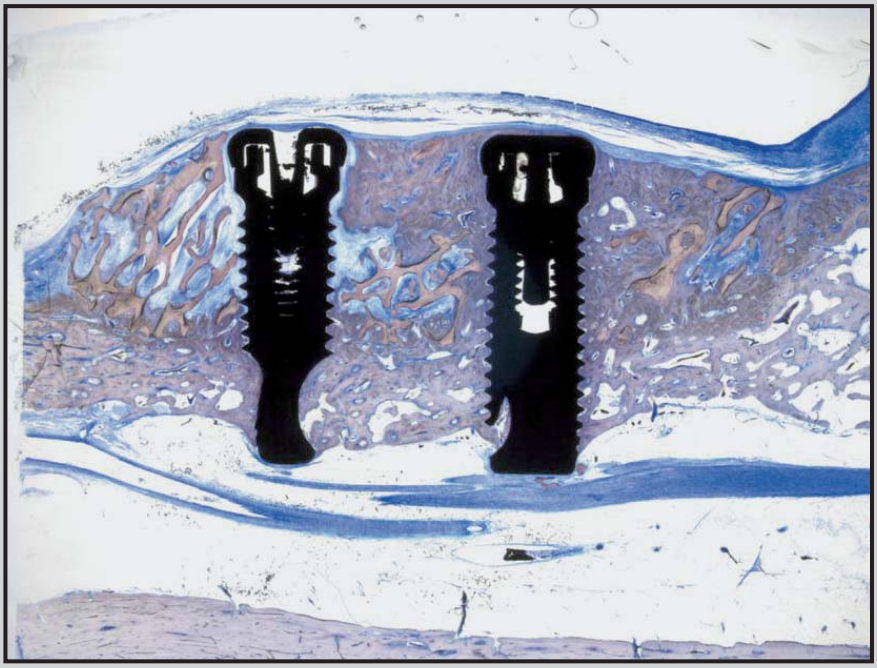
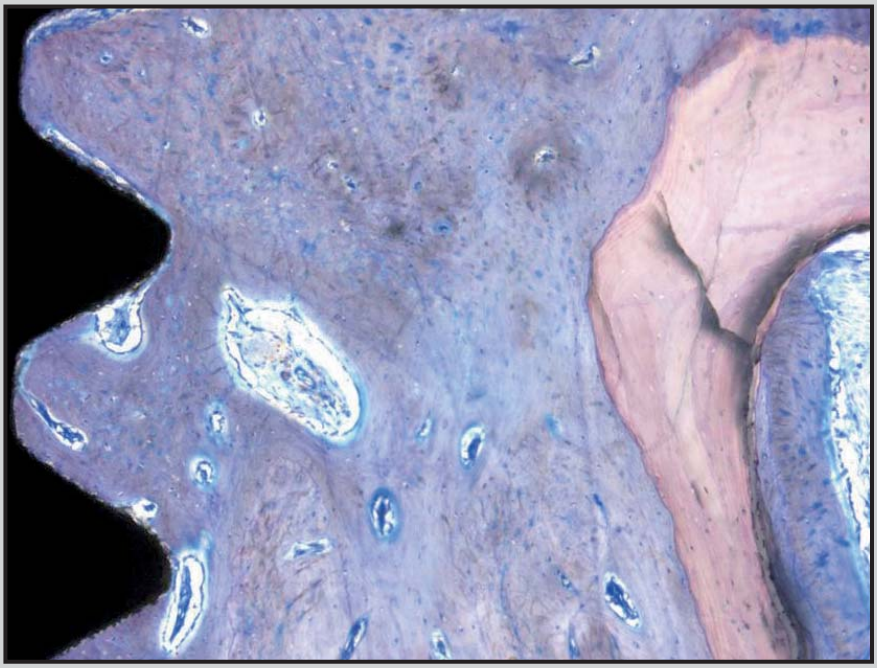
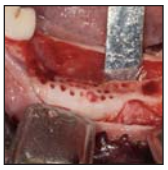




Vertical Ridge Augmentation by Means of Deproteinized Bovine Bone Block and Recombinant Human Platelet-Derived Growth Factor-BB: A Histologic Study in a Dog Model



Massimo Simion, MD, DDS[1] /Isabella Rocchietta, DDS[2]/ David Kim, DDS, DMSc[3]/Myron Nevins, DDS[4]/Joseph Fiorellini, DMD, DMSc[5]

