Case of Severe Bone Atrophy of the Posterior Maxilla Rehabilitated With Blocks of Equine Origin Bone: Histological Results

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Purpose: *To report the histological outcomes of a case of bilateral severely resorbed posterior maxilla augmented with the use of blocks of enzymatically deantigenated equine bone.*

Materials and Methods: *In conjunction with bilateral sinus lifts, blocks of enzymatically deantigenated equine bone were used bilaterally to augment the severely atrophic maxilla of a patient seeking a fixed implant-supported prosthesis. After 8 months, bone core samples were obtained from the augmentation sites and implants were placed.*

Results: *Six months after implant placement, the peri-implant bone levels were maintained. A prosthesis delivered 3 months after implant placement provided excellent rehabilitation. Histological analysis of the bone cores revealed that the graft material was still undergoing remodeling, with newly formed vital bone in all fields and osteoclasts included in the mineralized component.*

Conclusions: *The deantigenated equine bone was biocompatible and resorbed only minimally. This material seems to offer excellent potential for being incorporated in a procedure that increases the width of edentulous alveolar crests. (Implant Dent 2013;22:8–15)*

Key Words: bone grafting, heterologous bone, equine bone

Various methods have been developed to increase the amount of bone available at implant placement sites.[1–6] Guided bone regeneration (GBR) is one such technique, enabling a simultaneous increase in both vertical and horizontal bone.[4–6] A filler is generally used underneath the membrane. In most cases, the graft material used is autologous bone collected from intraoral sites such as the chin,[7] mandibular symphysis,[8–10] and branch [8–11] or from extraoral sites such as the iliac crest,[12] calvaria, or tibia.[13,14] Autologous bone can be ground or harvested in blocks that are fixed with osteosynthesis screws. Although the osteoconductive, osteoinductive, and osteogenic properties of autologous bone have led it to be considered the gold standard for bone regeneration,[15] the quantity that can be harvested from intraoral sites is limited. Harvest from extraoral sites takes longer and increases morbidity and the risk of intra- and postsurgical complications.[12,16,17]

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The use of bone substitutes for grafting is less invasive and may reduce the discomfort to patients. Such alternative materials, which are available in large quantities, include homologous bone, heterologous bone, and alloplastic materials, used alone or in combination.[5,18,19] These materials have low or no antigenic potential.[20]

Among heterologous materials, deproteinized bovine bone has been studied most extensively. Frequently used in combination with GBR membranes,[21,22] thermally deproteinized bovine bone particles have good osteoconductive properties but, depending on how they have been treated, may also have a low resorption capacity.[23] This material has been used successfully in association with collagen membranes for horizontal crest increases.[24,25] The same material in block form has a lower osteoconductive capacity when used in both vertical[26,27] and lateral[27,28] bone augmentation procedures. Histological examination of such blocks has found them to be surrounded by connective tissue, with only a small quantity of newly formed bone at the base of the graft.[27,29]

Recently, a form of equine bone that is enzymatically deantigenated at low temperatures (37°) was introduced for use as a scaffold in supporting bone regeneration of severe crestal defects.

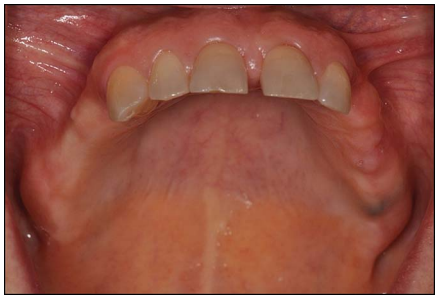

Fig. 1. Presurgical intraoral view.

