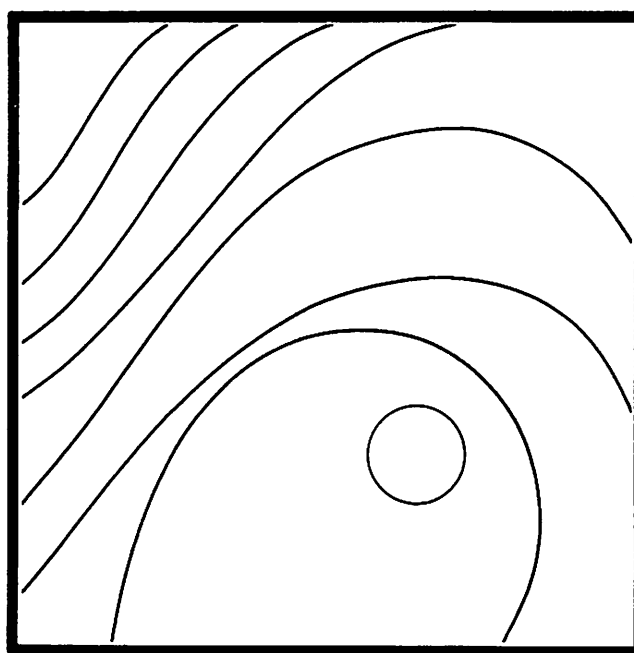


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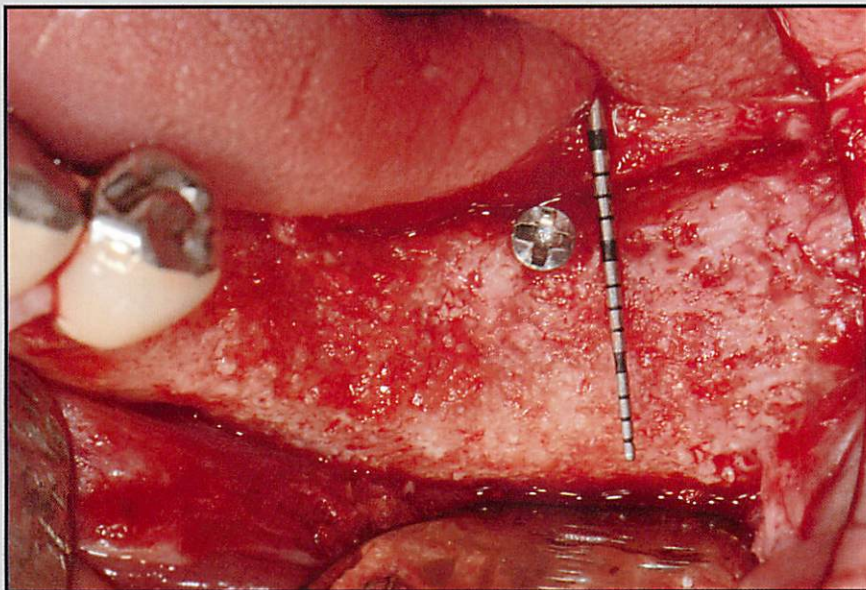
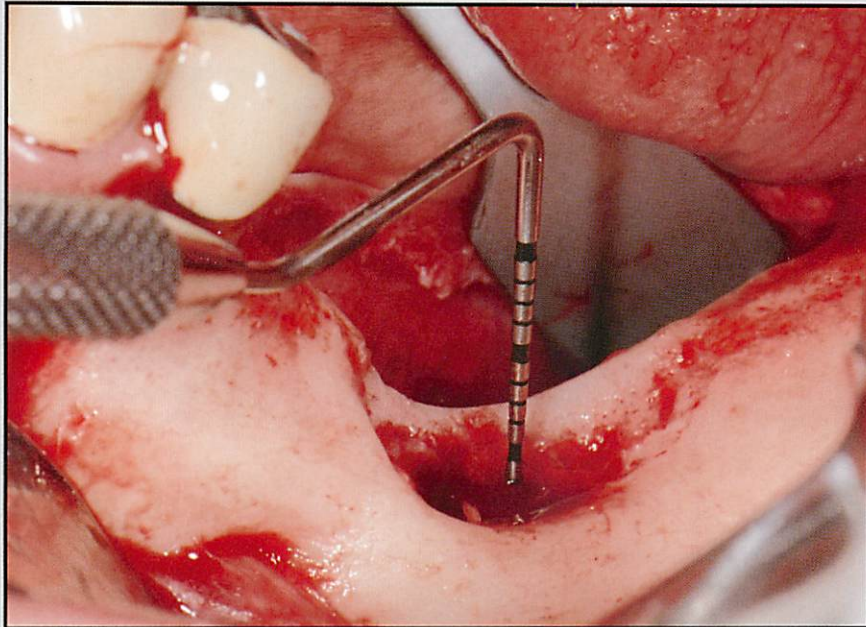
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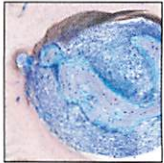


