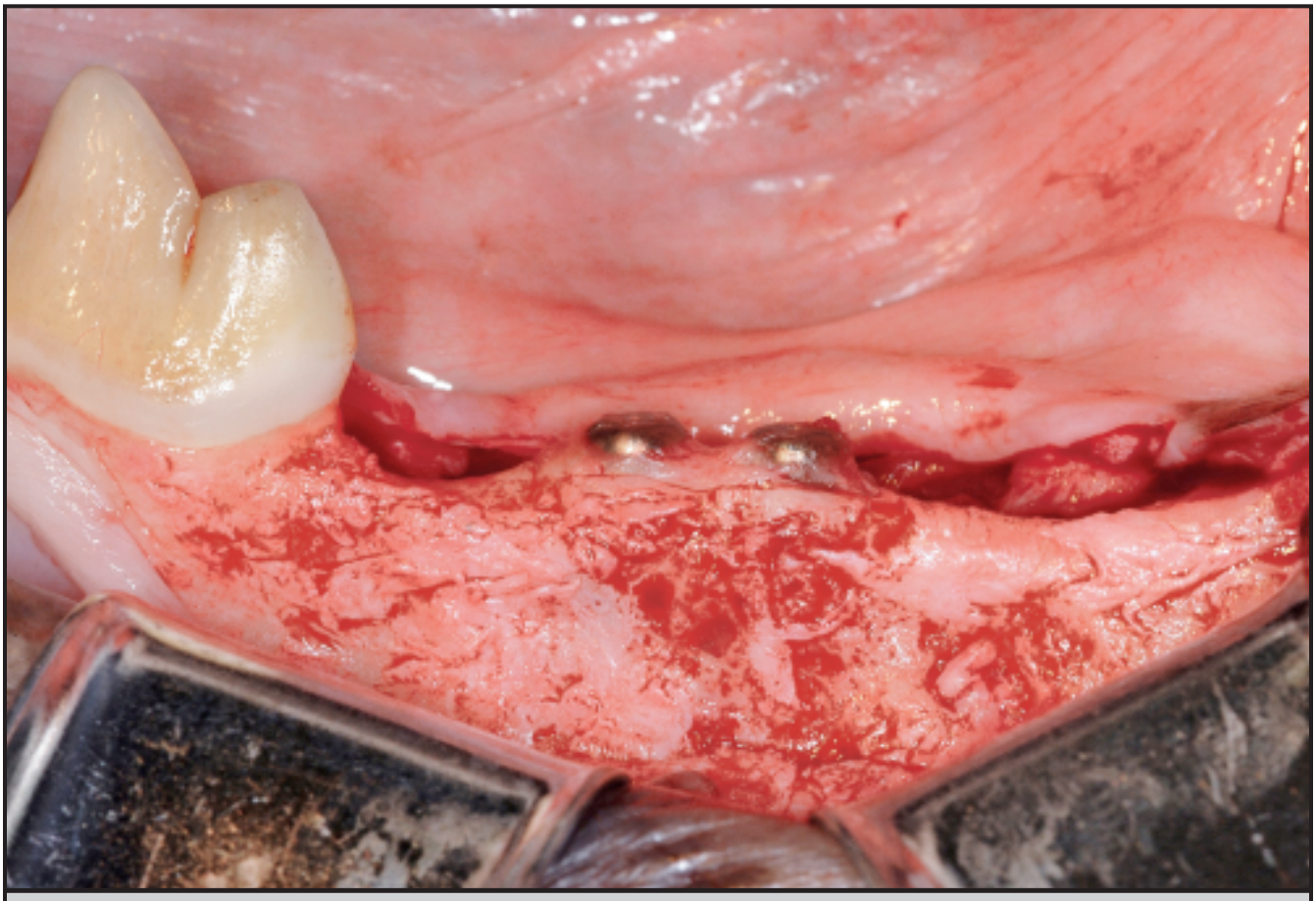
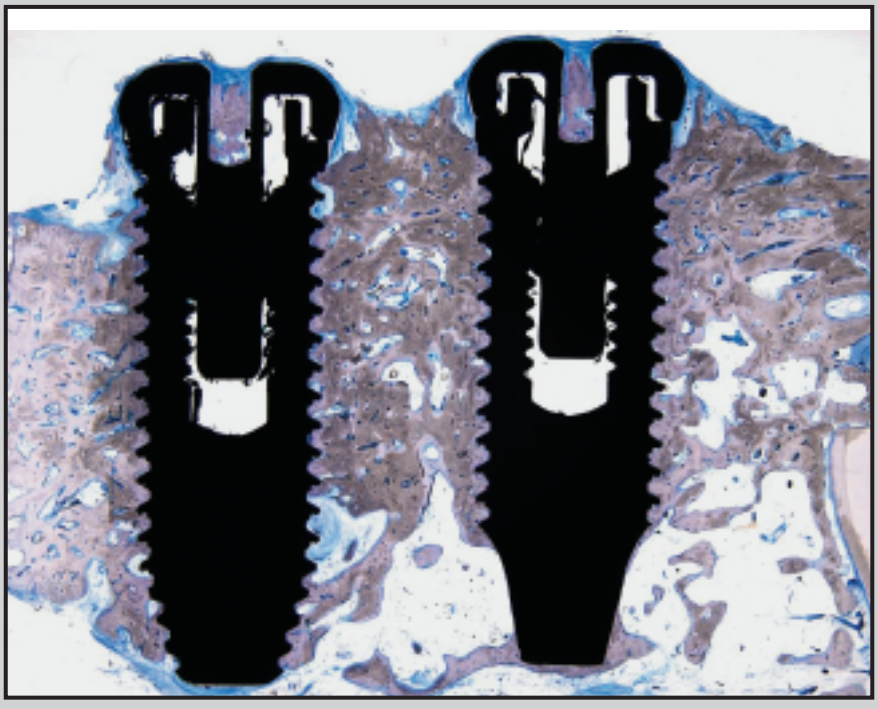
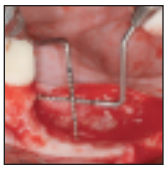