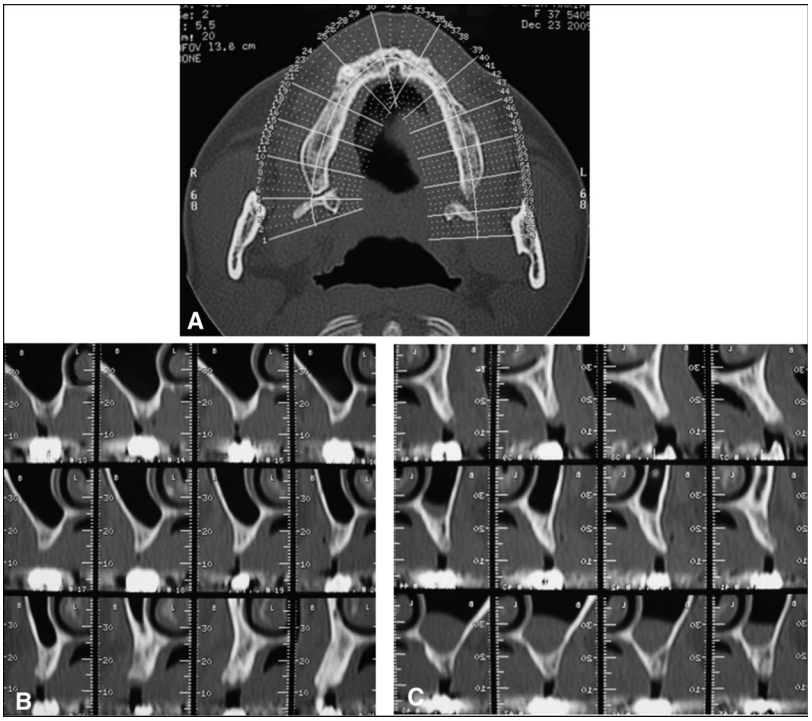

Fig. 2. A–C, Presurgical CT scans.

The enzymatic process used to deantigenate this material preserves the type I bone collagen component in its native nondenatured state, and this should allow for a better bone regeneration process, given the well-known biological properties of this molecule.[30–37]

When sites augmented with the equine bone alone were compared with others augmented with the same material added with autogenous bone,[43] immunohistochemical tests showed no differences between the 2 as far as the expression of some markers of bone regeneration (nitric oxide synthase [NOS] 1 and 2) and vascular endothelial growth factor) were concerned.

The present article describes the treatment of a patient to increase the transverse thickness of the atrophic edentulous posterior maxillary crests using enzymatically deantigenated equine bone xenograft material containing native type I collagen. Biopsy samples were obtained to enable histological analysis.

Materials and Methods

The patient was a healthy, nonsmoking, 50-year-old woman who presented seeking a fixed prosthesis to replace her ill-fitting full maxillary denture. The patient was missing teeth numbers 14 to 17 and 23 to 27.

Bilateral posterior maxillary atrophy (Seibert Class III[41]) was noted clinically (Fig. 1) and confirmed by radiographic examination and CT scanning (Fig. 2, A–C). A treatment plan was developed that called for placement of 6 implants after healing of transversal bone augmentation to be carried out simultaneously with bilateral sinus lift grafts. The patient provided informed consent.

An hour before surgery, the patient rinsed with 15 mL of 0.12% chlorhexidine gluconate for 60 seconds before receiving 2.2 g iv antibiotic (Augmentin; GSK, Brentford, United Kingdom). Local anesthetic was administered by means of an infiltration with 1% Articaine with adrenaline 1:100,000.

A releasing incision was created mesially from the canine, continuing first intrasulcularly around the canine itself and then at the top of the edentulous ridge to detach a full-thickness flap. The same procedure was performed on both sides. Two mucoperiosteal flaps were then elevated to expose the lateral-distal sectors of the superior maxilla, bilaterally. The antral floor was lifted in both maxillary sinuses using deproteinized bovine bone particles (0.25–1 mm diameter, Bio-Oss; Geistlich, Wolhusen, Switzerland) (Fig. 3, A–D).

A series of holes were made on the vestibular and crestal bone surfaces in zones 14 to 15 and 24 to 25, using osteosynthesis cutters with a diameter of 0.9 mm. This was done to obtain profuse bleeding from the marrow of the bone graft recipient sites. Two blocks of equine bone measuring 10 $\times$ 10 $\times$ 20 cm (Osteoplant Osteoxenon, OSP-OX52 Cancellous Block; Bioteck, Italy) were shaped in a sterile field, using diamond-tipped disks and steel laboratory cutters (Horico; Dental, Berlin, Germany). One block was cut in 2 pieces, and the 2 pieces were shaped to conform to the contours of the left. The second block, for the right side, did not require cutting but only proper shaping. The aim was to obtain blocks that would adapt perfectly to the recipient bone bed.

Holes were then made both on the bone blocks and, correspondingly, bilaterally on the patient's vestibular bone to accommodate 1.5-mm-long osteosynthesis screws (Modus; Medartis, Basel, Switzerland). Screws of that diameter with lengths ranging from 9 to 14 mm were then used to secure the blocks to the recipient beds (Fig. 4, A and B). The screw heads were slightly submerged. After having positioned and secured all the blocks, the graft material was profiled again, without irrigation, to remove all bone asperities.