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Three-Dimensional Ridge Augmentation with Xenograft and Recombinant Human Platelet-Derived Growth Factor-BB in Humans: Report of Two Cases



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The present paper reports on two patients who underwent three-dimensional ridge augmentation using a xenograft in combination with recombinant human platelet-derived growth factor-BB (rhPDGF-BB). Patient 1 received a deproteinized bovine block infused with PDGF and secured to the alveolar crest by two fixation screws to augment the crest horizontally. After 5 months, implants were successfully placed. Patient 2 underwent a vertical ridge augmentation procedure that combined deproteinized bovine bone particles embedded in a collagen matrix soaked in PDGF. Three titanium dental implants were placed in each patient 5 months later. Clinical and histologic results showed excellent soft and hard tissue healing. Bone had regenerated throughout the whole area and the xenograft particles were embedded in bone, which presented resorption lacunae close to areas with ongoing bone formation. This indicated that, in augmented areas, intense physiologic remodeling was ongoing. No data exist concerning three-dimensional bone augmentation using PDGF and a xenograft in humans. This report suggests that the use of rhPDGF-BB in combination with a deproteinized bovine graft may have the potential to regenerate large three-dimensional alveolar defects in humans. (Int J Periodontics Restorative Dent 2007;27:109–115.)

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Placement of dental titanium implants is a well-established treatment modality in edentulous areas of the jaws.¹ However, implant placement may not be possible in areas with limited alveolar bone height. For these situations, reconstructive surgical techniques have been developed to restore missing bone to allow the placement of dental implants in either simultaneous or staged approaches.^{2,3}

Techniques such as autogenous onlay bone grafts, distraction osteogenesis, and nerve transposition result in increased morbidity to the patient and often require two separate surgical procedures because bone must be harvested from intra-oral or extraoral sites. Moreover, resorption of up to 50% of grafted autogenous bone has been documented, especially when it is used for vertical ridge augmentation.⁴

Guided bone regeneration (GBR) techniques are based on the principle of compartmentalized wound healing. The technique implies that an enclosed space is created by the placement of a physical barrier, so that only bone-forming cells are

allowed to populate the healing wound.^{5,6} A reported drawback of these procedures is their technical complexity, with frequent premature membrane exposure resulting in bacterial contamination. This complication, which is generally attributed to insufficient periosteal releasing incisions and excessive tension in the sutures at closure, is particularly evident when nonresorbable membranes are used. To overcome these problems, less invasive surgical modalities that are technically less demanding and promote faster bone regeneration are sought by clinicians. Hence, a technique that eliminates the need for a barrier membrane and autogenous bone would be beneficial to reduce the incidence of complications and increase patient acceptance of alveolar ridge augmentation.

Tissue engineering is defined as any attempt to regenerate tissues of the body by combining the elements of cells, scaffolds, and signaling molecules. Platelet-derived growth factor (PDGF) is a signaling molecule contained in the alpha granules of blood platelets and bone matrix.⁷ Its efficacy in wound healing has been studied extensively in animals and humans. It is considered to be chemotactic, to be mitogenic for osteoblasts, and it initiates angiogenesis leading to capillary budding into the graft site.