The primary objective of this proof-of-principle study was to evaluate the outcome of vertical ridge augmentation in a standardized dog model by combining purified recombinant platelet-derived growth factor (rhPDGF-BB) and a block of deproteinized cancellous bovine bone. The secondary objective was to determine the value of a resorbable barrier membrane to improve the efficacy of the procedure. Six adult foxhounds were committed to bilateral surgical extraction of all four mandibular premolars. A vertical alveolar ridge defect was created at the time of the extractions. Three months later, the artificially created defects were grafted: Group A used a deproteinized bovine bone block in combination with a collagen barrier membrane, group B used a deproteinized bovine bone block infused with rhPDGF-BB only, and group C included a deproteinized bovine bone block infused with rhPDGF-BB, plus a collagen resorbable barrier membrane. After 4 months, the animals were sacrificed. Histologic examination of group B revealed a large amount of newly formed bone, and a large amount of bone-to-implant contact was visible in the areas of bone regeneration extending over the top of the implant cover screw. The results of this preclinical canine study provide proof-of-principle that rhPDGF-BB, used in combination with a deproteinized bovine block without placement of a barrier membrane, has the potential to regenerate significant amounts of new bone in severe mandibular ridge defects. In addition, the results seem to point to the importance of the periosteum as a source of osteoprogenitor cells in growth factor–mediated regenerative procedures. (Int J Periodontics Restorative Dent 2006;26:415–423.)

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Horizontal and vertical regeneration of severe localized edentulous atrophic alveolar ridges remains a challenging procedure in implant dentistry. Several contemporary treatment modalities are available, including autogenous onlay bone grafts, distraction osteogenesis, and guided bone regeneration (GBR). Autogenous onlay bone grafts have been considered the "gold standard" for bone regenerative procedures.[1,2] However, harvesting of autogenous bone is invasive, technically demanding, and often requires extraoral donor sites. Moreover, resorption of autogenous grafted bone by up to 50% has been demonstrated in cases of vertical ridge augmentation.[3]

Distraction osteogenesis is an alternative technique for vertical ridge augmentation that eliminates the need for bone harvesting.[4] It, too, is technically demanding and often unacceptable for patients who cannot tolerate intraoral distraction devices. In addition, distraction osteogenesis is generally limited to vertical bone augmentation, a problem when horizontal augmentation is also indicated.

GBR is used for ridge augmentation prior to or in conjunction with os-

seointegrated implant placement.[5,6] Whether with a barrier membrane alone or in combination with bone grafts or bone substitutes, GBR offers predictability in providing bone augmentation simultaneously in both horizontal and vertical directions.[7–9] However, it is technically complex, with frequent premature membrane exposure resulting in bacterial contamination.[10,11]

To overcome these problems, researchers and clinicians strive to develop less invasive surgical modalities that are technically less demanding and promote faster bone regeneration. Thus, a technique that would eliminate the need for a barrier membrane and/or autogenous bone grafts could be beneficial in reducing the incidence of complications and increasing patients' acceptance of the procedure.

Recent advances in tissue engineering may offer solutions that resolve bone volume deficits and periodontal defects while at the same time eliminating some of the concerns posed by current techniques. One signaling, wound-healing molecule that has been studied extensively in both animals and humans is platelet-derived growth factor (PDGF), which is contained in the alpha granules of blood platelets and bone matrix,[12] is now produced recombinantly, and was recently approved by the United States Food and Drug Administration in combination with b-tricalcium phosphate (GEM 21S, Osteohealth) for the treatment of periodontally related defects. PDGF is a natural hormone produced by the body at sites of soft tissue and bone injury. PDGF is both chemotactic and mitogenic for osteoblasts and also angiogenic, promoting capillary budding into the graft site.

The main purpose of this study was to evaluate the outcome of vertical ridge augmentation in a standardized dog model[13] by combining purified recombinant human PDGF-BB (rhPDGF-BB) and a deproteinized bovine bone block. The secondary objective of this study was to determine the value of a resorbable barrier membrane when used with this tissue-engineered approach to bone regeneration.

Method and materials

Six female foxhounds (average weight 25 to 30 kg) were used for this experimental study. Protocols for animal use were reviewed and approved by the Institutional Animal Care and Use Committee, in compliance with the Guide for the Care and Use of Laboratory Animals.