Additional bovine particulate material, mixed with saline, was used to fill the spaces created between the blocks and recipient sites. Resorbable collagen membranes (Bio-Gide; Geistlich) were

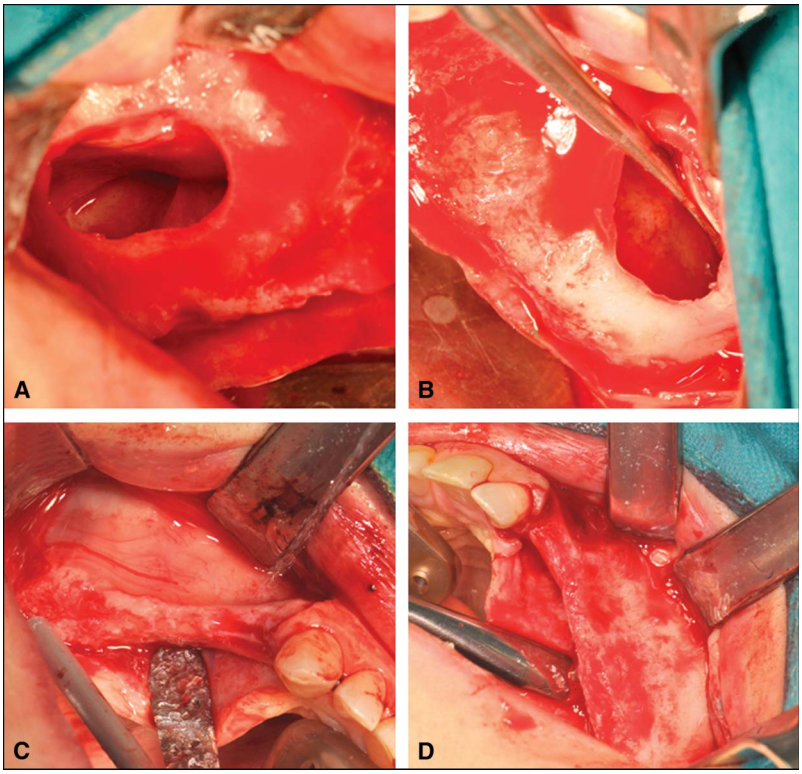

Fig. 3. **A**, Significant horizontal atrophy is evident in the exposed upper left alveolar ridge. **B**, The atrophic maxillary right ridge, after flap elevation. **C**, A bone window was created in the posterior left maxilla to enable performance of a sinus elevation. **D**, Sinus elevation also was performed in the posterior right maxilla.

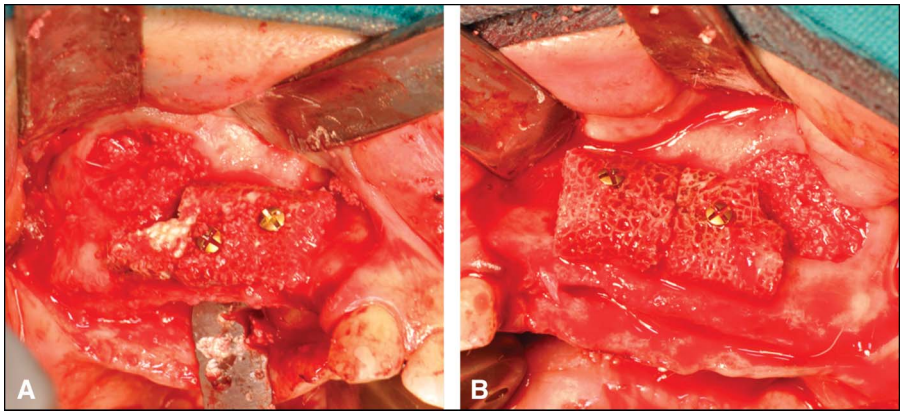

Fig. 4. **A**, In the upper right, a single block was shaped and fixed with osteosynthesis screws over the patient's vestibular bone. **B**, The same procedure in the left side.

positioned to cover the graft sites. To relieve any tension in the vestibular flap, an incision was made in the periosteum on each side. Complete flap closure was achieved using single-filament nylon 3/0 (Monosof; Covidien, Mansfield, MA) suture material.

The patient was instructed to rinse twice a day for 30 seconds with 15 mL of chlorhexidine 0.12% mouthwash, and the following was prescribed for 5 days: Augmentin 2 g twice daily, metronidazole 500 mg twice daily, and omeprazole 10 mg once daily (Antra; AstraZeneca S. p.A., Milan, Italy). In addition, the patient was told to take four 1-mg tablets of betamethasone disodium phosphate (Bentelan; Biofutura Pharma S.p.A., Rome, Italy) on the morning of surgery, 3 tablets the next morning, 2 tablets the third morning, and 1 tablet the fourth morning after surgery.

