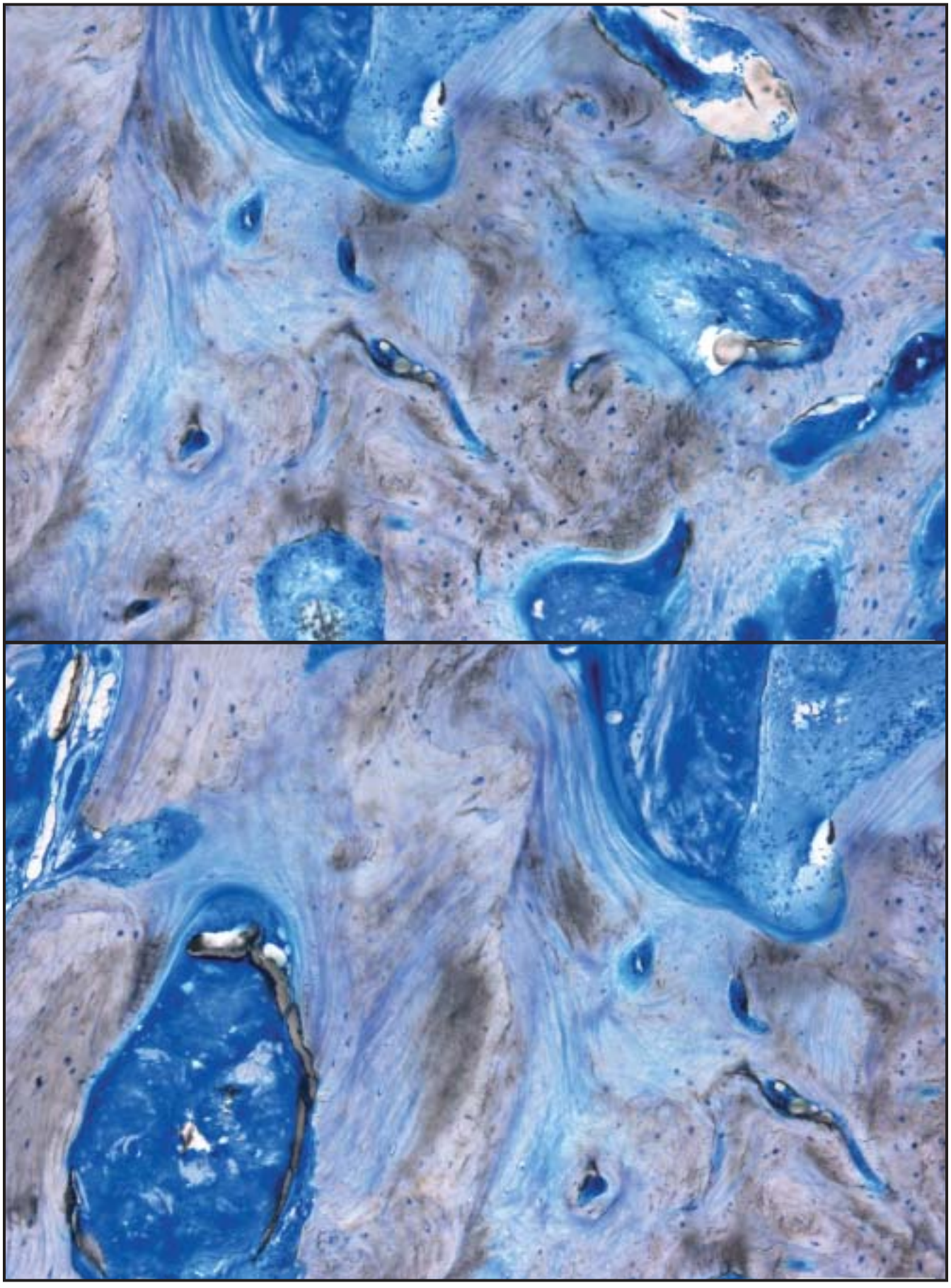


Human Histologic Evaluation of Anorganic Bovine Bone Mineral Combined with Recombinant Human Platelet-Derived Growth Factor BB in Maxillary Sinus Augmentation: Case Series Study

Myron Nevins, DDS[1]/David Garber, DMD[2]/James J. Hanratty, DDS[3]
Bradley S. McAllister, DDS[4]/Marc L. Nevins, DMD, MMSc[5]
Maurice Salama, DMD[6]/Peter Schupbach, PhD[7]
Steven Wallace, DDS[8]/Simon M. Bernstein, DDS, MS[3]
David M. Kim, DDS, DMSc[9]

