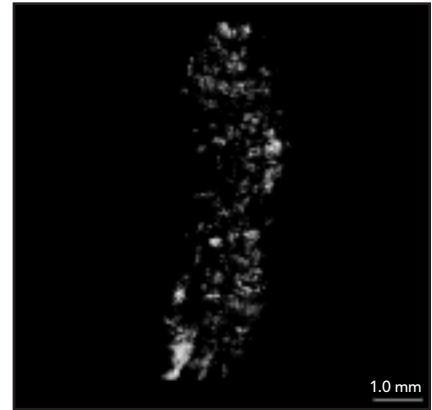
Subject	Tooth no. [†]	Defect dimensions*	
		Preop	Postop
Group A			
301	11	9.6	15.8
	22	15.8	16.2
305	12	6.2	10.7
	22	6.1	10.3
308	11	8.6	13.1
	22	6.3	11.9
310	11	5.0	13.2
	22	4.8	12.1
Group B			
302	12	4.5	3.9
	22	4.1	10.1
303	11	5.7	12.2
	22	3.2	9.8
307	11	6.4	12.8
	21	8.2	11.3
309	11	4.2	9.5
	22	3.4	9.5
Group C			
304	11	NA	NA
	21	NA	NA
306	13	9.6	8.0
	11	6.6	4.0
311	23	1.4	11.8
312	11	1.8	21.0
	21	2.1	18.7

*Width at 6 mm.

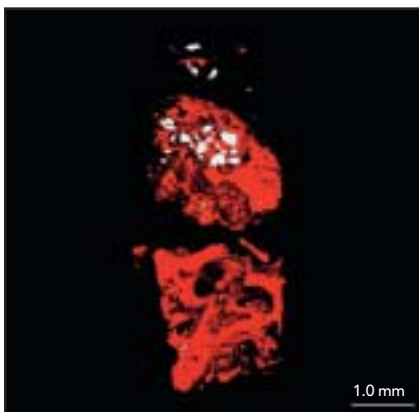
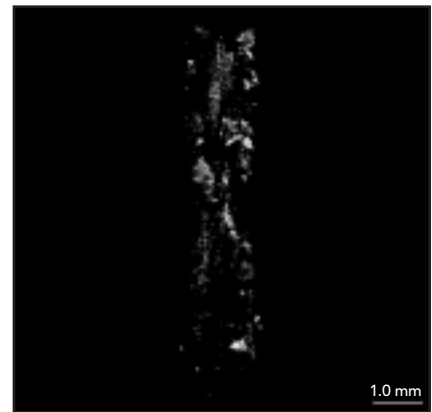
[†]FDI tooth-numbering system used.



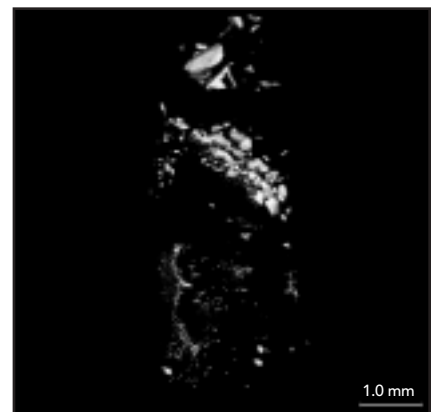
Figs 6a and 6b MicroCT representation of sample from patient 310 (group A). Red = host bone; white = FDDB particles.



Figs 7a and 7b MicroCT representation of sample from patient 302 (group B). Red = host bone; white = ABBG particles.



Figs 8a and 8b MicroCT representation of sample from patient 306 (group C). Red = host bone; white = ABBG/MCBS particles.



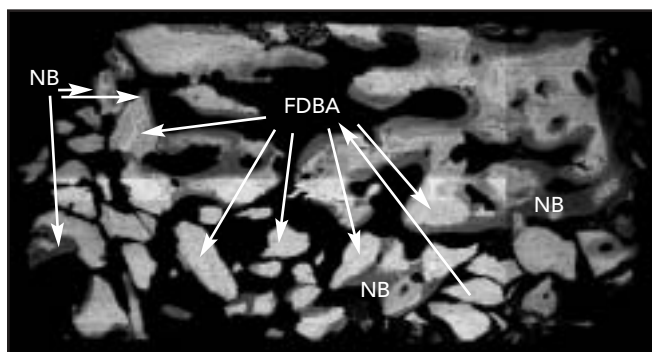


Fig 9a Compositing image of BE-SEM views of an FDDB core (patient 310/group A). The grey levels indicate different degrees of mineralization. The FDDB chips can be distinguished from newly formed bone (NB).