The patient returned for follow-up 1, 2, 4, 6, 12, and 16 weeks after surgery. Sutures were removed at the 2-week follow-up appointment, and at this time, an additional CT scan was taken.

Eight months later, the patient began another prophylactic course of Augmentin, and the next day, she returned for the reentry surgery. Clinical and radiographic examinations (both x-rays and an additional CT scan) showed healthy tissue and a substantial quantity of additional bone (Fig. 5, A–C). A crestal incision was made, and mucoperiosteal flaps were elevated to allow for careful clinical inspection of the alveolar ridge (Fig. 6, A and B). The fixing screws were still completely immersed up to their heads, indicating no loss of the graft volume during the healing period. The screws were removed, and a prefabricated surgical template was positioned to guide the implant placement at sites 14, 15, 16, 24, 25, and 26. Biopsy samples were taken at each of the implant placement sites, using a scalpel and a trephine with an external diameter of 3 mm (Fig. 7). Six titanium implants were then placed (Figs. 8 and 9), and the tissue was approximated over them.

The biopsy samples were fixed in formalin buffered at 10% and then decalcified. A decision was made to use a bland relatively slow decalcification process to preserve morphological detail and tissue immunogenicity to as great a degree as possible. To this end, a chelator normally employed for bone marrow biopsies was used (EDTA, Mielodec; Bio-Optica, Milan, Italy). The samples were subsequently placed in paraffin and cut with a microtome into 5-μm sections.[42] Later, the samples were stained with hematoxylin and eosin and Papanicolaou and observed under an optical microscope with transmitted light (Laborlux; Leitz, Wetzlar, Germany). Although widely used in cytology, this multichrome dye is not commonly used to stain decalcified bone biopsies. However, the authors

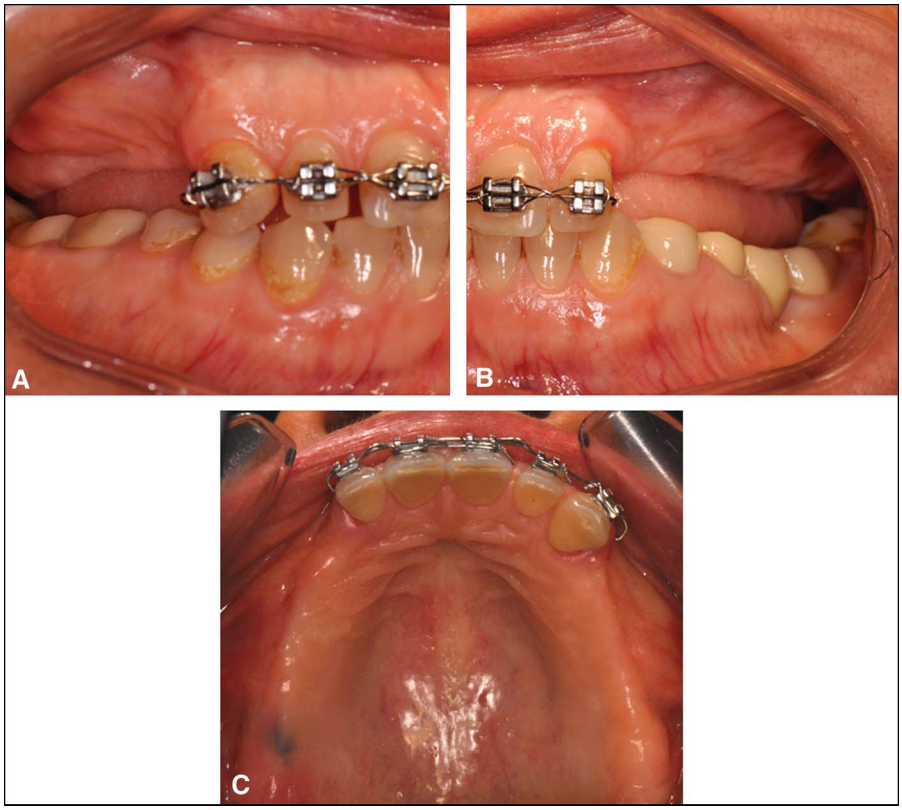

Fig. 5. A, B, Intraoral vestibular view after 8 months of healing. **C**, Intraoral occlusal view after 8 months of healing.