A histologic study⁸ conducted in dogs demonstrated that the combination of recombinant human (rh) PDGF-BB and a deproteinized bovine block, without placement of a barrier membrane, has the potential to regenerate significant amounts of new bone to overcome severe mandibular ridge

defects. However, there is no available data concerning the use of PDGF for three-dimensional alveolar bone augmentation in humans. The current report provides a clinical and histologic evaluation of two clinical human situations in which three-dimensional bone augmentation was performed using rhPDGF-BB in combination with a deproteinized bovine bone xenograft.

Clinical case descriptions

Both patients provided written informed consent regarding grafting procedures and implant placement. All surgeries were done under local anesthesia.

Patient 1

A 65-year-old female Caucasian patient presented for placement of osseointegrated implants in the mandibular left premolar and molar regions. Preoperative examination by means of panoramic radiographs and tomographs revealed inadequate bone volume. The edentulous ridge lacked bone height and width for correct implant placement.

A full-thickness incision was made within the keratinized mucosa from the distal aspect of the canine to the ascending ramus of the mandible. The incision was extended intrasulcularly to the mesial aspect of the canine. Vertical releasing incisions were made at the mesiobuccal angle and at the distal aspect of the crestal incision. After elevation of a full-thickness flap, an atrophic 2-mm-wide alveolar ridge

was detected, with a lack of bone height of about 3 mm (Fig 1a). The bone was curetted to remove all residual soft tissue and perforated to expose the medullary spaces and increase bleeding.⁹ A deproteinized bovine bone block (Bio-Oss, Geistlich) was adapted to the defect, soaked under vacuum conditions with rhPDGF-BB (Osteohealth), and fixed to the buccal bone wall of the defect with two screws (ACE Surgical Supply) (Fig 1b). The fragility of the xenograft block should be noted, because this could result in a fracture or disturbance of the integrity of the block. Releasing incisions were placed in the buccal and lingual periosteum to enhance elasticity of the flap and to achieve tension-free adaptation. The wound was closed with horizontal mattress and interrupted sutures (W. L. Gore).

After an uneventful healing period of 5 months, the flaps were reopened with a midcrestal incision, the regenerated tissue was exposed, and the fixation screws were removed. The radiographic appearance at the time of reentry suggested that the cancellous bovine block had integrated with the basal bone (Fig 1c).

The regenerated tissue appeared hard, and residual remnants of deproteinized bovine bone were evident at the surface (Fig 1d). Three implants (MKIII Ti-Unite regular platform, Nobel Biocare) were placed in the sites of the first and second premolar and the first molar (Figs 1e and 1f).

A biopsy was harvested from the regenerated bone with a 3-mm internal-diameter trephine bur. This sample was processed for histologic examination (Figs 1g and 1h).

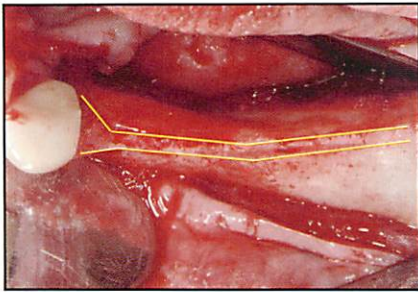


Fig 1a (left) Atrophic alveolar ridge of the edentulous site in patient 1. The very narrow bone crest (yellow lines) would not allow placement of implants.

Fig 1b (right) A deproteinized bovine block infused with rhPDGF-BB was placed over the buccal wall of the atrophic mandible and secured by means of two fixation screws mesially and distally.