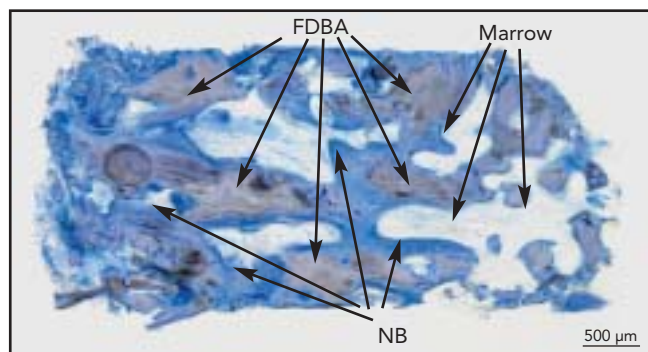
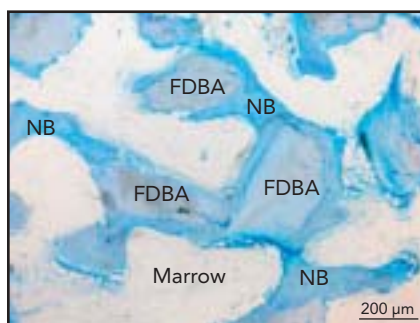
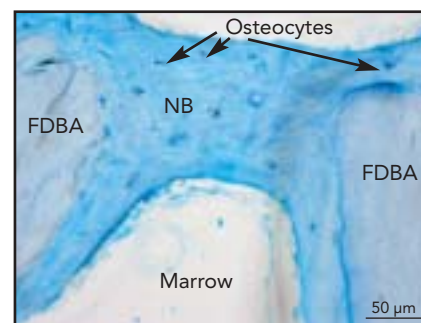


Fig 9b Light microscopic view of the same core shown in Fig 9a. Note the bridges of newly formed bone between the FDDB particles.



Figs 9c (left) and 9d (right) Higher magnifications of the same FDDB chips and newly formed bone surrounding them, indicating the osteoconductive bone formation.



MicroCT and histomorphometric analyses

The microCT evaluation could not differentiate between local bone, newly formed bone, and graft material because the grey levels were too similar (Figs 6 to 8). Thus, only the total amount of mineralized tissue per core was evaluated by microCT. The mean percentage (\pm SD) of mineralized tissue

in group A was $34.6\% \pm 8.7\%$; in group B it was $38.2\% \pm 8.7\%$; and in group C it was $52.9\% \pm 12.9\%$.

MicroCT evidenced intense osteogenesis with extensive new bone formation. It was possible to isolate remaining matrix. The graft particulate was apparent to varying degrees in all of the samples.

BE-SEM and histologic evaluation

Group A

BE-SEM and histologic evaluations showed FDDB chips and newly formed cancellous bone surrounding them and between them, indicating osteoconductive bone formation (Fig 9a). Bone formation was ongoing at the time of biopsy sampling (Figs 9b to 9d).

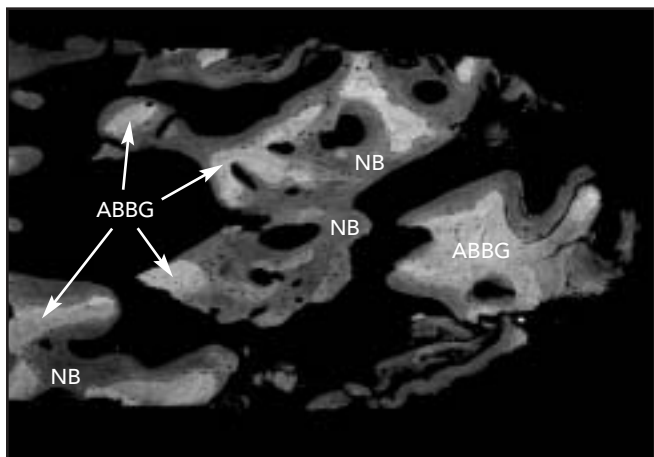


Fig 10a Compositd image of BE-SEM of an ABBG core (patient 302/group B). The grey levels indicate different degrees of mineralization. The brighter the signal, the denser the material; this distinguishes ABBG remnants from newly formed bone (NB).