Vertical Ridge Augmentation Using an Equine Block Infused with Recombinant Human Platelet-Derived Growth Factor-BB: A Histologic Study in a Canine Model



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This preclinical study evaluated the efficacy of purified recombinant human platelet-derived growth factor (rhPDGF-BB), combined with a novel equine hydroxyapatite and collagen (eHAC) bone block, in providing vertical bone regeneration in critical-size defects simulating localized mandibular alveolar bone atrophy. In addition, the impact of barrier membrane placement in growth factor–mediated bone regeneration was also studied. Bilateral posterior mandibular defects simulating severe localized bony atrophy were created in 12 adult foxhounds following removal of all four mandibular premolars. Three months later, the defects were grafted as follows: group A: eHAC block alone; group B: eHAC block + collagen membrane; group C: eHAC block + rhPDGF-BB; group D: eHAC block + rhPDGF-BB + membrane. The animals were sacrificed after 5 months and the grafted areas were examined histologically, radiographically, and clinically. Groups A and B (controls) exhibited little to no vertical bone regeneration. Group C demonstrated significant vertical bone regeneration, with dense, well-vascularized bone, high bone-to-implant contact, and accelerated replacement of graft particles with newly formed bone. In group D, with the imposition of a barrier membrane, robust bone regeneration was less evident when compared to group C. As in the first study in this series, the importance of the periosteum as a source of osteoprogenitor cells in growth factor–mediated regenerative procedures is examined. (Int J Periodontics Restorative Dent 2009;29:245–255.)

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Effective vertical regeneration of severely atrophied edentulous alveolar ridges often continues to elude surgeons' best efforts, in spite of significant advances in bone regenerative therapeutics. Restoration of vertical bone height in these atrophied jaw segments is critical to successful long-term implant survival and function. Numerous procedures, including bone splitting, distraction osteogenesis, forced tooth eruption, guided bone regeneration, and autogenous onlay bone grafting, provide surgeons with an array of alternative approaches to the management of severe alveolar bony atrophy.[1–6] Although viable, each of these procedures presents potential complications and has shown limited success when addressing severe alveolar bone loss. Autogenous bone grafts remain perhaps the mainstay of current approaches to augment severely atrophied alveolar bone.

Autogenous bone grafts represent an ideal matrix by providing an immunologically compatible source of bone complete with viable osteoprogenitor and mature osteoblastic cells, an effective osteoconductive scaffold, and abundant numbers of the

growth factor–signaling molecules required for optimal bone regeneration.[7] However, pain and morbidity at the donor site, as well as a limited supply of autogenous graft material, often preclude or discourage the use of autogenous grafts.[8]

Tissue engineering may offer a viable and attractive alternative to current treatment modalities for the surgical management of severe jawbone atrophy. Recombinantly produced human platelet-derived growth factor-BB (rhPDGF-BB), with its potent chemotactic and mitogenic effects on such target cells as periodontal ligament and alveolar bone, as well as its critical role in angiogenesis, may potentially play an effective role in the treatment of severe alveolar bone atrophy.[9–11] Simion et al achieved significant vertical bone regeneration in severe mandibular alveolar ridge defects using a deproteinized bovine block infused with rhPDGF-BB in a canine model.[12] A human case report produced similar results, with placement of an rhPDGF-BB–saturated deproteinized bovine block prior to implant placement in the posterior mandible.[13] Growth factor–mediated bone regeneration obviated the need for autogenous grafting in both studies.

Placement of a user-friendly matrix with appropriate growth factor attachment and release kinetics is critical to successful bone and tissue regenerative procedures. An equine hydroxyapatite and collagen bone block (eHAC) appeared to be biocompatible and easily managed when applied to atrophied bony surfaces in a canine model.[14] The purpose of the current study was to assess the safety and efficacy of the eHAC block, infused with rhPDGF-BB and applied with and without a collagen barrier membrane, to achieve vertical bone augmentation in severe mandibular critical-size defects in a standardized canine model.

Method and materials

The study protocol was approved and conducted in accordance with the Biomatech Ethical Committee in Lyon, France.