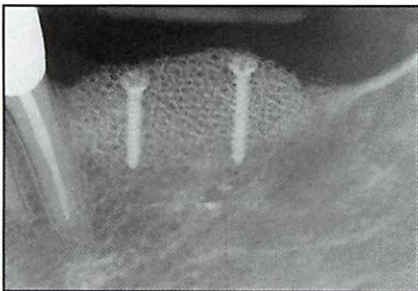
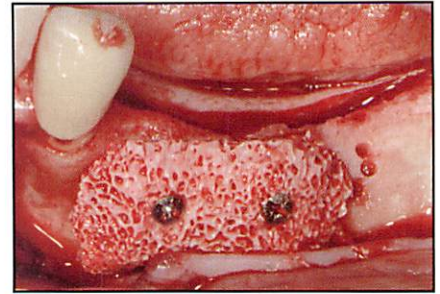


Fig 1c (left) Radiographic appearance at the time of site reentry (5 months).

Fig 1d (right) Occlusal view of the clinical appearance of the site at reentry. Remnants of the deproteinized bovine block are apparently well integrated into the native alveolar crest. Note the hard bleeding surface of the regenerated tissue.

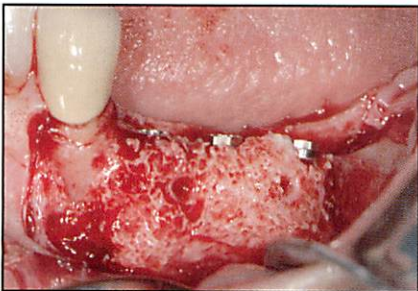
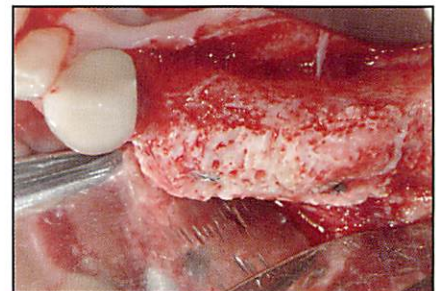


Fig 1e (left) Three implants are placed in the left posterior quadrant of the mandible.

Fig 1f (right) Radiographic appearance of the three implants in place following loading, which occurred 6 months after implant placement.

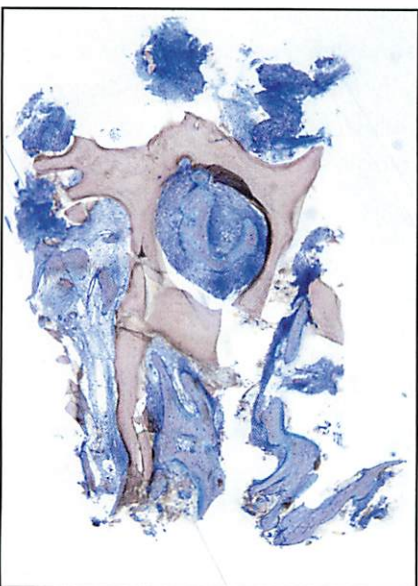
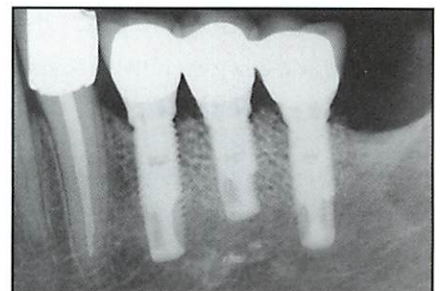


Fig 1g (left) Histologic overview of the specimen stained with toluidine blue/pyronine G. Note the formation of new bone throughout the sample. Resorption lacunae in the xenograft trabeculae, associated with ongoing bone formation, are detectable (original magnification $\times 12.5$).

Fig 1h (right) Higher magnification of a xenograft trabeculae embedded in regenerated bone. Ongoing bone formation in the central portion of the deproteinized bovine bone trabeculae. Note the lacuna in the central portion of the xenograft, filled with new bone and lined by osteoblasts (original magnification $\times 160$).



Patient 2

A 60-year-old man who required a fixed prosthesis presented with a deep vertical bone defect in the left posterior mandible. The defect was caused by the failure of two implants corresponding to the first and second molar areas (Fig 2a).