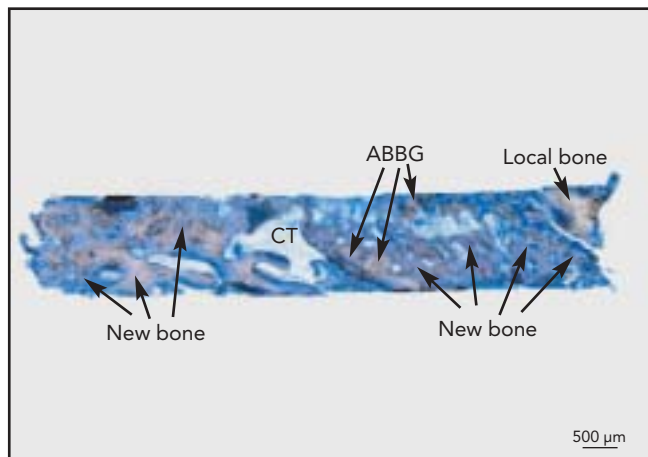


Fig 10b Light microscopic view of the same core shown in Fig 10a. CT = connective tissue.

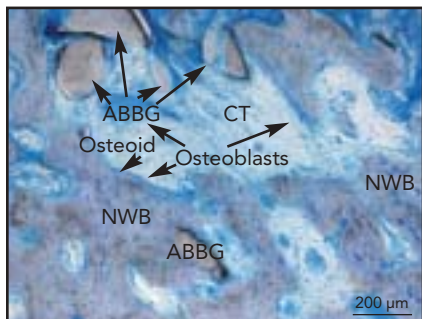
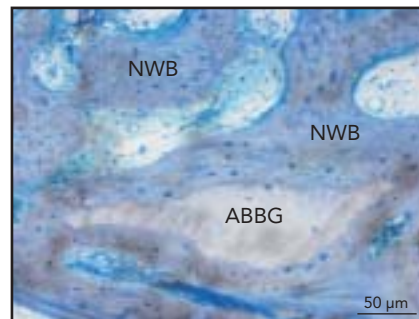


Fig 10c (left) Higher-power view. NWB = newly formed woven bone; CT = connective tissue.

Fig 10d (right) Newly formed woven bone (NWB) can be observed maturing into bone trabeculae completely surrounding ABBG particles.



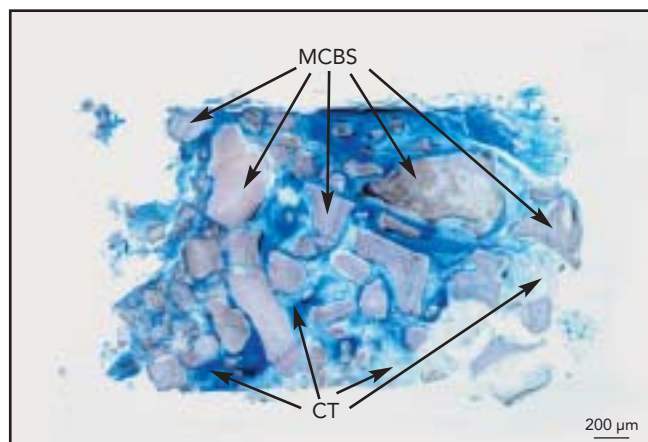
Group B

Group B specimens showed the presence of ABBG surrounded by new bone. BE-SEM grey levels indicated the degree of mineralization (Fig 10a). Light microscopy demonstrated intensive new bone formation with varying degrees of maturation, including newly formed woven bone and lamellar bone forming bridges between ABBG particles (Figs 10b to 10d). Some specimens demonstrated new bone formation within the ABBG particles.

Group C

Group C results varied with location of the bone core. Multiple cores demonstrated MCBS particles surrounded by connective tissue (Fig 11). Newly formed bone was observed in one specimen. Other specimens showed solid bone with limited remnants of MCBS particles.

Fig 11 Light microscopic view of the fibrous encapsulation of the mineralized collagen bone substitute (MCBS) particles. CT = connective tissue.