The objective of this proof-of-principle study was to examine the potential for improved bone regenerative outcomes in maxillary sinus augmentation procedures when recombinant human platelet-derived growth factor BB (0.3 mg/mL) is combined with particulate anorganic bovine bone mineral. The surgical outcomes in all treated sites were uneventful at 6 to 8 months, with sufficient regenerated bone present to allow successful placement of maxillary posterior implants. Large areas of dense, well-formed lamellar bone were seen throughout the intact core specimens in more than half of the grafted sites. Abundant numbers of osteoblasts were noted in concert with significant osteoid in all sites, indicating ongoing osteogenesis. A number of cores demonstrated efficient replacement of the normally slowly resorbing anorganic bovine bone mineral matrix particles with newly formed bone when the matrix was saturated with recombinant human platelet-derived growth factor BB. (Int J Periodontics Restorative Dent 2009;29:583–591.)

[1]Associate Clinical Professor, Division of Periodontology, Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, Massachusetts.
[2]Clinical Assistant Professor, Departments of Periodontics and Oral Rehabilitation, Medical College of Georgia School of Dentistry, Augusta, Georgia.
[3]Private Practice, Swampscott, Massachusetts.
[4]Assistant Clinical Professor, Department of Periodontology, Oregon Health Sciences University, Portland, Oregon.
[5]Assistant Clinical Professor, Division of Periodontology, Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, Massachusetts.
[6]Clinical Assistant Professor, Department of Periodontics, Medical College of Georgia, Augusta, Georgia.
[7]Adjunct Professor, Department of Periodontics, School of Dental Medicine University of Pennsylvania, Philadelphia, Pennsylvania.
[8]Associate Professor, Ashman Department of Implant Dentistry, New York University, New York, New York.
[9]Assistant Professor, Division of Periodontology, Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, Massachusetts.

Correspondence to: Dr Myron Nevins, Harvard School of Dental Medicine, 188 Longwood Avenue, Boston MA 02114; fax: 617-432-1897; email: nevinsperimp@aol.com.

The dental literature is replete with studies documenting vital bone regeneration following maxillary sinus augmentation. Multiple variables affecting bone regenerative outcomes have been examined, including the type of graft matrix,[1–10] length of time between subantral grafting and core biopsy,[1–11] presence or absence of occlusive membranes over the lateral wall osteotomy site,[12–16] and placement of resorbable versus nonresorbable membranes over the osteotomy site.[12] Anorganic bovine bone mineral (ABBM) has been used extensively, either alone or as a composite graft with other matrices, in sinus augmentation procedures. Reviews of survival rates for implants placed in the grafted maxillary sinus have demonstrated the efficacy and safety of anorganic bovine bone mineral as the graft material.[13,17] Multiple additional studies have further documented superior implant survival rates of ABBM-grafted sinuses when compared to autogenous bone or composite grafts of ABBM and autogenous bone.[18–20]

The quality and quantity of bone regeneration at numerous time points in ABBM-grafted sinuses have been

reported.[1–4,7,9,10] A histologic examination of bone cores harvested at the time of implant placement is essential for confirmation of vital bone formation. A number of case series studies have noted a range of vital bone formation that varies with chosen times for core biopsy. Reported values for the percent of bone formed varied from about 19% at 3 to 4 months to about 70% after 1 year or longer.[2,4,7,10] The percent of residual ABBM particles integrated with newly formed bone was generally reported as high because of its slow rate of resorption.

Recombinantly produced human platelet-derived growth factor BB (rhPDGF-BB), with its potent critical role in angiogenesis and its chemotactic and mitogenic effects on target cells such as periodontal ligament and alveolar bone cells, may potentially play an effective role in sinus augmentation procedures when combined with an appropriate carrier matrix. An in vitro study by Jiang et al confirmed proper attachment and release kinetics leading to enhanced osteogenesis when rhPDGF-BB was combined with ABBM.[21] A number of prospective studies have further confirmed significantly improved bony regenerative outcomes when rhPDGF-BB is combined with ABBM matrices.[22–25]

The purpose of the present proof-of-principle study was to examine the potential of improved bone regenerative outcomes in maxillary sinus augmentation procedures when rhPDGF-BB is combined with particulate ABBM.[1,3,4,12]

Method and materials

This investigation was designed as a prospective, open-label clinical study involving 10 patients recruited from four different centers. An informed consent document prepared according to the Declaration of Helsinki was reviewed and obtained. Male or female patients between 20 and 65 years of age, with less than 6 mm of alveolar bone height in the posterior edentulous maxilla and who requested implant-supported maxillary posterior prostheses, were included in the study. Subjects with acute or chronic sinus disease, untreated periodontal disease, or significant acute or chronic systemic disease were excluded from the study.