A full-thickness crestal incision was made in the keratinized mucosa of the edentulous ridge. During careful flap elevation, the lingual flap was gently reflected beyond the mylohyoid insertion of the omohyoid muscle to enhance its mobility and allow a subsequent coronal advancement of the flap. A horizontal releasing incision was made at the base of the buccal flap for the same purpose. After the flap was elevated, a vertical bone defect was visible extending to a depth of 11 mm. The crestal bone and the defect were carefully curetted and perforated with a round bur to increase bleeding (Fig 2b).

Deproteinized bovine bone particles embedded in a collagen matrix (Bio-Oss Collagen, Geistlich) were infused with rhPDGF-BB, positioned on top of the defect, and retained with a fixation screw (Fig 2c). Once wet, the collagen matrix tended to collapse both laterally and over the defect, thus reducing the space-maintaining capability of the graft. The flaps were closed with horizontal mattress and interrupted sutures (W. L. Gore).

After 5 months of healing, incisions were made, flaps were elevated, the fixation screw was removed, and the amount of new

bone was assessed. The 11-mm long fixation screw could be considered as a reference point, as it protruded 3 mm from the new bone crest. The bone defect appeared to be completely filled with hard tissue that clinically resembled bone, and the area exhibited a total vertical gain of about 8 mm (Fig 2d).

Three implants (MKIII Ti-Unite regular platform, Nobel Biocare) and healing abutments were positioned in the sites of the second premolar and first and second molars; the implants subsequently were loaded and restored (Fig 2e). A bone biopsy was obtained from the area of regenerated bone with a 3-mm trephine bur and processed for histologic examination (Fig 2f).

Histologic processing

The biopsy specimens were immersed in a solution of 10% formalin and 0.1 mmol/L phosphate-buffered saline fixative (pH 7.4) for 12 hours at room temperature. They were then dehydrated in a graded series of alcohols (from 70% to 100%) and embedded in Hardrock 554 (Remet). The specimens were cut longitudinally and ground (Micromet and LS2, Remet) to obtain two sections from each specimen, each 80 μ m thick, according to the technique described by Donath and Breuner.¹⁰ Finally, bone sections were stained using acid fuchsin and toluidine blue (Sigma-Aldrich) and observed with a Nikon Eclipse E600 optical microscope equipped with a Nikon digital camera DXM1200.

Histologic findings

Patient 1

Histologic examination demonstrated new bone formation through the whole bovine bone block trabeculae (see Fig 1g). Predominantly woven bone exhibiting active, ongoing bone formation at the periphery was diffused through the whole specimen. The xenograft particles were embedded in bone, presenting resorption lacunae close to areas with ongoing bone formation (see Fig 1h). This indicated that, in augmented areas, intense physiologic remodeling was ongoing, with alternately occurring demineralization and remineralization.

Patient 2

Mature, well-mineralized trabecular bone with a lamellar parallel-fibered structure was found in these specimens. The bovine bone particles were surrounded by newly formed bone, which seemed to have been fully integrated. A high degree of cellular activity was found in the histologic sections (see Fig 2f).

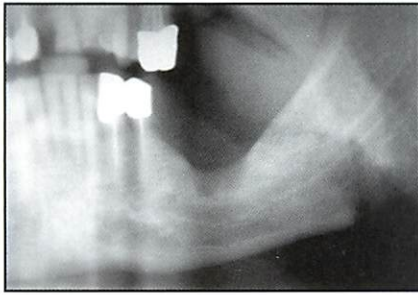


Fig 2a (left) Radiographic appearance of the severe defect prior to augmentation in patient 2, showing a deep vertical bone defect in the left posterior mandible.

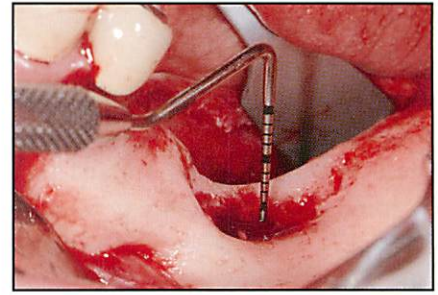


Fig 2b (right) Clinical appearance of the site after full-thickness flap elevation. A vertical bone defect was visible, extending to a depth of 11 mm.