Implant placement and success

Twenty implants were placed into the trephined biopsy sites at the 14-week postoperative visit (seven implants in group A, eight implants in group B, and five implants in group C). Implant placement was not possible in two patients in group C owing to immature bone quality and/or limited bone volume. Three implants in group A had to be removed because of mobility and buccal dehiscence at 6 months (one implant) and 8 months (two implants). The implants were removed prior to beginning the restorative phase of treatment. The patients used provisional removable partial denture appliances prior to restoration. The remaining implants were clinically and radiographically stable at the 12-month evaluation.

Discussion

There is a desire for minimally invasive surgery that offers a predictable and simple method to augment localized edentulous ridge defects.²¹ The present authors used a tunneling technique to obtain access to deficient ridges (maxillary anterior region) with minimally invasive incisions and minimal tissue elevation. This technique reduces the possibility of postoperative soft tissue loss or deformity.

All patients enrolled in the study presented with ridge defects that prohibited implant placement. They were segregated into three different treatment groups based on combinations of scaffolds and rhPDGF-BB. All eight patients in groups A (FDBA and rhPDGF-BB) and B (ABBG and rhPDGF-BB) demonstrated consistent increases in ridge dimension and graft maturity, and implant placement proceeded in all potential implant sites.

The concept of stabilization and intimate adaptation of a membrane to the surrounding bone to create a secluded space and to prevent the ingrowth of competing nonosteogenic cells into the defect area has been challenged recently by the results that have been obtained with rhPDGF-BB therapy.^{5,53,55,57,59} Preclinical and clinical studies have supported the use of xenografts without a membrane for significant ridge augmentation in both horizontal and vertical defects.^{53,55,56,57} It is thought that a membrane may inhibit progenitor cell migration and angiogenesis by presenting a physical barrier to chemotaxis. To achieve graft stability without a membrane, there was a need for a large volume of the graft material in each treatment site (2 to 3 g), compared to that reported by Hasson (1 to 1.5 g).²⁹

New bone formation was consistently observed with BE-SEM and histologic analysis for groups A and B

specimens. Newly formed woven and lamellar bone were consistently observed in close contact with graft particles in groups A and B specimens. The FDBA and ABBG particulate grafts appear to act as sufficient scaffolds to deliver the rhPDGF-BB signaling molecules to the recipient site and allow for normal new bone growth to enhance the edentulous ridge. In contrast, the MCBS specimens (group C) had more variable results, with fibrous encapsulation of graft particles and limited histologic evidence of new bone formation.

On reentry, the augmented ridges presented with variable contours and undercuts beyond the new bone. It appears that the use of a membrane might be reconsidered for the benefit of improving graft containment and the contour of the final grafted sites with this technique.

Qualitative assessment of the sites via CT scan revealed findings consistent with the clinical results but lacked the capability to discern radiolucent encapsulation of opaque graft materials. Therefore, in this study CT scans were able to measure bone augmentation quantitatively, but their ability to assess bone quality was limited. It appears that a combination of qualitative and quantitative assessment is necessary when using opaque grafting materials.

The time point of reentry for this study was only 14 weeks postoperative. This is an early time point compared with other clinical studies of particulate bone grafting.³⁻⁶ Nevins et al reentered extraction sites grafted with MCBS and rhPDGF-BB at 16 weeks and reported mature bone healing.⁵⁹

Fiorellini et al demonstrated mature healing of extraction sites treated with recombinant human bone morphogenetic protein-2 after 16 weeks.¹⁵ The present study demonstrated clinically immature tissue in some sites upon reentry. The histologic results demonstrated ongoing bone formation and remodeling, and it is possible that a longer healing period would enhance implant site development.

The limitations of the present study were the small sample size for each group and a lack of control groups (grafting group without the rhPDGF-BB or grafting group with the use of membrane). However, several previously reported studies have already investigated these control groups and have obtained similar results.²⁶⁻³⁰ Future studies are needed with larger sample sizes to verify the scientific value of these promising preliminary results.

Conclusion

Within the limits of this study, the freeze-dried bone allograft and anorganic bovine bone graft carriers appeared to be appropriate scaffolds to deliver recombinant human platelet-derived growth factor BB for ridge augmentation using minimally invasive surgical techniques.

Acknowledgment

This study was supported by a grant from Osteohealth.