The defects were created by the bilateral extraction of all four mandibular premolars. The edentulous ridge was then surgically reduced, resulting in a defect of 7 to 10 mm apicocoronally and 30 mm mesiodistally. Both buccal and lingual bony plates were removed to mimic a flat atrophic ridge. Primary wound closure was achieved by means of interrupted 4/0 expanded polytetrafluoroethylene sutures (Gore-Tex suture CV5, W. L. Gore). Control of postsurgical infection and swelling was accomplished with 1.0 mg dexamethasone administered the day after surgery and amoxicillin 500 to 750 mg twice daily for 10 days.

Following a healing period of 3 months, full-thickness mucoperiosteal flaps were carefully elevated, extending from the distal aspect of the canine to the mesial aspect of the first molar. All soft tissue remnants were carefully removed from the defect surface. Cortical perforations were made with a round carbide bur, exposing the underlying medullary spaces (Fig 1). A deproteinized bovine block was then closely adapted to the bony defect site and stabilized by means of two titanium implants (Nobel Biocare MKIII, 3.3 × 10 mm, one Ti-Unite surface in the distal site and one machined-surface in the mesial site) placed 10 mm apart (Fig 2).

Three cohorts were included in the study design. Group A (control) used a deproteinized bovine block (Bio-Oss cancellous [spongiosa] block, 20 × 10 × 10 mm, Geistlich Pharma) in combination with a resorbable bilayer collagen membrane (Bio-Gide, Geistlich Pharma). Group B used a deproteinized bovine block infused with rhPDGF-BB (BioMimetic Therapeutics). Group C included a deproteinized bovine block saturated with rhPDGF-BB and a resorbable collagen barrier membrane. Four sites were randomly assigned and included in each group.

The bovine cancellous block used in groups B and C was inserted in an empty sterile syringe, infused with rhPDGF-BB under pressure, and left to sit for 5 minutes. Using the aforementioned implants, the fragile cancellous block in all three groups was fixed to the atrophic ridge. As noted, a resorbable collagen membrane was placed over the grafted sites in groups A and C.

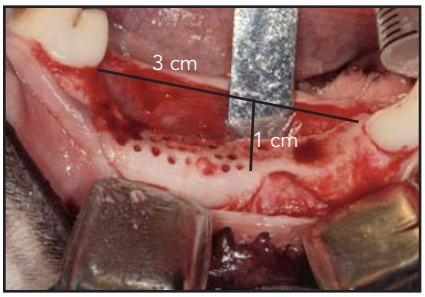


Fig 1 (left) *A full-thickness mandibular flap has been elevated and cortical perforations have been performed to encourage bleeding. The measurements of the defect are shown.*

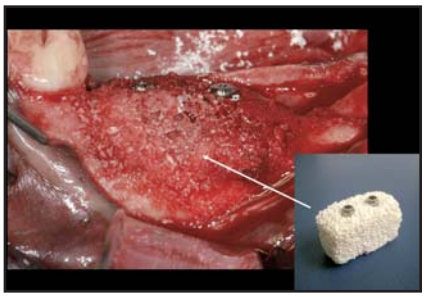


Fig 2 (right) *A deproteinized bovine block (inset) was placed over the atrophic mandible and secured by means of two titanium dental implants in mesial and distal positions. In eight sites (groups B and C) the block was infused by rhPDGF-BB.*

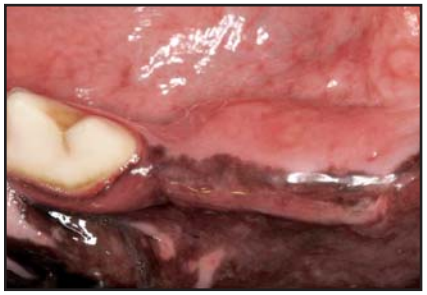


Fig 3 (left) *Deproteinized bovine block + rhPDGF-BB site (group 2) after 4 months of submerged healing. Note the volume of the ridge and the complete closure of the overlying soft tissues.*

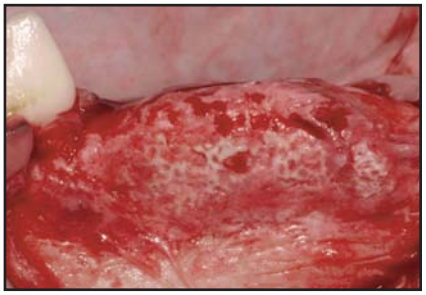


Fig 4 (right) *Clinical re-entry of the same site shown in Fig 3. The implants are covered with tissue resembling bone. Note the hard bleeding surface and the volume regenerated. Residual particles of the deproteinized bovine bone are visible at the surface.*