Tooth extraction and defect creation

Twelve foxhounds (each weighing at least 25 kg) were selected for the study. A critical-size alveolar bone defect with both the buccal and lingual bony plates removed was created by bilateral extraction of all four mandibular premolars and surgical reduction of the ridge height and width with rotary and hand instrumentation. The dimensions of the standardized defects were 20 to 25 mm mesiodistally, 7 to 8 mm apicocoronally, and 10 mm buccolingually (Fig 1a). Primary wound closure was achieved by means of interrupted sutures (CV-5, W. L. Gore & Associates). Periapical radiographs were obtained prior to and immediately following surgery. Sutures were removed after 10 to 14 days.

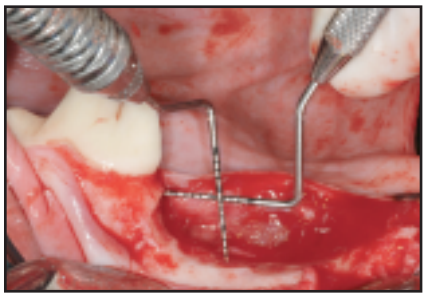


Fig 1a *Initial critical-size defect created 3 months prior to augmentation.*

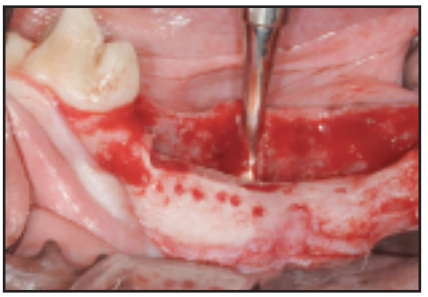


Fig 1b *Full-thickness mandibular flaps were elevated prior to the regenerative procedure. Cortical perforations were performed to encourage bleeding.*

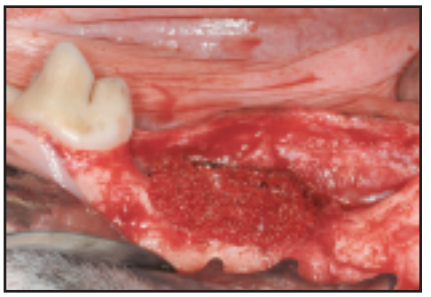


Fig 2 *An equine hydroxyapatite and collagen (eHAC) bone block was adapted and placed onto the atrophic mandibular surface and secured by means of two mesial and distal titanium dental implants. In 14 sites (groups C and D), the block was saturated with rhPDGF-BB prior to placement in the defect.*

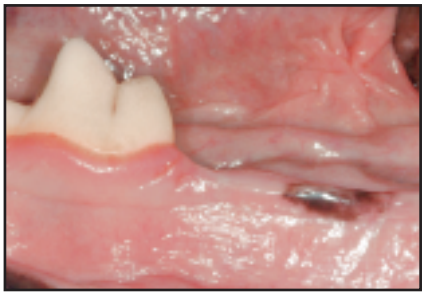


Fig 3 *An eHAC + rhPDGF-BB (group C) site after 5 months of submerged healing. Note the volume of the ridge and the complete closure of the overlying soft tissues.*

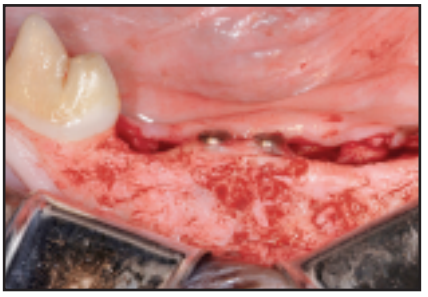


Fig 4a *Clinical reentry of group C site. The implants are completely covered with tissue resembling bone.*

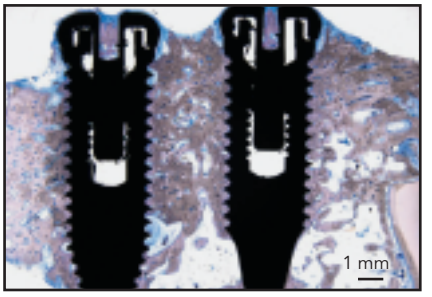


Fig 4b *Group C specimen demonstrates new bone formation within the cover screws.*

Vertical ridge augmentation procedure

To create a chronic bony defect, a healing period of 3 months preceded the augmentation procedure. At that time, bilateral mandibular full-thickness buccal and lingual mucoperiosteal flaps were elevated, extending from the mesial aspect of the canine to the distal aspect of the first molar. All soft tissue remnants were removed from the defect surface, and cortical perforations were made with a round carbide bur, exposing the underlying medullary spaces (Fig 1b). An eHAC bone block (Geistlich Pharma) was closely adapted to the bony defect site and stabilized with two titanium implants (3.3 × 10 mm, Speedy Groovy, Nobel Biocare) that penetrated both the block and the native mandibular cortical bone (Fig 2). Cover screws were then placed over the implants.