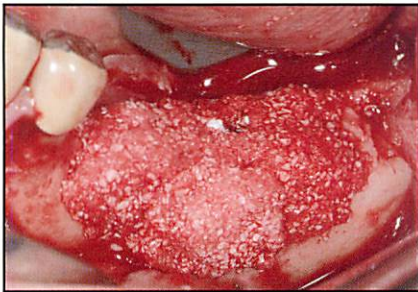


Fig 2c (left) Deproteinized bovine bone particles embedded in a collagen matrix were infused with rhPDGF-BB, positioned on top of the defect, and retained with a fixation screw. The xenograft was extended supracrestally.

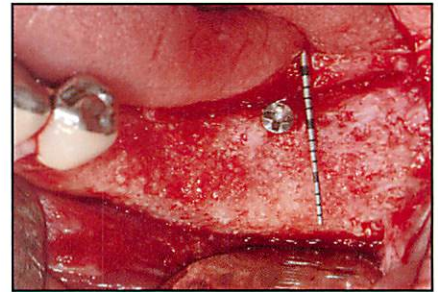


Fig 2d (right) Clinical appearance of the site after 5 months of healing. The bone defect appeared completely filled with a hard tissue, clinically resembling bone and exhibiting a total vertical gain of about 8 mm.

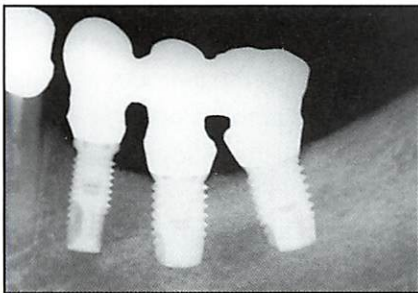


Fig 2e (above) Radiographic appearance following loading and restoration of three titanium dental implants placed in the regenerated site.

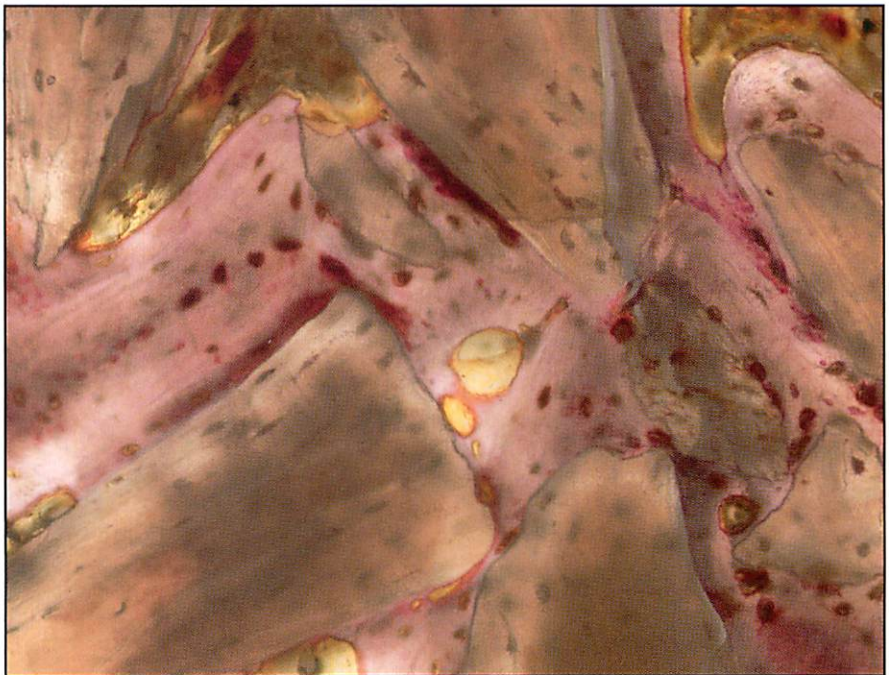


Fig 2f (right) Histologic overview of the specimen stained with acid fuchsin. Mature lamellar regenerated bone is exhibited in contact with the xenograft (original magnification $\times 200$).