Using periosteal releasing incisions with Gore-Tex horizontal mattress and interrupted sutures, primary tension-free wound closure was achieved. Periapical radiographs were taken prior to and immediately following surgery. To control infection, each animal was given 0.5 mg buprenorphine hydrochloride (Buprenex, Reckitt Benckiser) preoperatively and postoperatively, followed by 1 mg/day for an additional 3 days. Penicillin G benzathine (Bicillin L-A, Wyeth-Ayerst); 600,000 units intramuscularly) was also administered every other day for 1 week following grafting.

Two weeks postoperatively, the sutures were removed. Throughout the study, each dog was maintained on a soft diet. Following a healing period of 4 months, the animals were sacrificed. Two sites were re-entered clinically for macroscopic evaluation (Figs 3 and 4).

Histologic processing

Block sections were dissected free, fixed in 10% neutral buffered formalin, dehydrated, and processed for light microscopy without demineralization. The blocks were embedded in Kulzer Technovit 7200 VLC-resin and sliced on an Exakt cutting unit. The slices were reduced using an Exakt grinding unit to an even thickness of 30 to 40 µm, stained with toluidine blue/pyronine G, and examined with a Leica DM6000B light microscope. Ground sections were prepared in mesiodistal and buccolingual directions.

Results

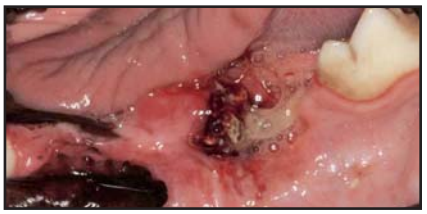
Clinical observations

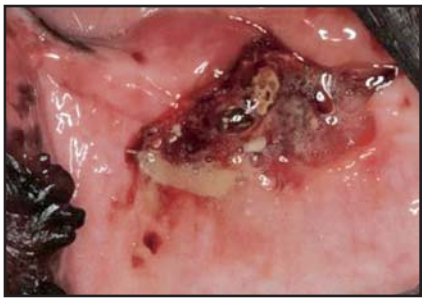
Healing proceeded uneventfully at all 12 surgical sites during the 3 months following creation of the mandibular defects. The mandibular alveolar ridges appeared as flat, no-wall defects (see Fig 1), simulating localized, highly resorbed, atrophic posterior mandibles.

The 4-month postgrafting healing phase was uneventful for 7 of the 12 sites, with 4 sites exhibiting soft tissue dehiscence and a fifth showing fistula formation (Table 1) (Figs 5a and 5b). Three of the five failures occurred in sites that received a deproteinized bovine block in combination with a resorbable membrane but without rhPDGF-BB (group A). Hence, a clinically uneventful healing rate of 75% was visible in sites treated with

Table 1 **Clinical outcomes of the specimens during the 4-month healing phase**

Sample	Clinical outcome				
	14 days	24 days	1 month	3 months	Sacrifice
Group C (block + rhPDGF-BB + membrane)					
498688R	Uneventful	Uneventful	Uneventful	Uneventful	Uneventful
496197L	Uneventful	Edema	Fistulas	Uneventful	Uneventful
496201R	Uneventful	Uneventful	Uneventful	Uneventful	Uneventful
497193R	Edema	Edema	Uneventful	Uneventful	Fistula
Group B (block + rhPDGF-BB)					
498688L	Edema	Edema	Uneventful	Fistulas	Uneventful
496197R	Uneventful	Uneventful	Fistulas	Uneventful	Uneventful
496219R	Uneventful	Uneventful	Uneventful	Uneventful	Uneventful
496189R	Edema	Edema	Uneventful	Exposure	Exposure
Group A (block + membrane)					
496201L	Uneventful	Fistulas	Fistulas	Exposure	Exposure
497193L	Edema	Edema	Uneventful	Uneventful	Uneventful
496219L	Edema	Exposure	Exposure; block removed	Exposure of implants	Exposure
496189L	Edema	Exposure	Exposure	Exposure	Exposure





Figs 5a and 5b *Clinical images of control sites (bovine block + membrane) shortly after suture removal. The soft tissue dehiscences precluded further healing.*

rhPDGF-BB, compared to 25% in sites that did not receive the growth factor.