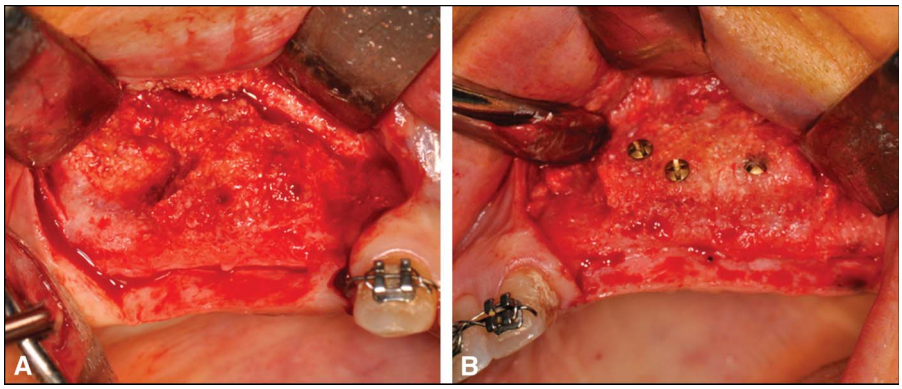

Fig. 6. A, Exposure of the upper left arch showed the equine bone blocks to be well integrated. **B**, The upper right arch at second stage surgery.

chose it because it allowed for a particularly clear and detailed highlighting of the ratio of graft and newly formed autologous bone as well as of cell morphology.

Three months later, the implants were uncovered and a fixed metal-ceramic prosthesis was fabricated and delivered. Follow-up radiographs taken at 6 months after implant placement showed complete maintenance of the peri-implant bone levels. The prostheses enabled excellent patient rehabilitation (Fig. 10).

Histological Results

As the bone cores were harvested completely in the vestibular area of the grafted blocks, the histological findings of vital bone must be considered as newly formed bone. Such newly formed vital bone was found in all

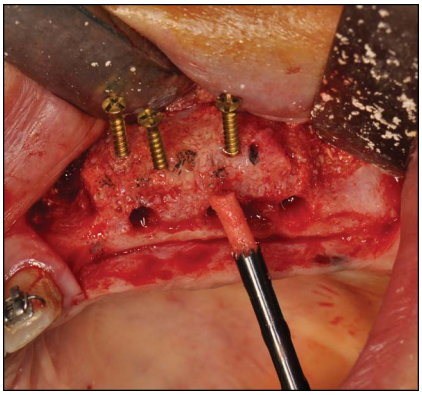

Fig. 7. Removal of the screw and sample-trephine retrieval.

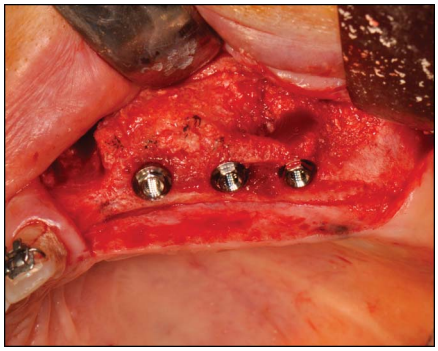

Fig. 8. Three implants in place in the upper left.

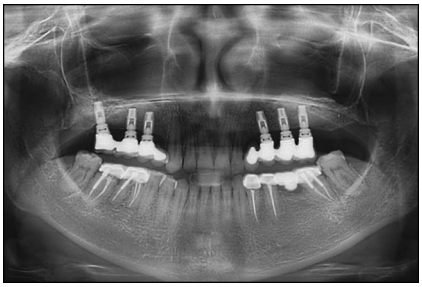

Fig. 9. Panoramic radiograph of the 6 implants immediately after placement.

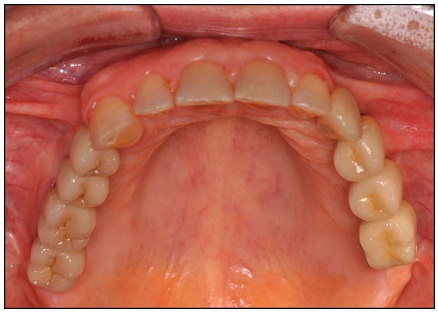

Fig. 10. The implant-supported prosthesis after delivery.