Appropriate dental examination, full-mouth periapical radiographs, clinical photographs, and maxillary computed tomography (CT) scans were performed at baseline. A traditional maxillary lateral wall osteotomy approach to the sinus, following elevation of a full-thickness mucoperiosteal flap, was performed under local anesthesia (2% lidocaine with 1:100,000 epinephrine). Piezosurgical instrumentation was used to create the lateral window osteotomy. Prior to grafting, 2 g of ABBM particles (Bio-Oss, Osteohealth Company) were thoroughly saturated with 1 mL of 0.3 mg/mL rhPDGF-BB (Gem21S, Osteohealth Company) for a minimum of 10 minutes to allow for adequate attachment of the PDGF molecules to the graft matrix. After the sinus membrane was elevated to form a new sinus floor, incremental quantities of the composite graft were placed carefully

into the newly created subsinus space. A resorbable collagen barrier membrane (Bio-Gide, Osteohealth Company) was then positioned over the lateral window, and the mucoperiosteal flap was coapted with multiple expanded polytetrafluoroethylene sutures (CV-5, Gore-Tex, WL Gore & Associates). Patients were instructed to rinse with 0.12% chlorhexidine and not to brush or floss the surgical sites until sutures were removed.

Subjects were seen for postoperative evaluations at 1, 2, 4, 8, and 12 weeks and every 6 weeks thereafter until biopsies were obtained at 6 to 8 months postaugmentation. There were no serious adverse events during the course of the study. Core biopsies of 2 mm in diameter were harvested at implant placement from the reconstructed alveolar ridge, preserved, and prepared for histologic evaluation. One to four implants were placed in each augmented area.

Ground sections for light microscopy

Fixed samples were dehydrated in a graded series of ethanols using a dehydration system with agitation and vacuum. The blocks were infiltrated with Kulzer Technovit 7200 VLC resin. Infiltrated specimens were placed into embedding molds, and polymerization was performed under ultraviolet light. Polymerized blocks were sliced longitudinally on an Exakt cutting unit (Exakt). The slices were reduced by microgrinding and polishing using an Exakt grinding unit to an even thickness of 30 to 40 µm. Sections were stained with toluidine blue/pyronine G and examined using a both a Leica MZ16 stereomicroscope and a Leica 6000DRB light microscope.

Microcomputed tomographic analysis

The specimens were scanned using a high-resolution microcomputed tomography (micro-CT) system (µCT 40, Scanco Medical) in multislice mode. Each image data set consisted of approximately 600 micro-CT slice images. The specimens were scanned in high-resolution mode with an x-, y-, and z-resolution of 16 µm. The image data sets were used to produce three-dimensional views of the specimens using a special software (Scanco Medical).

Results

Thirteen maxillary sinus augmentation sites were treated in this proof-of-principle case series. Surgical outcomes in all cases were uneventful, and sufficient regenerated bone was present in all sites for successful implant placement. Two bone cores were harvested from each treated sinus 6 to 8 months after treatment. No serious adverse events occurred during the course of this study. Seven specimens exhibited robust histologic and micro-CT evidence of new bone formation and resorption of ABBM. The other specimens demonstrated clinically significant bone regeneration but continued to show considerable unresorbed matrix. The following figures

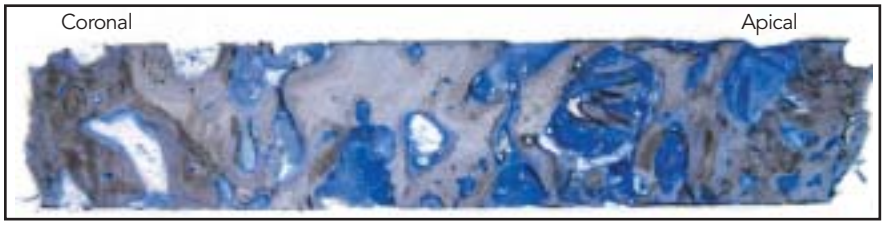


Fig 1a *An intact core specimen from the time of implant placement demonstrates robust bone formation throughout the augmented area, with little evidence of remaining ABBM matrix particles.*