Surgical re-entry of two sites (in group B and group C) prior to animal sacrifice 4 months following grafting revealed implants that were completely covered by tissue resembling bone (see Fig 4).

Radiographic observations

All four sites in the control group (bovine cancellous block + membrane, group A) exhibited no evidence of additional radiopacity superior to the defect surfaces, indicating a lack of bone regeneration. Radiographic evidence of new bone formation was observed in sites treated with the deproteinized bovine block and rhPDGF-BB (group B) at the time of sacrifice. The areas of interest appeared radiopaque, with the implants completely embedded in bone. The grafted cancellous block appeared to be integrated with both newly regenerated bone and pre-existing basal bone. No radiographic evidence of separation between the block and the alveolar crest was apparent. In contrast, sites that received the deproteinized bovine block infused with rhPDGF-BB plus a resorbable membrane (group C) demonstrated a radiographic separation between the grafted block and the alveolar ridge (Fig 6).

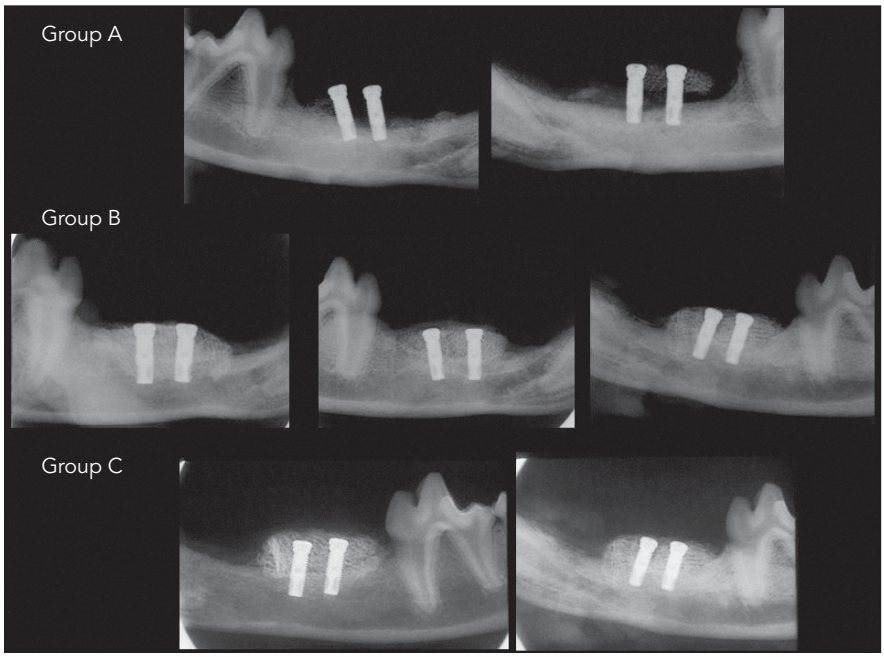
Histologic observations

Block sections of the mandible containing the implants and surrounding tissues were obtained after the animals were euthanized. These were dissected free, fixed in 10% neutral buffered formalin, and prepared in the usual manner for light microscopy. Ground sections were prepared in both mesiodistal and buccolingual directions.

Specimens that exhibited flap dehiscences or infection were not included in the histologic evaluation.

Group A

Only one site without infectious complications (specimen 497193L) was available in group A (bovine cancellous block + membrane). This site demonstrated a very thin layer of newly formed bone in continuity with the native basal bone, but no bone regeneration was detectable in the whole bovine block area. The block appeared to be embedded in healthy connective tissue, with no signs of inflammation (Fig 7).