Four cohorts were included in the study design. Group A, one of two control groups, received an eHAC bone block alone. Group B, the second control group, received the eHAC bone block in conjunction with an overlying resorbable collagen membrane (Bio-Gide, Geistlich Pharma). Group C animals were treated with an eHAC block infused with rhPDGF-BB (Osteohealth) and group D animals were treated with an eHAC block infused with rhPDGF-BB and covered with a resorbable collagen membrane. Ten defect sites were randomly allocated to groups A and B, whereas 14 sites were randomly allocated to groups C and D, for a total of 24 sites.

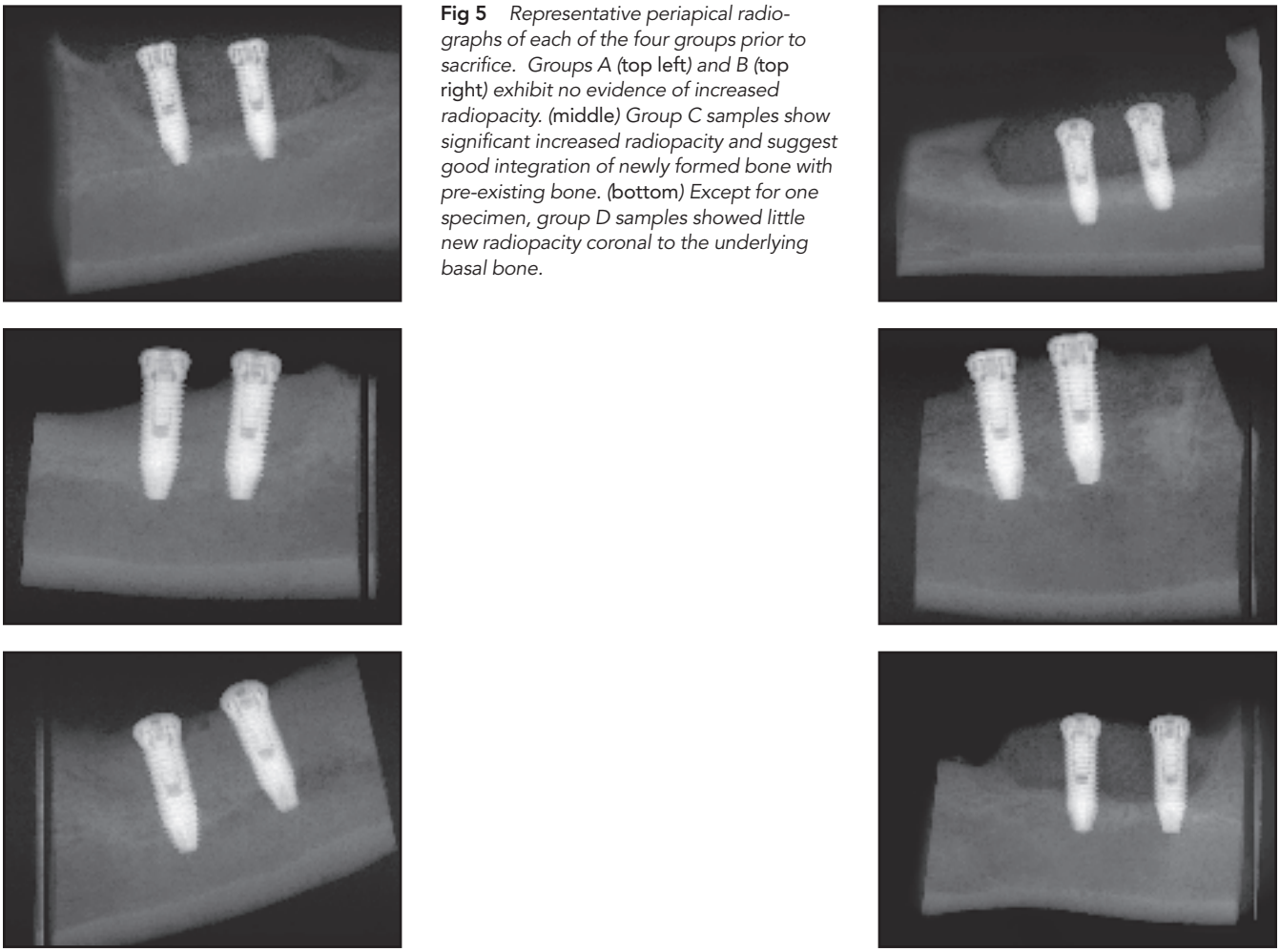


Fig 5 *Representative periapical radiographs of each of the four groups prior to sacrifice. Groups A (*top left*) and B (*top right*) exhibit no evidence of increased radiopacity. (*middle*) Group C samples show significant increased radiopacity and suggest good integration of newly formed bone with pre-existing bone. (*bottom*) Except for one specimen, group D samples showed little new radiopacity coronal to the underlying basal bone.*

The eHAC bone block used in groups C and D was inserted into an empty sterile syringe and infused under pressure with rhPDGF-BB (three units) for 5 minutes. In all surgical sites, tension-free primary soft tissue closure was achieved by means of periosteal releasing incisions, followed by horizontal mattress and interrupted sutures. Periapical radiographs were obtained prior to and immediately following surgery. Antimicrobial prophylaxis consisted of spiramycin 750,000 IU and metronidazole (125 mg, 1 tablet/10 kg per day per os) beginning at least 5 days before and continuing for at least 14 days after surgery (Stomorgyl, Merial). Oral hygiene was maintained with chlorhexidine digluconate wipes three times a week for 2 weeks. Sutures were removed after a healing period of 15 days. Following a healing period of 5 months after grafting, periapical radiographs were obtained and the animals were then killed.