Discussion

Horizontal and vertical augmentation of the severely atrophic alveolar ridge continues to be a significant surgical challenge. Although various successful techniques have been described in the literature, these often present difficulties and/or patient morbidity, as previously described. Vertical bone augmentation with GBR has been demonstrated to be an efficacious technique in atrophic maxillae and mandibles.^{11,12} However, premature exposure of the nonresorbable membrane because of poor soft tissue management continues to be a common complication.¹³

A challenging goal would be to investigate a less invasive treatment technique and compare it to the traditional techniques. If the less invasive regimen were successful, this would increase patient acceptance and tolerance, and successful outcomes would be more common. For this purpose, the principles of tissue engineering were applied to three-dimensional ridge augmentation. Recombinant human PDGF is a wound-healing "hormone" that plays an important role in the normal healing of bone and skin.¹⁴ A review addressing the role of growth factors in periodontal tissue engineering noted their effects on the mitosis, migration, matrix synthesis, and differentiation of periodontal ligament cells and osteoblasts.¹⁵ In addition, the use of a xenograft in the present patients eliminated the need for intraoral or extraoral harvesting of autogenous bone.

Studies have shown that PDGF promotes faster healing of soft tissues.¹⁶ The noticeable positive effect that rhPDGF-BB may produce on soft tissue has particular clinical relevance in advanced vertical defects, where the management of soft tissues is crucial for successful wound healing.

The aim of this report was to evaluate the potential of a well-documented growth factor in horizontal and vertical bone regeneration in humans. The first histologic study that reported on vertical ridge augmentation using a combination of rhPDGF-BB and a deproteinized bovine block was conducted recently by Simion et al.⁸ The rationale was to eliminate the use of a nonresorbable membrane to decrease or eliminate the soft tissue complications of wound dehiscence with consequent membrane exposure and to eliminate the need for an extra surgical site to obtain the autogenous graft, thereby avoiding patient morbidity and decreasing postoperative discomfort. This study was conducted in dogs, in which the extraction of premolars and surgical creation of a 3 × 1-cm defect had been performed 3 months prior to regeneration. The results demonstrated that vertical augmentation of severely atrophic ridges could be successfully performed by combining PDGF and a deproteinized bovine bone block.

No data are available on human studies of three-dimensional bone regeneration that combined PDGF with a xenograft. The encouraging results of the aforementioned canine

study⁸ led the authors to apply the same principles in humans.

The two patients presented here provide evidence of the successful use of deproteinized bovine bone as a scaffold for rhPDGF-BB. The results can be appreciated through clinical and histologic observations. In both cases, there was a considerable amount of newly formed bone, which surrounded the xenograft trabeculae, with a high amount of cellular activity. No membrane was applied in either patient, with evidence from the early results of the canine study⁸ that rhPDGF-BB in combination with a deproteinized bovine block, without placement of a barrier membrane, had the potential to regenerate significant amounts of new bone in severe mandibular ridge defects. Furthermore, it appeared that the membrane would reduce the amount of bone regenerated when the xenograft was combined with PDGF.

The scaffold properties of the deproteinized bovine block and the deproteinized bovine particles embedded in collagen do not completely satisfy clinical needs because of the fragility of the block and the tendency of the bovine bone/collagen to collapse. The block appears to be brittle and could easily fracture. The collagen embedded with the xenograft particles, on the other hand, was too soft for proper space maintenance. Hence, a scaffold with the ideal physical properties still needs to be found.

This report has provided histologic evidence in humans regarding the potential of rhPDGF-BB com-

bined with deproteinized bovine to regenerate bone. This was performed in severely atrophic alveolar ridges. Further studies with larger numbers of patients are needed to draw any conclusions; nevertheless, these results seem extremely promising for bone regeneration in patients with severely atrophic ridges.

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