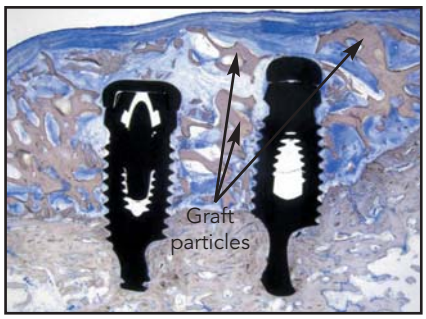


Fig 6 (left) *Periapical radiographs of all the specimens in the three groups prior to sacrifice. Group A: The radiolucency between the implants reflects the absence of new bone formation. Group B: The block appears to be integrated with the surrounding bone. A radiolucent area is noted at the center of the site. This pattern is consistent with the histologic image of the same site. Group C: A radiographic distinction can be appreciated between the block and the native bone.*

Fig 7 (right) *Control specimen (deproteinized bovine block + membrane). Overview of the mesiodistal ground section. The block is embedded in healthy connective tissue with no bone regeneration (toluidine blue/pyronine G stain; magnification ×8).*

Group B

In general, the mesiodistal ground sections of Group B (bovine cancellous block + rhPDGF-BB; specimens 498688L, 496197R, 496219R) demonstrated a significant amount of new bone formation, particularly at the coronal portion of the regenerated tissue facing the periosteum and the soft tissues. Bone formation was also evident in the apical third of the specimens, in direct continuity with the native lamellar bone. There was a relatively small area in the center of the specimens without new bone formation, where remnants of the deproteinized bovine block structure appeared to be embedded in healthy connective tissue. The amount of intact bovine block remnants was greater in the areas that showed no new bone formation than in areas with regenerated bone (Figs 8 and 9). In the latter areas, the bovine block appeared to have been actively resorbed and partially replaced by newly formed bone.

The xenograft particles embedded in bone showed typical bright seams of demineralization and numerous resorption lacunae adjacent to areas of ongoing bone formation (Figs 10 and 11), indicating the occurrence of intense physiologic remodeling, with alternating demineralization and remineralization. Osteoclastic lacunae were also present in the xenograft particles embedded in connective tissue.

All transient stages of woven to lamellar bone were detected in the newly formed bone. The most striking feature in the regenerated bone was the presence of intense osteoblastic activity and an unusually large number of bone remodeling units (BRU), together with the formation of mature osteons (Fig 12). Both transverse and longitudinal sections through BRU and osteons were visible. The formation of haversian systems or osteons was identified in transverse sections by the presence of osteoblasts and osteoid

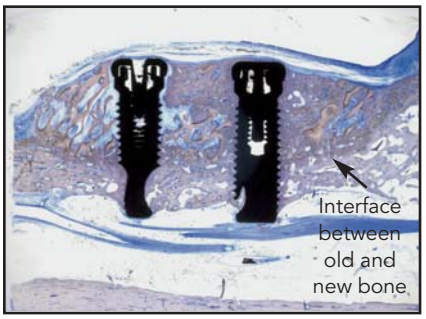


Fig 8 (left) *Group B specimen (deproteinized bovine block + rh-PDGF-BB). Overview of the mesiodistal ground section. Note the formation of new bone around the two implants. Note that the deproteinized bovine block is replaced by new bone. Inflammation-free connective tissue is present in the bovine block areas where bone formation has occurred (toluidine blue/pyronine G stain; magnification ×12.5).*

Fig 9 (right) *Group B specimen. Overview of the mesiodistal ground section. Note the formation of new bone over the implant cover screws and its integration with the native bone (toluidine blue/pyronine G stain; magnification ×12.5).*

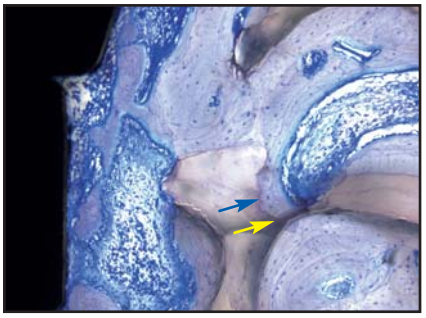


Fig 10 (left) *Group B specimen. Remodeling involving a deproteinized bovine block particle* (yellow arrow). *The same area was remodeled before* (blue arrow) *(toluidine blue/pyronine G stain; magnification ×160).*

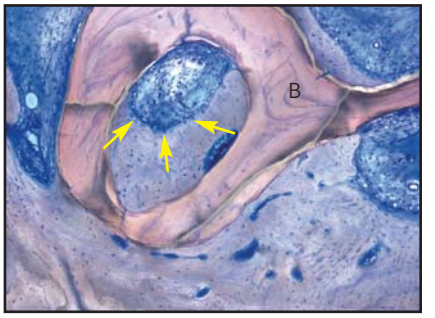


Fig 11 (right) *Group B specimen. Ongoing bone formation in the central portion of a deproteinized bovine bone trabecula. Note the lacunae in the central portion of the xenograft* (yellow arrows) *filled with new bone and an osteoblast lining (toluidine blue/pyronine G stain; magnification ×160). B = deproteinized bovine bone trabeculae.*