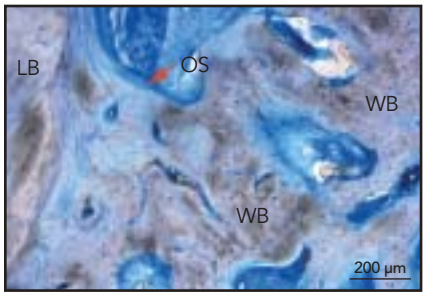


Fig 1b (top left) *At higher power, well-vascularized lamellar (LB) and woven (WB) bone are seen, along with active osteoid (OS) formation.*

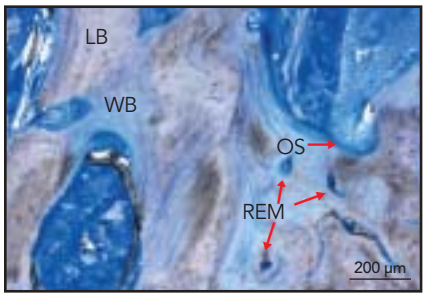


Fig 1c (bottom left) *In this high-power view, all phases of bone regeneration are seen, from ongoing osteoid secretion by osteoblasts, to woven bone, to dense lamellar bone. REM = remodeling.*

Fig 1d (right) *A micro-CT scan of the core reveals almost 100% bone* (red)*, with little evidence of remaining ABBM particles* (white).

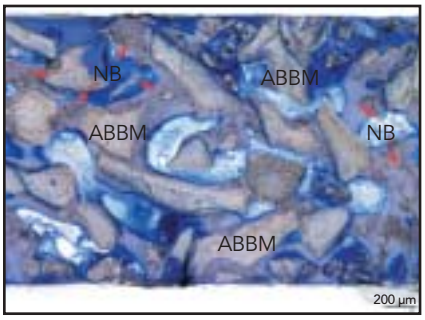
are representative biopsy samples demonstrating the range of regenerative results seen in this case series study.

Figure 1a represents an intact core specimen obtained at the time of implant placement. Robust bone regeneration was seen throughout the augmented area, with significant resorption of ABBM particles. Well-vascularized lamellar bone and woven bone were present, along with osteoblasts and osteoid, which could be observed at a higher magnification (Figs 1b and 1c). The progression from woven to lamellar bone, along with ongoing osteoid secretion by osteoblasts, is especially evident in Fig 1c. A micro-CT scan of the trephine core biopsy revealed almost all bone in the sample, with only a small amount of ABBM remaining (Fig 1d).

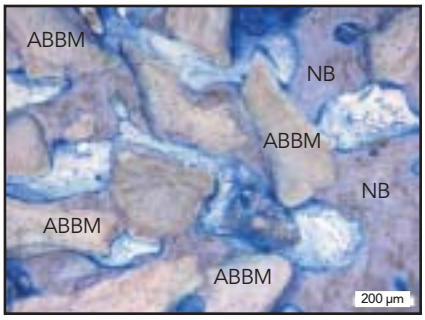
The intact bone specimen of core no. 2 exhibited significant quantities of dense, mostly lamellar, newly regenerated bone (Fig 2a). However, this core demonstrated intact ABBM particles surrounded by an abundance of well-formed newly regenerated bone. Magnified views emphasized both the quantity and maturation of the regenerated bone bridging the intact ABBM graft particles (Figs 2b and 2c). The intense osteogenesis evident in histologic examination was further supported by backscatter electron microscopy, in which large quantities of regenerated bone were seen connecting ABBM particles (Figs 2d and 2e).

In core no. 3, the intact specimen revealed large areas of regenerated lamellar bone surrounded by abundant amounts of less mature woven bone. Regenerated bone within the core biopsy was surrounded by significant amounts of marrow (Fig 3a). Findings seen at low power were confirmed at higher magnification, where regenerated woven bone surrounded more mature areas of lamellar bone (Fig 3b). Few, if any, intact ABBM particles remained within the biopsy field. A micro-CT scan of the trephine core biopsy revealed almost all bone, with scant amounts of ABBM remaining (Fig 3c).