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 with an Exakt cutting unit. The slices were reduced using an Exakt grinding unit to an even thickness of 30 to 40 µm and stained with toluidine blue/ pyronine G and examined with

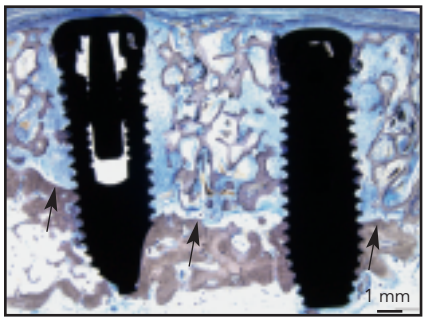
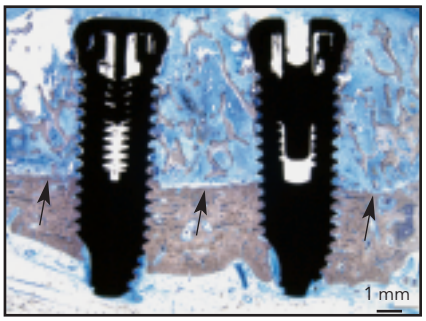


Fig 6 (left) *Group A specimen (eHAC block alone) in a coronal ground section demonstrates abundant amounts of connective tissue with no evidence of bone regeneration. Intact matrix material remains evident (magnification ×8).*

Fig 7 (right) *Group B specimen (eHAC block + membrane) demonstrates the implant embedded in connective tissue with no evidence of vertical bone regeneration. Unresorbed matrix material is seen throughout the section (magnification ×8).*



a Leica DM6000B light microscope. Ground sections were prepared in a mesiodistal direction.

Results

Clinical observations

Healing proceeded uneventfully for all 24 surgical sites during the 3 months following creation of the mandibular defects. The mandibular alveolar ridges appeared flat with no-wall defects, simulating localized atrophic posterior mandibles.

The 5-month postgrafting healing phase was uneventful for 13 of the 24 sites, with four sites exhibiting small soft tissue fistulae and six sites exhibiting soft tissue dehiscences. One site in group A (eHAC block alone) lost the implanted block and the associated implants. Four of the six soft tissue dehiscences occurred in sites that received the block with or without the membrane but without the rhPDGF-BB (groups A and B). Hence, an uneventful clinical healing rate of 85.7% was seen in sites treated with rhPDGF-BB, compared to 50% in sites that did not receive the growth factor.

Surgical reentry of one of the group C sites (eHAC block + rhPDGF-BB) revealed implants that were completely covered with bonelike tissue (Figs 3 and 4).

Radiographic observations

All ten sites in groups A (eHAC block alone) and B (eHAC block + membrane), which did not receive rhPDGF, demonstrated no evidence of additional radiopacity superior to the grafted defect surface, indicating no new bone formation (Fig 5). Group C sites (eHAC block + rhPDGF-BB) exhibited radiographic evidence of new bone regeneration, with a number of implants completely embedded in bone. Radiographically, the newly regenerated bone appeared well integrated with both the underlying basal bone and the grafted eHAC block. In all but one specimen, group D (eHAC block + rhPDGF-BB + membrane) sites demonstrated little to no increased radiopacity coronal to the grafted defect surface. In one group D specimen, increased radiopacity extended coronally to the level of the cover screws, indicating significant new bone regeneration (Fig 5).

Histologic observations

Group A (eHAC block)

Sites grafted with the eHAC block alone demonstrated little to no bone regeneration coronal to the native basal bone (Fig 6). Abundant amounts of unresorbed HA particles were embedded in healthy connective tissue that was devoid of inflammation. An intact epithelium covered the grafted areas.

Group B (eHAC block + membrane)

Group B sites demonstrated histologic findings similar to those seen in group A. Little to no bone regeneration was evident coronal to the apical native bone. Healthy, inflammation-free connective tissue surrounded the implants. As in group A, unresorbed matrix material was seen throughout the specimens (Fig 7).