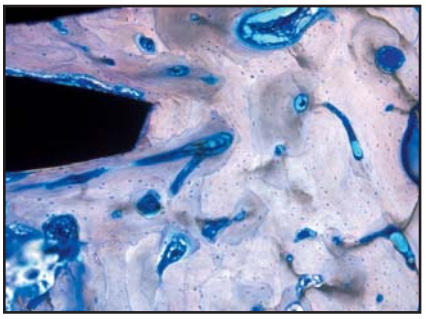


Fig 12 (left) *Group B specimen. Intense osteoblastic activity and remodeling with mature osteons (toluidine blue/pyronine G stain; magnification ×160).*

Fig 13 (right) *Group B specimen. Deproteinized bovine bone trabecula embedded in bone. Bone formation continues around the bovine particle and in areas adjacent to it Note the high degree of bone-to-implant contact of the newly formed bone (toluidine blue/pyronine G stain; magnification ×160).*

(Fig 13). Longitudinal sections through BRU demonstrated the typical cutting cones. Newly formed lamellar bone demonstrated bone density that was even higher than that present in the native alveolar ridge.

A high rate of bone-to-implant contact was visible in the areas of regenerated bone, even superior to that seen at the implant cover screw. Intense bone-forming activity continued at the bone-to-implant interface on both the oxidized and the machined titanium surfaces. The small number of specimens precluded any histomorphometric comparison.

Group C

The mesiodistal ground sections from group C (bovine cancellous block + rhPDGF-BB + membrane; specimens 496197L, 498688R, 496201R) showed some new bone formation in both the coronal portion facing the periosteum and at the apical portion in continuity with the native bone. The amount of

Fig 14 *Group C specimen (deproteinized bovine block + rhPDGF-BB + membrane). Overview of the mesiodistal ground section. Only a thin layer of new bone is visible at the coronal aspect of the specimen (toluidine blue/pyronine G stain; magnification ×8).*

newly formed bone appeared to be significantly lower than in the specimens treated with PDGF but not covered with a membrane (Fig 14). Only a thin layer of new bone was seen at the coronal aspect of the specimen. A large area of intact deproteinized bovine block embedded in healthy connective tissue was present between the coronal and apical layers of newly formed bone. Evidence of bovine block resorption and substitution was observed in the areas with new bone.

Discussion

Despite the evolution of numerous surgical approaches, vertical augmentation of the severely atrophic alveolar ridge continues to be a significant surgical challenge. The primary purpose of this proof-of-principle study was to evaluate the efficacy of vertical ridge augmentation in a standardized dog model by combining purified rhPDGF-BB and a deproteinized cancellous bovine bone block. A secondary objective was to determine the need for a resorbable barrier membrane when using a growth factor–mediated regenerative procedure.

Current procedures for treatment of the atrophic alveolar ridge often require intraoral or extraoral bone harvesting, inferior alveolar nerve transposition, or distraction osteogenesis. These procedures often involve increased morbidity and discomfort for the patient.[14] In the present study, an alternative based on recent advances in tissue engineering was used in place of current therapies in the treatment of the atrophic alveolar ridge. If effective, the use of a recombinantly produced growth factor, rhPDGF-BB, with a readily available xenograft block graft would avoid many of the difficulties associated with current therapies.

In the current study, critical-size defects were created to simulate the highly resorbed, posterior atrophic alveolar ridge often seen in human mandibles. A 3-month timeframe was selected between defect creation and bone augmentation to replicate a chronic alveolar ridge defect. Dental implants were used to fix a deproteinized cancellous bovine bone block to the superior mandibular surface. Three cohorts, as previously noted, were included, with four sites randomly assigned and included in each group. Following a healing period of 4 months, the animals were sacrificed and specimens prepared for histologic examination.

PDGF, the primary growth factor within the alpha granules of platelets, is a 30-kDa glycoprotein that is present in the bone matrix and actively secreted during early fracture repair.[15] When produced recombinantly for clinical application, PDGF 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 upregulates vascular endothelial growth factor, increasing blood supply to the defect site.[15–19]

The extent of the artificially created defects in this study, 30 mm in mesiodistal length and requiring 7 to 10 mm of supracrestal bone regeneration, posed a significant challenge to both soft and hard tissue healing. Clinically, group A (deproteinized bovine block + membrane), demonstrated early soft tissue wound dehiscence with subsequent infection and no bone formation in three of four sites. The only site without dehiscence formed mainly dense, fibrous connective tissue with little to no new bone.