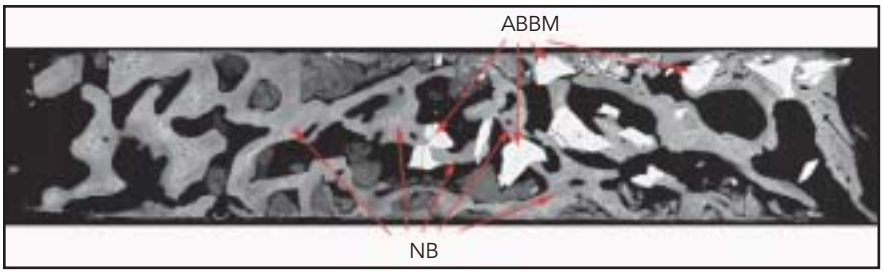
Fig 2a *An intact core specimen demonstrates ABBM particles surrounded by and interconnected with abundant amounts of well-formed, newly regenerated bone (NB). LB = local (old) bone.*

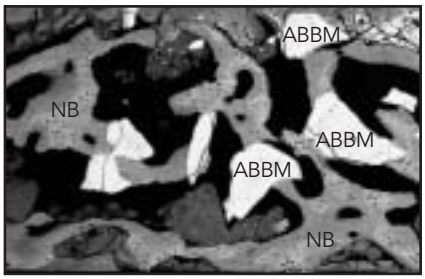


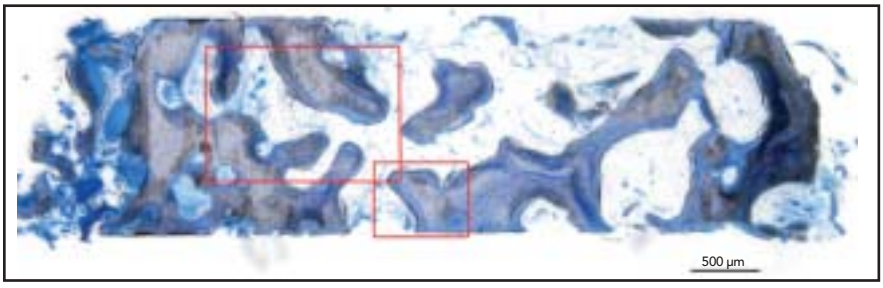
Figs 2b (left) **and 2c** (right) *Dense, mature lamellar bone (NB) is seen surrounding and interconnecting intact ABBM particles.*



Figs 2d (bottom left) **and 2e** (bottom right) *The intense osteogenesis evident in the histologic sample is further supported by backscatter electron microscopy, in which large quantities of regenerated bone (NB) are seen connecting ABBM particles.*







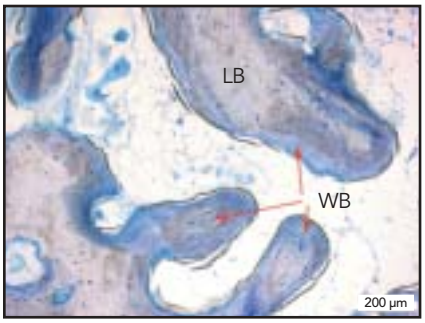


Fig 3a (above) *Large areas of regenerated lamellar bone are seen surrounded by less mature woven bone in this core biopsy specimen. Few remaining ABBM particles are seen in this core.*

Fig 3b (left) *At higher power, regenerated lamellar bone (LB) and woven bone (WB) are clearly seen (large boxed area from Fig 3a).*

Fig 3c (right) *A micro-CT scan of the trephine core biopsy reveals almost all bone* (red)*, with only small amounts of ABBM particulate* (white) *remaining.*

Fig 4a (left) *A micro-CT scan of the trephine core reveals a composite specimen of mainly bone* (red) *integrated with lesser amounts of ABBM matrix* (white).

Fig 4b (right) *In this micro-CT image reformatted without the remaining ABBM graft matrix, the core biopsy is seen to be composed primarily of bone.*

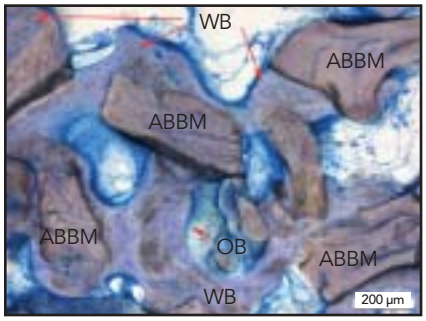
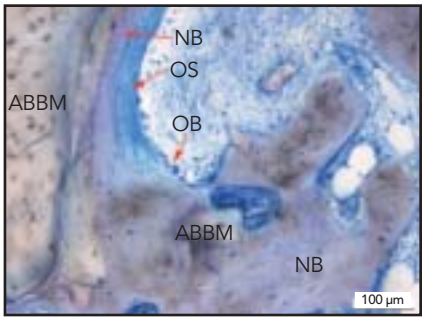