References

1. Hämmerle CH, Karring T. Guided bone regeneration at oral implant sites. *Periodontol 2000* 1998;17:151–175.
2. Salama H, Salama M. The role of orthodontic extrusive remodeling in the enhancement of soft and hard tissue profiles prior to implant placement: A systematic approach to the management of extraction site defects. *Int J Periodontics Restorative Dent* 1993;13:312–333.
3. Buser D, Dula K, Belser UC, Hirt HP, Berthold H. Localized ridge augmentation using guided bone regeneration. I. Surgical procedure in the maxilla. *Int J Periodontics Restorative Dent* 1993;13:29–45.
4. Nevins M, Mellonig JT. The advantages of localized ridge augmentation prior to implant placement: A staged event. *Int J Periodontics Restorative Dent* 1994;14:96–111.
5. Buser D, Dula K, Belser UC, Hirt HP, Berthold H. Localized ridge augmentation using guided bone regeneration. II. Surgical procedure in the mandible. *Int J Periodontics Restorative Dent* 1995;15:10–29.
6. Buser D, Dula K, Hirt HP, Schenk RK. Lateral ridge augmentation using autografts and barrier membranes: A clinical study with 40 partially edentulous patients. *J Oral Maxillofac Surg* 1996;54:420–432.
7. Oda T, Sawaki Y, Ueda M. Experimental alveolar ridge augmentation by distraction osteogenesis using a simple device that permits secondary implant placement. *Int J Oral Maxillofac Implants* 2000;15:95–102.
8. Sethi A, Kaus T. Maxillary ridge expansion with simultaneous implant placement: 5-year results of an ongoing clinical study. *Int J Oral Maxillofac Implants* 2000;15:491–499.
9. Lam RV. Contour changes of the alveolar processes following extraction. *J Prosthet Dent* 1960;10:25–32.
10. Pietrokovski J, Massler M. Alveolar ridge resorption following tooth extraction. *J Prosthet Dent* 1967;17:21–27.
11. Johnson K. A study of the dimensional changes occurring in the maxilla following closed face immediate denture treatment. *Aust Dent J* 1969;14:370–376.
12. Cardaropoli G, Araújo M, Lindhe J. Dynamics of bone tissue formation in tooth extraction sites. An experimental study in dogs. *J Clin Periodontol* 2003;30:809–818.
13. Kao RT. Periodontal regeneration and reconstructive surgery. In: Rose LF, Mealey BL, Genco RJ, Cohen DW (eds). *Periodontics: Medicine, Surgery, and Implants*. St Louis: Elsevier Mosby, 2004:572–609.
14. Araújo MG, Lindhe J. Dimensional ridge alterations following tooth extraction. An experimental study in the dog. *J Clin Periodontol* 2005;32:212–218.
15. Fiorellini JP, Howell TH, Cochran D, et al. Randomized study evaluating recombinant human bone morphogenetic protein-2 for extraction socket augmentation. *J Periodontol* 2005;76:605–613.
16. Nevins M, Camelo M, De Paoli S, et al. A study of the fate of the buccal wall of extraction sockets of teeth with prominent roots. *Int J Periodontics Restorative Dent* 2006;26:19–29.
17. Buser D, Brägger U, Lang NP, Nyman S. Regeneration and enlargement of jaw bone using guided tissue regeneration. *Clin Oral Implants Res* 1990;1:22–32.
18. Schenk RK, Buser D, Hardwick WR, Dahlin C. Healing pattern of bone regeneration in membrane-protected defects: A histologic study in the canine mandible. *Int J Oral Maxillofac Implants* 1994;9:13–29.
19. Aghaloo TL, Moy PK. Which hard tissue augmentation techniques are the most successful in furnishing bony support for implant placement? *Int J Oral Maxillofac Implants* 2007;22:49–70.
20. Harrel SK. A minimally invasive surgical approach for periodontal bone grafting. *Int J Periodontics Restorative Dent* 1998;18:161–169.
21. Harrel SK, Nunn ME, Belling CM. Long-term results of a minimally invasive surgical approach for bone grafting. *J Periodontol* 1999;70:1558–1563.
22. Harrel SK, Wilson TG, Nunn ME. Prospective assessment of the use of enamel matrix proteins with minimally invasive surgery. *J Periodontol* 2005;76:380–384.
23. Cortellini P, Tonetti MS. Minimally invasive surgical technique and enamel matrix derivative in intra-bony defects. I: Clinical outcomes and morbidity. *J Clin Periodontol* 2007;34:1082–1088.
24. Cortellini P, Tonetti MS. A minimally invasive surgical technique with an enamel matrix derivative in the regenerative treatment of intra-bony defects: A novel approach to limit morbidity. *J Clin Periodontol* 2007;34:87–93.
25. Cortellini P, Nieri M, Prato GP, Tonetti MS. Single minimally invasive surgical technique with an enamel matrix derivative to treat multiple adjacent intra-bony defects: Clinical outcomes and patient morbidity. *J Clin Periodontol* 2008;35:605–613.
26. Kent JN, Quinn JH, Zide MF, Guerra LR, Boyne PJ. Alveolar ridge augmentation using nonresorbable hydroxylapatite with or without autogenous cancellous bone. *J Oral Maxillofac Surg* 1983;41:629–642.
27. Vanassche BJ, Stoelinga PJ, de Koomen HA, Blijdorp PA, Schoenaers JH. Reconstruction of the severely resorbed mandible with interposed bone grafts and hydroxylapatite. A 2- to 3-year follow-up. *Int J Oral Maxillofac Surg* 1988;17:157–160.
28. Block MS, Degen M. Horizontal ridge augmentation using human mineralized particulate bone: Preliminary results. *J Oral Maxillofac Surg* 2004;62:67–72.
29. Hasson O. Augmentation of deficient lateral alveolar ridge using the subperiosteal tunneling dissection approach. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007;103:e14–e19.
30. Kfir E, Kfir V, Eliav E, Kaluski E. Minimally invasive guided bone regeneration. *J Oral Implantol* 2007;33:205–210.
31. Jeong SM, Choi BH, Li J, Xuan F. Simultaneous flapless implant placement and peri-implant defect correction: An experimental pilot study in dogs. *J Periodontol* 2008;79:876–880.