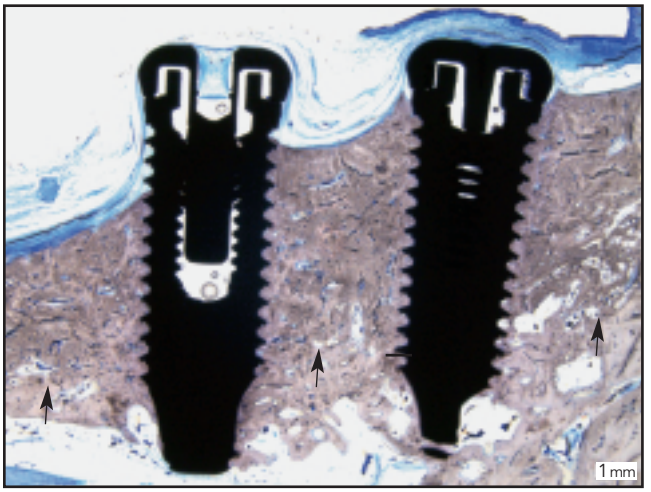


Fig 8 *Group C test specimen (eHAC block + rhPDGF-BB) exhibits new bone formation up to the implant cover screw. Dense, well-vascularized bone surrounds the entire implant. Few, if any, residual matrix particles remain at 5 months postgrafting (magnification ×8).*

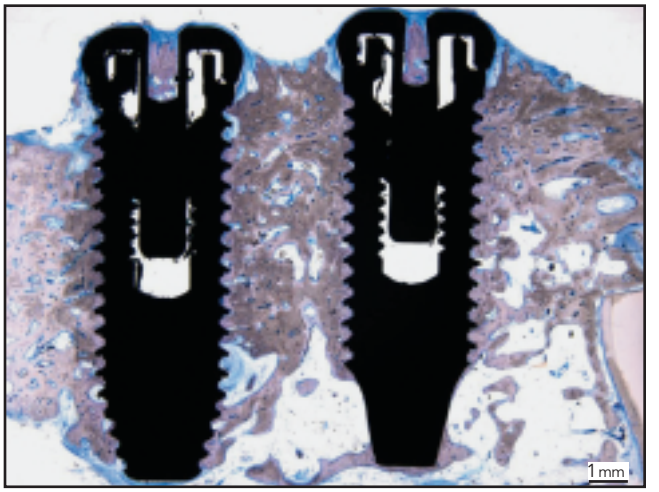


Fig 9 *Group C specimen demonstrates new bone formation within the cover screws.*

Group C (eHAC block + rhPDGF-BB)

Five of seven group C sites demonstrated histologic evidence of new bone formation. Complete bone regeneration up to the implant cover screws was evident in three of the five sites that exhibited new bone formation (Fig 4). Dense, well-vascularized bone was seen surrounding the entire body of the implant in a typical example within this cohort (Fig 8). Of particular importance was the amount of bone-to-implant contact seen histologically. Indeed, the density of the regenerated bone exceeded that seen in the apical native bone. Additionally, few if any residual matrix particles were found within the grafted area, which was almost completely replaced by newly formed bone. Interestingly, in one of the specimens, new bone had formed within the cover screws themselves (Fig 9).

High-power magnification provided further insight into the intense pro-osteogenic and angiogenic effects mediated by rhPDGF-BB. In Fig 10, intense osteogenic activity can be seen at the advancing margin of new bone formation, mediated by chemotactically recruited osteoblasts. Active remodeling and resorption of eHAC matrix particles by multinucleated giant cells was noted throughout the rhPDGF-BB–grafted sites but was largely missing in sites where the growth factor was excluded (Fig 11). Importantly, intimate, seamless integration occurred between well-formed newly regenerated bone and the underlying native bone (Fig 12). Osseointegration occurred between newly formed bone and the implant threads, allowing for high bone-to-implant contact (Fig 13). Finally, the occasional residual eHAC matrix particle seen under high magnification likewise showed intimate, seamless integration with the surrounding newly regenerated bone (Fig 14).

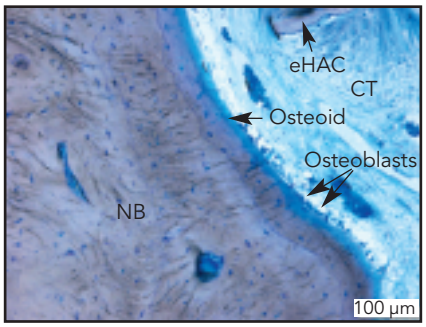


Fig 10 *Light microscopic view (group C specimen) of ongoing bone formation in areas previously occupied by eHAC matrix particles. Note new bone formation and the seam of osteoid lined with osteoblasts (ground section, toluidine blue). NB = new bone; CT = connective tissue.*

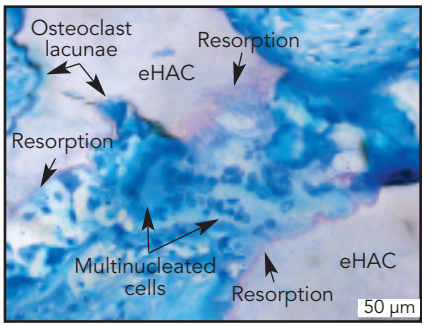


Fig 11 *Light microscopic view (group C specimen) of ongoing resorption phenomena in eHAC particle areas. Note the presence of multinucleated giant cells forming resorption seams, as well as osteoclastic activity resulting in osteoclastic lacunae (ground section, toluidine blue).*