Fig 4c (left) *High-power histologic view demonstrates mature, robust bone surrounding and interconnecting residual ABBM particles. WB = woven bone; OB = osteoblasts.*

Fig 4d (right) *Ongoing active bone formation is readily seen in a higher-power view in the form of rims of osteoblasts (OB) secreting abundant quantities of nonmineralized osteoid (OS). NB = new bone.*



In core no. 4, micro-CT scans of the trephine core revealed a composite of mostly bone integrated with lesser amounts of interspersed ABBM particles (Figs 4a and 4b). Backscatter electron microscopy demonstrated extensive new bone surrounding a number of intact ABBM particles, with the newly formed bone bridging gaps between the particulate graft matrix. High-power histologic images confirmed the accuracy of both the three-dimensional micro-CT scans and the backscattered electron microscopy, emphasizing robust bone formation within a matrix of ABBM particles (Fig 4c). Finally, a magnified histologic view revealed the dynamic juxtaposition of an intact ABBM particle with well-formed lamellar bone, the latter lined by recently secreted osteoid from an adjacent monolayer of osteoblasts (Fig 4d).

The fifth specimen contained intact ABBM particles throughout the core biopsy that were surrounded by regenerated bone. The overall quantity of new bone appeared to be somewhat lower than that seen in the other specimens (Fig 5a). Increased magnification revealed intact ABBM particles rimmed by newly formed bone interconnecting adjacent particles. Numerous osteoblasts were seen rimming the leading edge of secreted osteoid (Fig 5b).

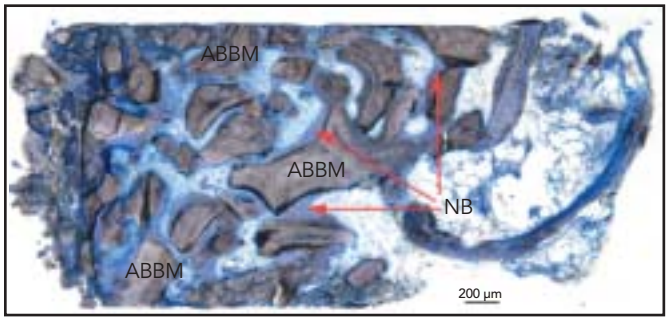


Fig 5a *This intact core reveals ABBM graft particles surrounded by and interconnected with newly formed bone (NB). The quantity of bone is lower than that seen in other specimens.*

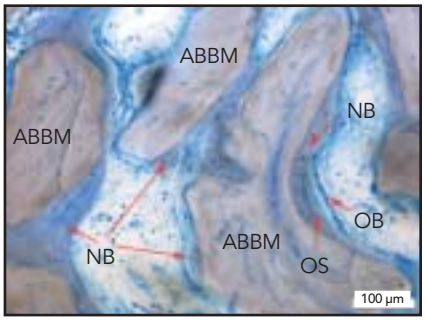


Fig 5b *Abundant numbers of osteoblasts (OB) throughout this higher-power view reveal continuing bone regeneration within this grafted site. OS = osteoid.*

Discussion

The long-term clinical success of maxillary sinus augmentation procedures is measured by the formation of vital, well-vascularized bone.[1–17] The inclusion of a tissue-engineered protein in the sinus grafting protocol provides opportunities to improve long-term clinical outcomes for this procedure. This proof-of-principle study represents a first attempt to examine the potential impact of rhPDGF-BB in sinus augmentation.

PDGF, the primary growth factor within the alpha granules of platelets, is a 30-kDa glycoprotein present in the bone matrix and actively secreted during early fracture repair.[26–28] It is chemotactic and mitogenic for a number of cell types, including osteoblasts, cementoblasts, and periodontal and gingival fibroblasts. In addition, during early stages of wound healing, PDGF up-regulates vascular endodothelial growth factor, increasing blood supply to the defect site.[29]

ABBM has shown well-documented effective clinical outcomes, especially in terms of implant survival, when used either alone or in combination with other matrices.[13,16–20] The evidence further documents a range of values for percent vital bone formation, dependent upon multiple examined variables, when ABBM is used in sinus augmentation procedures.[1–4,7,9,10]

Jiang et al confirmed the needed attachment and release kinetics for enhanced osteogenesis when rhPDGF-BB was added to ABBM in an in vitro study.[21] Subsequent studies have confirmed improved osteogenesis when ABBM is combined with rhPDGF-BB.[22–25]

The current study clearly suggests the possibility of improved bone regenerative results when rhPDGF-BB is added to the ABBM matrix.[1,3,4,12] In more than half of the grafted areas studied in the present case series, large areas of dense, well-formed lamellar bone were seen throughout the intact core specimens, with obvious resorption of ABBM. Abundant numbers of osteoblasts were noted secreting significant quantities of osteoid, indicating continuing active osteogenesis.