32. Heldin CH, Ostman A, Rönstrand L. Signal transduction via platelet-derived growth factor receptors. *Biochim Biophys Acta* 1998;1378:F79–F113.
33. Rosenkranz S, Kazlauskas A. Evidence for distinct signaling properties and biological responses induced by the PDGF receptor alpha and beta subtypes. *Growth Factors* 1999;16:201–216.
34. Cooke JW, Sarment DP, Whitesman LA, et al. Effect of rhPDGF-BB delivery on mediators of periodontal wound repair. *Tissue Eng* 2006;12:1441–1450.
35. Heldin CH. Platelet-derived growth factor—An introduction. *Cytokine Growth Factor Rev* 2004;15:195–196.
36. Lynch SE, Genco RJ, Marx RE (eds). *Tissue Engineering: Applications in Maxillofacial Surgery and Periodontics*. Chicago: Quintessence, 1999:1–297.
37. Lynch SE. Introduction. In: Lynch SE, Marx RE, Nevins M, Wisner-Lynch LA (eds). *Tissue Engineering: Applications in Oral and Maxillofacial Surgery and Periodontics*, ed 2. Chicago: Quintessence, 2008:xi–xvi.
38. Sato N, Beitz JG, Kato J, et al. Platelet-derived growth factor indirectly stimulates angiogenesis in vitro. *Am J Pathol* 1993;142:1119–1130.
39. Bouletreau PJ, Warren SM, Spector JA, Steinbrech DS, Mehrara BJ, Longaker MT. Factors in the fracture microenvironment induce primary osteoblast angiogenic cytokine production. *Plast Reconstr Surg* 2002;110:139–148.
40. Guo P, Hu B, Gu W, et al. Platelet-derived growth factor-B enhances glioma angiogenesis by stimulating vascular endothelial growth factor expression in tumor endothelia and by promoting pericyte recruitment. *Am J Pathol* 2003;162:1083–1093.
41. Lynch SE, Wisner-Lynch LA, Nevins M. Use of rhPDGF to improve bone and periodontal regeneration. In: Lynch SE, Marx RE, Nevins M, Wisner-Lynch LA (eds). *Tissue Engineering: Applications in Oral and Maxillofacial Surgery and Periodontics*, ed 2. Chicago: Quintessence, 2008:87–102.
42. Cho MI, Lin WL, Genco RJ. Platelet-derived growth factor-modulated guided tissue regenerative therapy. *J Periodontol* 1995;66:522–530.
43. 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-like growth factor-I in patients with periodontal disease. *J Periodontol* 1997;68:1186–1193.
44. Mumford JH, Carnes DL, Cochran DL, Oates TW. The effects of platelet-derived growth factor-BB on periodontal cells in an in vitro wound model. *J Periodontol* 2001;72:331–340.
45. Papadopoulos CE, Dereka XE, Vavouraki EN, Vrotsos IA. In vitro evaluation of the mitogenic effect of platelet-derived growth factor-BB on human periodontal ligament cells cultured with various bone allografts. *J Periodontol* 2003;74:451–457.
46. Camelo M, Nevins ML, Schenk RK, Lynch SE, Nevins M. Periodontal regeneration in human Class II furcations using purified recombinant human platelet-derived growth factor-BB (rhPDGF-BB) with bone allograft. *Int J Periodontics Restorative Dent* 2003;23:213–225.
47. Nevins M, Camelo M, Nevins ML, Schenk RK, Lynch SE. Periodontal regeneration in humans using recombinant human platelet-derived growth factor-BB (rhPDGF-BB) and allogenic bone. *J Periodontol* 2003;74:1282–1292.
48. Nevins M, Giannobile WV, McGuire MK, et al. Platelet-derived growth factor stimulates bone fill and rate of attachment level gain: Results of a large multicenter randomized controlled trial. *J Periodontol* 2005;76:2205–2215.
49. McGuire MK, Kao RT, Nevins M, Lynch SE. rhPDGF-BB promotes healing of periodontal defects: 24-month clinical and radiographic observations. *Int J Periodontics Restorative Dent* 2006;26:223–231.
50. Sarment DP, Cooke JW, Miller SE, et al. Effect of rhPDGF-BB on bone turnover during periodontal repair. *J Clin Periodontol* 2006;33:135–140.