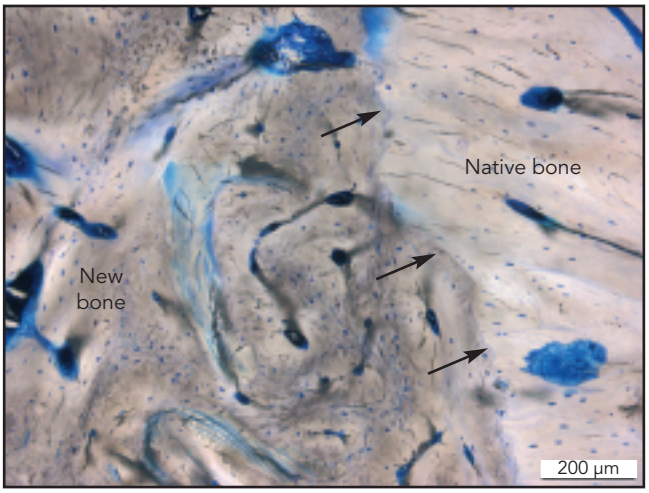
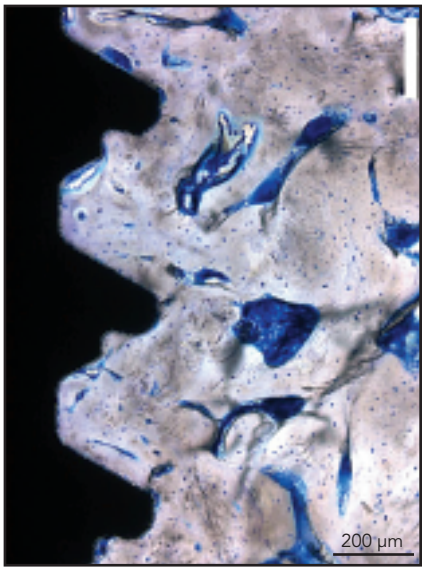


Fig 12 (above) *Light microscopic view (group C specimen) of the border (arrows) between native bone and newly formed bone in an eHAC specimen with rhPDGF-BB without membrane. Note the high remodeling activity in newly formed bone (ground section, toluidine blue).*

Fig 13 (above right) *Light microscopic view (group C specimen) of the osseointegration of the implant by newly formed woven bone (NB) formation in an eHAC specimen with rhPDGF-BB without membrane. Note the high remodeling activity in woven bone (ground section, toluidine blue).*



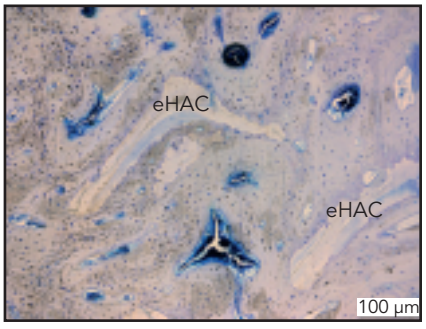


Fig 14 *Light microscopic view (group C specimen) of residues of the eHAC block embedded in newly formed bone. Note the high remodeling activity in newly formed bone (ground section, toluidine blue).*

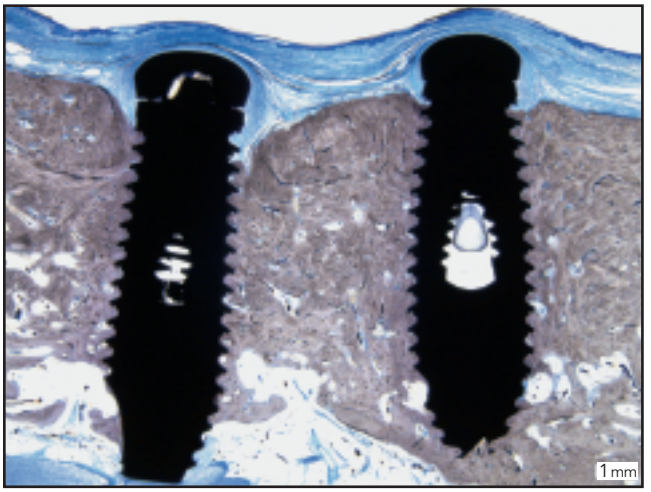


Fig 15 *Group D test specimen (eHAC block + rhPDGF-BB + membrane) demonstrates complete new bone formation surrounding both implants. Bone density and bone-to-implant contact are comparable to similar images seen in group C (magnification ×8).*

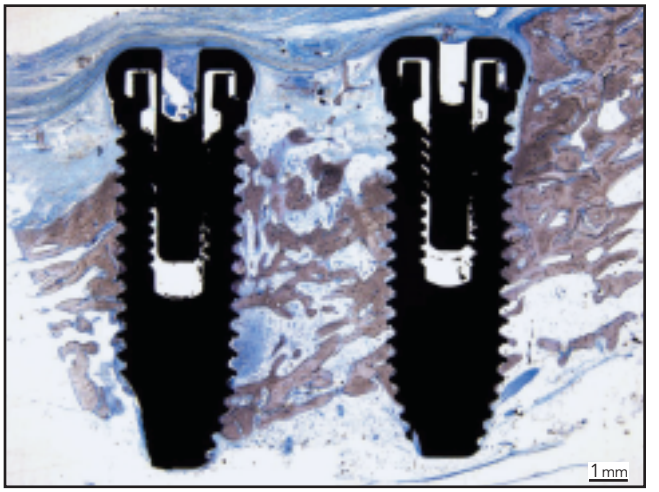


Fig 16 *In this group D specimen, new vertical bone growth has occurred, with good density and bone-to-implant contact. However, the vertical bone regeneration is incomplete, with a portion of the implant threads surrounded by dense connective tissue (magnification ×8).*

Group D (eHAC block + rhPDGF-BB + membrane)

Although group D sites demonstrated new bone regeneration, the vertical bone growth was generally less than that seen in group C specimens. The bone quantity and density in one specimen were comparable to that seen in group C specimens (Fig 15). Other specimens demonstrated less newly formed bone than was seen in group C sites (Fig 16).