In two preclinical canine studies, efficient replacement resorption of the matrix particles with newly formed bone occurred when the matrix was saturated with rhPDGF-BB.[25,30] The same phenomenon of accelerated replacement resorption of matrix particles saturated with rhPDGF-BB seems to be operative in human subjects as well.[22]

Although improved bone regenerative results were clearly evident in the present case series, histologic outcomes were not uniform across all grafted sites. Many specimens demonstrated broad areas of dense, well-formed bone. However, other specimens did not exhibit the same robust bone regenerative results; nor was accelerated replacement resorption of matrix particles always seen. Further investigations are clearly required to better understand those variables required for predictable outcomes in growth factor–mediated sinus augmentation procedures.

Conclusion

Regeneration of vital, well-vascularized bone in the posterior maxilla frequently must precede implant placement in the posterior maxilla. Maxillary sinus augmentation procedures have become a clinically accepted modality to achieve this goal. The recent introduction of tissue-engineered biomolecules, ie, recombinant human platelet-derived growth factor BB, for clinical use provides opportunities for significantly improved bone regenerative results in maxillary sinus augmentation procedures.

Acknowledgment

Special thanks to Dr Stuart Kay, science writer and consultant (Huntington, NY), for his help with the organization and production of this manuscript.

References

1. Froum SJ, Wallace SS, Cho SC, Elian N, Tarnow DP. Histomorphometric comparison of a biphasic bone ceramic to anorganic bovine bone for sinus augmentation: 6- to 8-month postsurgical assessment of vital bone formation. A pilot study. Int J Periodontics Restorative Dent 2008;28: 273–281.
2. Iezzi G, Degidi M, Scarano A, Petrone G, Piattelli A. Anorganic bone matrix retrieved 14 years after a sinus augmentation procedure: A histologic and histomorphometric evaluation. J Periodontol 2007;78: 2057–2061.
3. Froum SJ, Wallace SS, Elian N, Cho SC, Tarnow DP. Comparison of mineralized cancellous allograft (Puros) and anorganic bovine bone matrix (Bio-Oss) for sinus augmentation: Histomorphometry at 26 to 32 weeks after grafting. Int J Periodontics Restorative Dent 2006;26:543–551.
4. Lee YM, Shin SY, Kim JY, Kye SB, Ku Y, Rhyu IC. Bone reaction to bovine hydroxyapatite for maxillary sinus floor augmentation: Histologic results in humans. Int J Periodontics Restorative Dent 2006;26: 471–481.
5. Gapski R, Neiva R, Oh TJ, Wang HL. Histologic analyses of human mineralized bone grafting material in sinus elevation procedures: A case series. Int J Periodontics Restorative Dent 2006;26:59–69.
6. Cammack GV 2nd, Nevins M, Clem DS 3rd, Hatch JP, Mellonig JT. Histologic evaluation of mineralized and demineralized freeze-dried bone allograft for ridge and sinus augmentations. Int J Periodontics Restorative Dent 2005;25:231–237.
7. John HD, Wenz B. Histomorphometric analysis of natural bone mineral for maxillary sinus augmentation. Int J Oral Maxillofac Implants 2004;19:199–207.
8. Zerbo IR, Zijdervels SA, de Boer A, et al. Histomorphometry of human sinus floor augmentation using a porous beta-tricalcium phosphate: A prospective study. Clin Oral Implants Res 2004;15:724–732.
9. Tadjoedin ES, de Lange GL, Bronckers AL, Lyaruu DM, Burger EH. Deproteinized cancellous bovine bone (Bio-Oss) as bone substitute for sinus floor elevation. A retrospective, histomorphometrical study of five cases. J Clin Periodontol 2003;30: 261–270.
10. Fugazzotto PA. GBR using bovine bone matrix and resorbable and nonresorbable membranes. Part 1: Histologic results. Int J Periodontics Restorative Dent 2003;23: 361–369.
11. Yamamichi N, Itose T, Neiva R, Wang HL. Long-term evaluation of implant survival in augmented sinuses: A case series. Int J Periodontics Restorative Dent 2008;28: 163–169.
12. Wallace SS, Froum SJ, Cho SC, et al. Sinus augmentation utilizing anorganic bovine bone (Bio-Oss) with absorbable and nonabsorbable membranes placed over the lateral window: Histomorphometric and clinical analyses. Int J Periodontics Restorative Dent 2005;25:551–559.
13. Wallace SS, Froum SJ. Effect of maxillary sinus augmentation on the survival of endosseous dental implants. A systematic review. Ann Periodontol 2003;8: 328–343.
14. Tarnow DP, Wallace SS, Froum SJ, Rohrer MD, Cho SC. Histologic and clinical comparison of bilateral sinus floor elevations with and without barrier membrane placement in 12 patients: Part 3 of an ongoing prospective study. Int J Periodontics Restorative Dent 2000;20:117–125.
15. Tawil G, Mawla M. Sinus floor elevation using a bovine bone mineral (Bio-Oss) with or without the concomitant use of bilayered collagen barrier (Bio-Gide): A clinical report of immediate and delayed implant placement. Int J Oral Maxillofac Implants 2001;16:713–721.
16. Froum SJ, Tarnow DP, Wallace SS, Rohrer MD, Cho SC. Sinus floor elevation using anorganic bovine bone matrix (Osteo-Graf/N) with and without autogenous bone: A clinical, histologic, radiographic, and histomorphometric analysis—Part 2 of an ongoing prospective study. Int J Periodontics Restorative Dent 1998;18: 528–543.