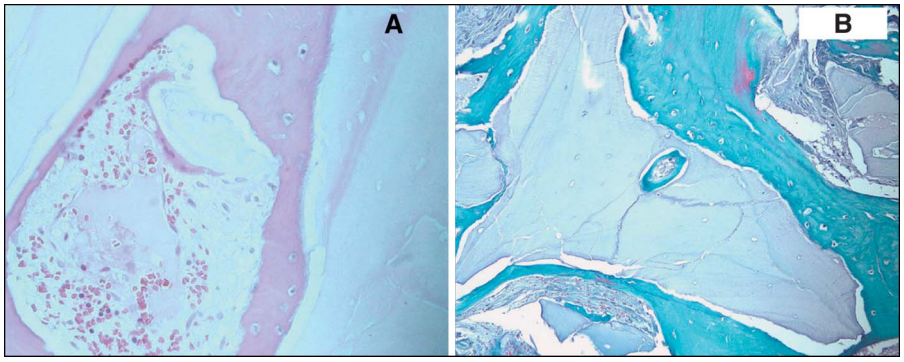

Fig. 11. A, In this bone sample, stained with hematoxylin and eosin, the presence of vital bone is evident with some osteocytes and graft material, characterized by empty cell gaps in the mineralized component. At the center, syncytial polynuclear cells can be seen to embrace a graft spicule during reabsorption. A stroma is also visible with a vessel and numerous fibroblasts. The lower part shows approximately 6 isolated lymphocytes, which can be recognized by their characteristic small rounded nucleus and the scarcity of cytoplasm, originating from a blood vessel, arranged as normally occurs in tissues in physiological conditions. **B**, In this sample, stained with Papanicolaou, part of the graft is completely surrounded and in close contact with the autologous bone (the small space between them is an artifact of the slide preparation). In the upper right, a medullary cavity with stroma can be seen.

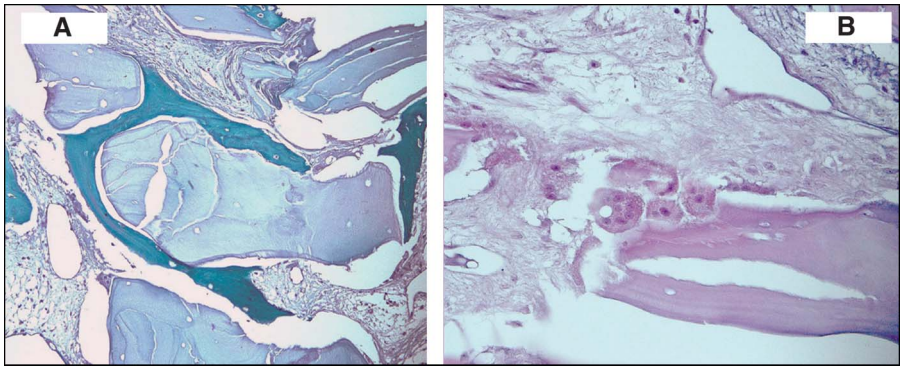

Fig. 12. A, This slide contains stroma components with newly formed vessels, graft, and autologous bone in close contact. **B**, This sample, stained with hematoxylin and eosin, shows 4 polynuclear cells in the center, with clear cytoplasm, slightly eosinophil. Morphologically speaking, these cells have the classic appearance of osteoclasts and are actively reabsorbing the graft. Newly formed autologous bone is in continuity with a stromal cavity and the lumen of a blood vessel, probably newly formed.

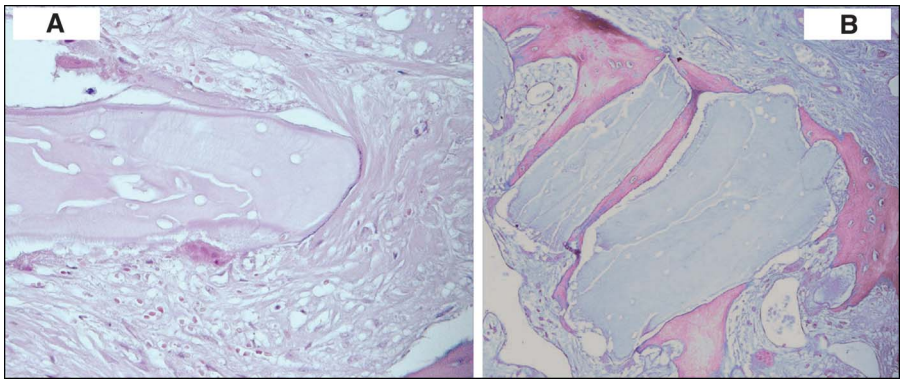

Fig. 13. A, This slide displays graft material, abundant fibrillary stroma, and an osteoclast-like cell that reabsorbs the graft. **B**, This sample, stained with Papanicolaou, shows graft material in close contact with autologous bone, medullary stroma, osteoclast-like cells, and no sign of inflammation.

fields, with osteoclasts included in the mineralized component and new bone deposition areas. Graft material was always present but in close contact with the vital bone, indicating that the graft material was biocompatible. The histological figures show that 8 months after grafting, the bone blocks were still undergoing remodeling. No complete substitution had occurred, indicating that the blocks were still acting as a space-maintaining material.