Discussion

Vertical ridge augmentation, especially in the severely atrophic mandible, continues to pose significant clinical challenges. A number of current procedures provide different approaches to the correction of vertical alveolar ridge deficiencies. Recent systematic reviews emphasize the lack of predictable outcomes and frequent complications associated with current therapies for vertical ridge deficiencies.[15,16] In an effort to reduce the complication rates, especially those related to donor surgery associated with autogenous grafts, as well as to improve efficacy outcomes, growth factor–mediated regenerative procedures may offer attractive alternatives to current procedures. The present study is the second in a series of proof-of-principle studies designed to investigate the potential of rhPDGF-BB when placed on appropriate bone substitute matrices to regenerate well vascularized, dense bone in severe localized posterior mandibular defects.

As in the first study in the current series,[12] critical-size defects were created to simulate the highly resorbed posterior alveolar ridges that are frequently seen in human mandibles. To replicate a chronic bone defect, a 3-month waiting period was chosen between the time of defect creation and the bone augmentation procedure. Unlike the earlier study, an additional control cohort, ie, bone substitute block graft alone, was added to this investigation to provide a parallel comparison to test group C (block + rhPDGF-BB).

In addition to the extra control group, a different bone substitute matrix was used in the current study.[14] In the earlier study, a brittle cancellous deproteinized bovine block (Bio-Oss cancellous block, Osteohealth), designed for indications other than mandibular vertical ridge augmentation, was prone to fracture during implant insertion. In addition, the block's friability made proper shaping difficult.[12] In the current investigation, a new equine HA and collagen bone block (eHAC) appeared to be more malleable, less prone to fracture, and easier to trim, and it had diminished sharp angles and was more readily adapted to the shape of the native mandibular basal bone defect. Fewer dehiscences occurred in this second investigation, perhaps as a result of the fact that this more malleable block material exerted less pressure on the coronally repositioned soft tissues, with fewer sharp angles and corners.

The clinical and histologic results of the current investigation parallel those seen in the prior study in this series. In both studies, control groups, with or without barrier membranes and without the addition of rhPDGF-BB, failed to produce new vertical bone growth. In all instances, inflammation-free connective tissue surrounded the implant bodies, with little evidence of surrounding new bone regeneration. In addition, although the matrices were different in each investigation, matrix particles persisted in all control groups, showing little tendency toward remodeling during the time frames of each study.

Similar, although not identical, results were seen in the test groups of the current investigation when compared to the earlier study. Vertical bone regeneration was clearly more predictable and consistent when rhPDGF-BB was added to each block matrix without the imposition of a barrier membrane. In the current study, five of seven sites in group C (eHAC + rhPDGF-BB) exhibited vertical bone growth, with three sites showing complete bone regeneration up to the implant cover screws. In one case, bone regenerated within the cover screws. In addition, the regenerated bone was dense and well-vascularized, with excellent bone-to-implant contact. In those sites where bone regeneration occurred, the newly formed bone appeared denser than the native apical bone. As in the prior study, efficient replacement resorption of the matrix particles with newly formed bone occurred when the matrix was saturated with rhPDGF-BB. Accelerated bone graft substitute resorption, with subsequent replacement with dense new bone, occurred in both groups C and D in those specimens exhibiting new bone regeneration.

Finally, both studies continue to highlight the importance of the periosteum as a source of progenitor cells during bone regenerative procedures.[17–19] Growth factor–mediated bone regeneration benefited when access to the periosteum was not prohibited by a barrier membrane. The superior regenerative results seen in group C without barrier membranes emphasize the importance of allowing chemotaxis to osteogenic periosteal cells.

Conclusion

Vertical ridge augmentation, especially in the severely atrophic mandible, continues to pose significant surgical challenges for the clinician. This proof-of-principle study, which combined recombinant human platelet-derived growth factor-BB with a novel equine hydroxyapatite and collagen bone block without the use of a barrier membrane, resulted in significant vertical bone regeneration, with dense, well-vascularized bone; high bone-to-implant contact; and accelerated replacement of graft particles with newly formed bone. Five of seven sites in the group that received bone blocks infused with growth factor exhibited substantial bone regeneration, with three of the sites showing complete vertical restoration of the atrophied mandible. The current study provides further evidence of the potential for tissue-engineered solutions in the management of difficult-to-treat jawbone defects.

Acknowledgment

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