17. Del Fabbro M, Testori T, Francetti L, Weinstein R. Systematic review of survival rates for implants placed in the grafted maxillary sinus. Int J Periodontics Restorative Dent 2004;24:565–577.

18. Hallman M, Sennerby L, Lundgren S. A clinical and histologic evaluation of implant integration in the posterior maxilla after sinus floor augmentation with autogenous bone, bovine hydroxyapatite, or a 20:80 mixture. Int J Oral Maxillofac Implants 2002;17:635–643.

19. Hising P, Bolin A, Branting C. Reconstruction of severely resorbed alveolar ridge crests with dental implants using a bovine mineral for augmentation. Int J Oral Maxillofac Implants 2001;16:90–97.

20. Valentini P, Abensur D. Maxillary sinus floor elevation for implant placement with demineralized freeze-dried bone and bovine bone (Bio-Oss): A clinical study of 20 patients. Int J Periodontics Restorative Dent 1997;17:232–241.

21. Jiang D, Dziak R, Lynch SE, Stephan EB. Modification of an osteoconductive anorganic bovine bone mineral matrix with growth factors. J Periodontol 1999;70: 834–839.

22. Nevins ML, Camelo M, Schupbach P, Kim DM, Camelo JMB, Nevins M. Human histologic evaluation of mineralized collagen bone substitute and recombinant platelet-derived growth factor-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.

23. Nevins ML, Camelo M, Nevins M, Schupbach P, Friedland B, Camelo JMB, Kim DM. Minimally invasive alveolar ridge augmentation procedure (tunneling technique) using rhPDGF-BB in combination with three matrices: A case series. Int J Periodontics Restorative Dent 2009;29: 371–383.

24. 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.

25. Simion M, Rocchietta I, Kim D, Nevins M, Fiorellini J. Vertical ridge augmentation by means of a 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.

26. Andrew JG, Hoyland JA, Freemont AJ, Marsh DR. Platelet-derived growth factor expression in normally healing human fractures. Bone 1995;16:455–460.

27. Joyce ME, Jingushi S, Scully SP, Bolander ME. Role of growth factors in fracture healing. Prog Clin Biol Res 1991;365:391–416.

28. Fujii H, Kitazawa R, Maeda S, Mizuno K, Kitazawa S. Expression of platelet-derived growth factor proteins and their receptor alpha and beta mRNAs during fracture healing in the normal mouse. Histochem Cell Biol 1999;112:131–138.

29. Lynch SE. Introduction. In: Lynch SE, Genco RJ, Marx RE (eds). Tissue Engineering: Applications in Maxillofacial Surgery and Periodontics. Chicago: Quintessence, 1999:xi–xviii.

30. Simion M, Nevins M, Rocchietta I, et al. Vertical ridge augmentation using an equine block infused with recombinant human platelet-derived growth factor-BB: A histologic study in a canine model. Int J Periodontics Restorative Dent 2009;29: 245–255.