In histological terms, a restructuring process appeared to be underway, with osteoclast-like polynuclear cells reabsorbing spicules of biomaterial. In view of the morphology and histotopographical detail (the cells surrounding spicules of material undergoing reabsorption), the osteoclast-like function of these elements was clear, with no further histoimmune/histochemical investigation necessary.

All magnifications showed a complete lack of inflammation. Vasal lumens, some of which appeared to be newly formed, were present, but the typical cells of inflammatory infiltration, such as granulocytes and lymphocytes, were absent. Newly formed medullary cavities containing stroma, fibroblasts, and vessels also appeared to be free of any inflammatory infiltration (Figs. 11–14).

DISCUSSION

Onlay grafts have been successfully used either in treatment of wide alveolar defects or when it is necessary to increase the horizontal diameter of the alveolar crest to insert the implants in the correct position.[2,40] Xenografts are highly attractive for this purpose because they have a reduced risk of contamination from infectious diseases, do not compromise the patients remaining tissues, and may have a similar structure as the component to be replaced.[39]

The equine xenograft,[42] because of its elasticity and flexibility, allows easy handling and adaptation to the bone surface. Furthermore, this material is not friable and is simple to secure with osteosynthesis screws. These mechanical properties are related to the high percentage of the collagen component.

The lack of inflammatory reaction in the adjacent tissues indicates that the

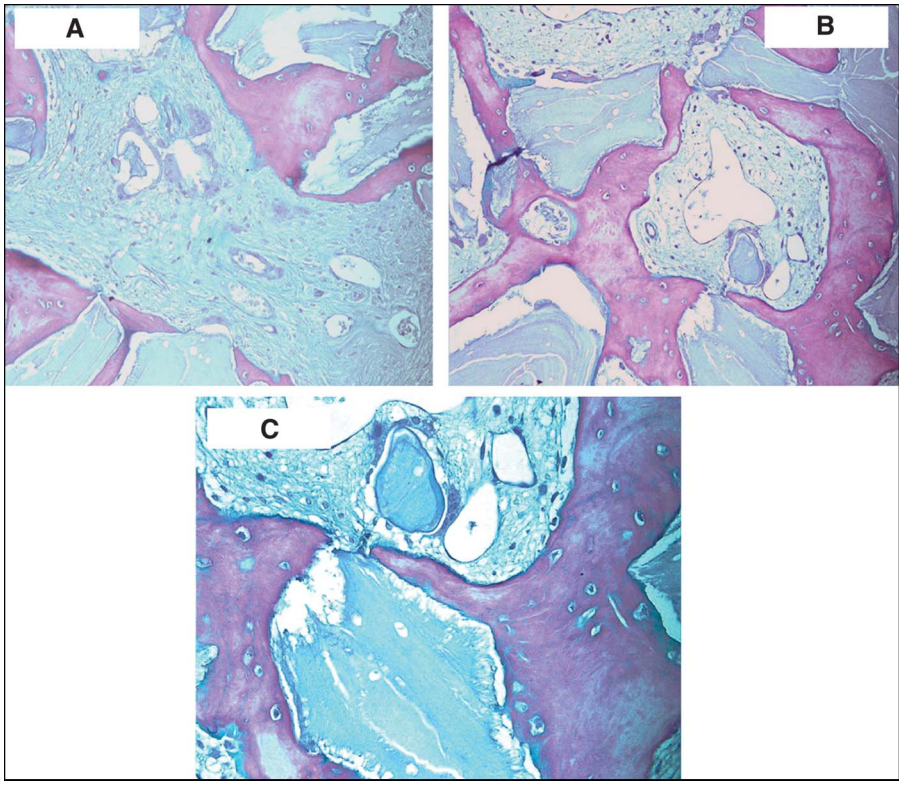

Fig. 14. A, At the center of this sample, stained with Papanicolaou, can be seen polynuclear osteoclast-like cells that reabsorb particles of the graft material. Autologous bone is in continuity and with an apposition line on the graft. **B**, Papanicolaou staining. Notable are the autologous bone, graft material, and close-up of 2 giant polynuclear osteoclast-like cells reabsorbing the graft. **C**, Papanicolaou staining. At higher magnification, 2 osteoclast-like cells can clearly be seen surrounding and reabsorbing a fragment of graft material.

equine bone block material has good biocompatibility. This confirms earlier results of studies carried out on animal models.[38,39,41] Schwarz et al,[47] for example, assessed bovine and equine blocks used to augment alveolar defects created in dogs. Three months after placement, no material appeared to provoke any foreign body reactions. No sign of inflammation has been noted in our specimens.