51. Nevins M, Hanratty J, Lynch SE. Clinical results using recombinant human platelet-derived growth factor and mineralized freeze-dried bone allograft in periodontal defects. *Int J Periodontics Restorative Dent* 2007;27:421–427.
52. Nevins M, Lynch SE, Cappetta EG. Treatment of advanced periodontal defects using bioactive therapies. In: Lynch SE, Marx RE, Nevins M, Wisner-Lynch LA (eds). *Tissue Engineering: Applications in Oral and Maxillofacial Surgery and Periodontics*, ed 2. Chicago: Quintessence, 2008:67–86.
53. Nevins ML, Mellonig JT. Site development for implant placement: Regenerative and esthetic techniques in oral plastic surgery. In: Lynch SE, Marx RE, Nevins M, Wisner-Lynch LA (eds). *Tissue Engineering: Applications in Oral and Maxillofacial Surgery and Periodontics*, ed 2. Chicago: Quintessence, 2008:119–131.
54. Ridgway HK, Mellonig JT, Cochran DL. Human histologic and clinical evaluation of recombinant human platelet-derived growth factor and beta-tricalcium phosphate for the treatment of periodontal intraosseous defects. *Int J Periodontics Restorative Dent* 2008;28:171–179.
55. Simion M, Rocchietta I, Kim D, Nevins M, Fiorellini J. Vertical ridge augmentation by means of deproteinized bovine bone block and recombinant human platelet-derived growth factor-BB: A histologic study in a dog model. *Int J Periodontics Restorative Dent* 2006;26:415–423.
56. Rocchietta I, Dellavia C, Nevins M, Simion M. Bone regenerated via rhPDGF-BB and a deproteinized bovine bone matrix: Backscattered electron microscopic element analysis. *Int J Periodontics Restorative Dent* 2007;27:539–545.
57. Simion M, Rocchietta I, Dellavia C. Three-dimensional ridge augmentation with xenograft and recombinant human platelet-derived growth factor-BB in humans: Report of two cases. *Int J Periodontics Restorative Dent* 2007;27:109–115.
58. Simion M, Rocchietta I, Monforte M, Maschera E. Three-dimensional alveolar bone reconstruction with a combination of recombinant human platelet-derived growth factor BB and guided bone regeneration: A case report. *Int J Periodontics Restorative Dent* 2008;28:239–243.
59. Nevins ML, Camelo M, Schupbach P, Kim DM, Camelo JM, Nevins M. Human histologic evaluation of mineralized collagen bone substitute (MCBS) and recombinant platelet-derived growth factor-BB (rhPDGF-BB) to create bone for optimal implant placement in extraction socket defects at 4 and 6 months: A case series. *Int J Periodontics Restorative Dent* 2009;29:129–139.