Six of eight sites in groups B (deproteinized bovine block + PDGF) and C (deproteinized block + rhPDGF-BB + membrane) healed uneventfully. In Group B, one site developed a soft tissue wound dehiscence that precluded further healing. In Group C, infection occurred, and a fistula formed at one site, which also precluded further healing.

Clinically, therefore, 75% of the sites treated with the addition of rhPDGF-BB healed uneventfully, versus 25% of sites without the growth factor. The role of PDGF as a "wound-healing hormone" and its essential role in the normal healing of soft tissue and bone is well documented and seems to be borne out in this study by significantly fewer clinically observed soft tissue complications when rhPDGF-BB was included as part of the graft.[15] Uneventful early soft tissue healing was a necessary factor in treating critical-size vertical defects seen in this study and is an absolute prerequisite for subsequent bone regeneration.

The fragility of the xenograft block should be noted; 8 of the 12 procedures resulted in some fracture or disturbance of the block integrity. The possibility of microscopic fractures may very well be an explanation for the differences in osteogenic behavior.

In the three groups tested, clinical and histologic evidence of significant amounts of bone regeneration with good bone-to-implant contact occurred only in group B, treated with deproteinized cancellous block with rhPDGF-BB without a membrane. Of the four sites within this group, two exhibited sufficient bone volume to restore the artificially created atrophic alveolar ridge to its previous dimensions.

Bone regeneration in two of the four group B sites was impressive, with a large amount of bone-to-implant contact and augmented bone superior to the implant cover screws. The density of the regenerated bone appeared to be greater than that of the surrounding native alveolar ridge. In addition, intense osteoblastic activity was histologically evident, suggesting ongoing bone regeneration within the grafted sites.

Equally noteworthy was the intense remodeling of the bovine deproteinized bone block that occurred in group B: xenograft particles, embedded in newly formed bone, exhibited numerous resorption lacunae and bright seams of demineralization. Such accelerated remodeling of xenograft particles is not normally seen, suggesting further influence of the rhPDGF-BB on the original graft.

Clearly important in this study are the superior results obtained with the addition of the growth factor but without placement of an overlying collagen barrier membrane. Unlike traditional GBR procedures, where placement of an appropriate barrier membrane is integral to the success of the procedure, the growth factor–mediated regeneration method examined in this study suggests that barrier membranes are not only not required, but may prevent an adequate regenerative response. Similar results without barrier membranes have also been seen in preclinical and clinical studies with bone morphogenetic protein-2.[20–22]

The histologic finding of lack of bone formation in the central portion of the deproteinized bovine block suggests that bone could regenerate from the periphery to the center of the wound. A difficulty in the infusion process, which resulted in incomplete soaking of the central portion of the block by PDGF, could be the cause of this.

The importance of the periosteum in growth factor–mediated regenerative procedures is also suggested by the results of this study. The role of periosteum in osteogenesis, in which it serves as a source for pluripotential mesenchymal cells and osteoblasts, especially at fracture sites, is well documented.[23–25] To effectively induce chemotaxis in bone regenerative procedures, PDGF requires an adequate supply of locally available osteoblastic-type cells—which are found in the undersurface of an intact periosteum. Imposition of a barrier membrane, as required in current GBR procedures, between the periosteum and graft appears to block PDGF's access to periosteally derived osteogenic cells, and therefore would appear to be contraindicated in PDGF-mediated regenerative procedures.

Conclusion

Vertical augmentation of the severely atrophic alveolar ridge continues to be a significant surgical challenge for the profession. In this preclinical proof-of-principle canine study, rhPDGF-BB, when combined with a deproteinized cancellous bovine bone block without the use of a barrier membrane, evidenced significant new bone regeneration, with high bone-to-implant

contact, accelerated remodeling of the xenograft carrier, and, in two of four sites, restoration of atrophic ridges back to normal anatomic form. Proof-of-principle that the use of the active growth factor rhPDGF-BB, when placed on an appropriate carrier, may regenerate bone in severe canine atrophic alveolar ridge defects was thus achieved.

Acknowledgments

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