Even if the slow resorption of bovine and porcine xenografts did not influence the implant outcomes in grafted sites, a material that allows the bone apposition in conjunction with its rapid and progressive degradation can improve our grafting procedures.

Equine eterologous bone appears to stimulate the activity of human osteoclasts, with the formation of resorption lacunae. The attachment and resorptive activity of the osteoclastic cells implies the formation of links between the cells and the proteins of the organic matrix that result when very low proteins are absent, just as in cases with anorganic bone. The equine bone tested in this study has been subjected to a low temperature (37°) deantigenation process, with no alteration of its organic component. This matrix of bone is composed mainly of type I collagen (70% in weight). Type I native bone collagen, in fact, has been shown to be a positive activator of many biological processes that lead to bone regeneration, involving both osteoblast and osteoclast adhesion and differentiation, growth factor coactivation, and others.[30–37] When osteoclasts were cultured over such equine, enzymatically deantigenated and collagen-preserving bone substitutes, their adhesion and activity were significantly higher than that found for osteoclasts grown over deproteinized bovine bone.[35,39]

Another characteristic of this equine xenograft is the particular range of the pore size of 430 to 750 μm that seems to be ideal not only for bone ingrowth but also for osteoclastic cell development and function.[38]

The cell-mediated resorption mechanism has been confirmed in our study in which a great quantity of polynuclear cells reabsorbing spicules of biomaterial was observed.

The resorption process described may be considered the preliminary condition of the osteoblastic bone formation, which can be promoted by the cell to cell contact, the cell-matrix interaction, and the action of cytokines released by osteoclasts. Bone formation is closely linked to new blood vessel invasion (angiogenesis).[40] Vascular endothelial growth factor seems to be the angiogenic mediator major involved in this process. It has been demonstrated in the literature that equine bone has a higher capacity to support a vessel formation than bovine bone, with newly formed bone always found in very close contact with the newly formed blood vessels,[41] as has been observed in our specimens.

When partially demineralized blocks of this material were placed in 5 patients,[40] lateral ridge augmentation was achieved successfully. No bone loss was observed on CT scans, with respect to the grafted volume. Histological tests comparing bone cores collected after 6 months from the grafted sites and from nonregenerated adjacent sites showed no difference in newly formed bone quantity. At that time, the material was still undergoing remodeling (the remaining quantity was approximately 30% of the core volume). The blocks, however, were covered by nonresorbable titanium-reinforced Gore-Tex membranes, which have been shown to promote bone regeneration when used alone.

Another recent article presented a case in which blocks of equine bone were placed in the atrophic maxilla of one patient, fixed with screws, and covered with resorbable collagen membranes. The resulting increase in bone volume was sufficient to enable placement of implants. Histological assessment of the regenerated bone confirmed the positive results.[43] Our case report,

similar from a clinical point of view, better demonstrates the presence of osteoclastic-like cells and the contact between the graft and the newly formed bone thanks to the Papanicolaou method of coloration.

Our study demonstrated that 8 months after placement, the graft material revealed wide areas of bone remodeling with the osteoclastic cell actively reabsorbing fragments of the graft. Actually, to establish the real percentage of newly formed bone and state the real entity of resorption, it needs a histomorphometrical analysis and controlled studies for comparison of different materials.

Nevertheless, earlier studies of both bovine bone grafts[8,23,25,45] and equine bone grafts[26,46] reported contrasting results with regard to the potential resorption of both materials. These differences may be due to the type of membrane used to cover the grafts. The use of nonresorbable membranes may, in fact, protect the graft from resorption,[46,47] whereas resorbable collagen membranes may, as they deteriorate, facilitate the resorption process.[26]

The authors have initiated a randomized, controlled, clinical trial to compare the results obtained with the equine material to those obtained using autologous bone.

CONCLUSIONS

This clinical case demonstrated the successful reconstruction of a bilaterally atrophic maxilla using bone blocks of equine origin. This enzymatically deantigenated graft material seems to offer excellent potential for being incorporated as a part of native bone, increasing the width of edentulous alveolar crests. It seems to stimulate the osteoclastic activity and remodeling process.

DISCLOSURE

All authors of this case report are aware of not being involved in any pertinent consultancies, stock ownership, or other equity interests, and there are no patent licensing arrangements regarding the surgical procedures and technical apparatus presented.

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