



## Preserving the Bone Profile in Anterior Maxilla using an Equine Cortical Bone Membrane and an Equine Enzyme-treated Bone Graft: A Case Report with 5-year Follow-up

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### ABSTRACT

**Aim:** This study aimed to report a well-succeeded use of equine enzyme-deantigenic cortical membrane and bone granules for guided bone regeneration (GBR) in the esthetic zone concomitant with implant placement.

**Background:** In the anterior maxilla, where patients typically have the highest expectations for implant-supported restorations, bone resorption in the wake of tooth loss often leads to a lack of soft tissue support and ultimately to unsatisfactory esthetic results. Buccal bone augmentation at the time of implant placement has thus become common. This is usually accomplished following GBR principles and employing membranes made from various materials to serve as a barrier between the soft tissue and graft material. One of the more recently introduced membrane materials is made from thin, flexible equine cortical bone, i.e., rendered nonantigenic in an enzymatic process that preserves native bone collagen.

**Case report:** This report describes the treatment of a patient who received an equine enzyme-deantigenic graft and membrane in conjunction with placement of an implant in the right maxillary lateral incisor site.

**Conclusion:** After 5 years of follow-up, the patient was very satisfied with her appearance. A cone-beam computed tomography scan showed that the peri-implant bone levels and ridge thickness had been maintained, and the cortical layer in the pristine ridge had also undergone remodeling.

**Clinical significance:** Guided bone regeneration with the concomitant use of enzyme-deantigenic membrane and graft is a valuable and suitable option for effective implant-supported prosthetic rehabilitation in the esthetic zone.

**Keywords:** Cortical bone membrane, Guided bone regeneration, Horizontal ridge augmentation, Xenograft.

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**Conflict of interest:** None

### BACKGROUND

Implant-supported prosthetic rehabilitation in the esthetic zone presents one of the hardest challenges for oral surgeons, as several factors may compromise the final esthetic result. Among these, bone resorption caused by a previous tooth loss or extraction<sup>1</sup> and the consequent lack of soft tissue support may lead to unsatisfying results. A second issue is how the choices undertaken at the moment of implant placement will condition the preservation of the esthetic result over time. For this purpose, the stability of both the hard and soft tissues is of paramount importance. The two are interconnected, as only a bone volume, i.e., stable over time may guarantee adequate support to soft tissues and preservation of their long-term stability.

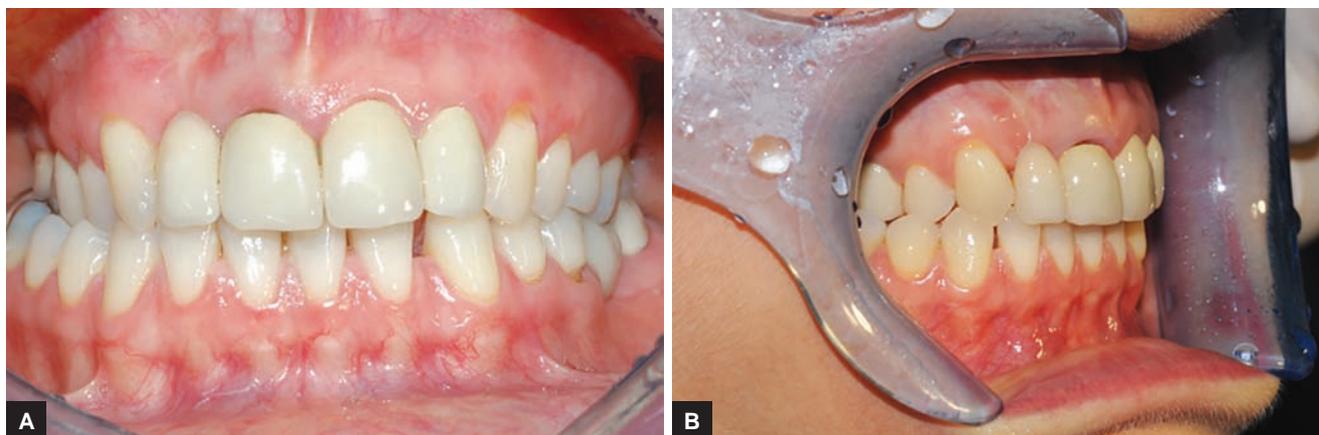
Even when the dimensions of the alveolar bone are sufficient to allow for placement of osseointegrated fixtures, buccal bone augmentation is often advised to

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**Figs 1A and B:** Appearance of the patient's prosthesis with esthetic concerns

achieve a stable and satisfying esthetic restorative result. Augmentation at the time of implant placement has thus become a common technique for enhancing implant survival and success or increasing soft tissue support in demanding esthetic cases.<sup>2,3</sup> When the available bone volume enables implant placement, buccal bone augmentation is usually performed following the principles of guided bone regeneration (GBR)<sup>4,5</sup> (i.e., interposing a barrier between the soft tissue and graft material that works as an osteoconductive support for bone regeneration). The first such barriers were nonresorbable and had to be removed after healing of the grafted site.<sup>6,7</sup> Later, resorbable membranes were introduced that allowed effective bone regeneration while reducing the complication rates associated with membrane exposure and avoiding a second surgery for barrier removal. Membranes currently used to perform GBR interventions range from resorbable collagen or synthetic membranes<sup>8,9</sup> to nonresorbable expanded or high-density polytetrafluoroethylene (PTFE) barriers<sup>10</sup> to nonresorbable titanium meshes.<sup>11</sup> Whatever the material used and its resorption properties, barrier membranes for GBR still must meet the five criteria stated by Scantlebury in 1993,<sup>12</sup> i.e., tissue integration, cell occlusivity, clinical manageability, space making, and biocompatibility.

Recently, an equine bone membrane was made available in the market that consists of a thin layer of flexible cortical bone. For its manufacture, equine cortical bone is first made nonantigenic by the use of digestive enzymes that eliminate equine antigens. Then, the bone is made thin by mechanical abrasion. Finally, the bone sheet undergoes partial demineralization to make it flexible. The enzymatic process preserves bone collagen in its native form. In a previous case, such a membrane was found to allow effective bone regeneration when horizontal bone augmentation was performed in combination with enzyme-deantigenic bone granules.<sup>13</sup> When a portion of the membrane was retrieved 3.5 months later, histologic examination showed that it was still occlusive,

even though it was undergoing osteoclastic remodeling. Preservation of occlusivity for some months, if confirmed, would make this membrane a possible candidate for effective GBR aimed at preserving bone profiles in the esthetic zone concomitant with implant placement.

The present report describes a case in which this kind of membrane was used in association with equine enzyme-deantigenic bone granules for this specific application.

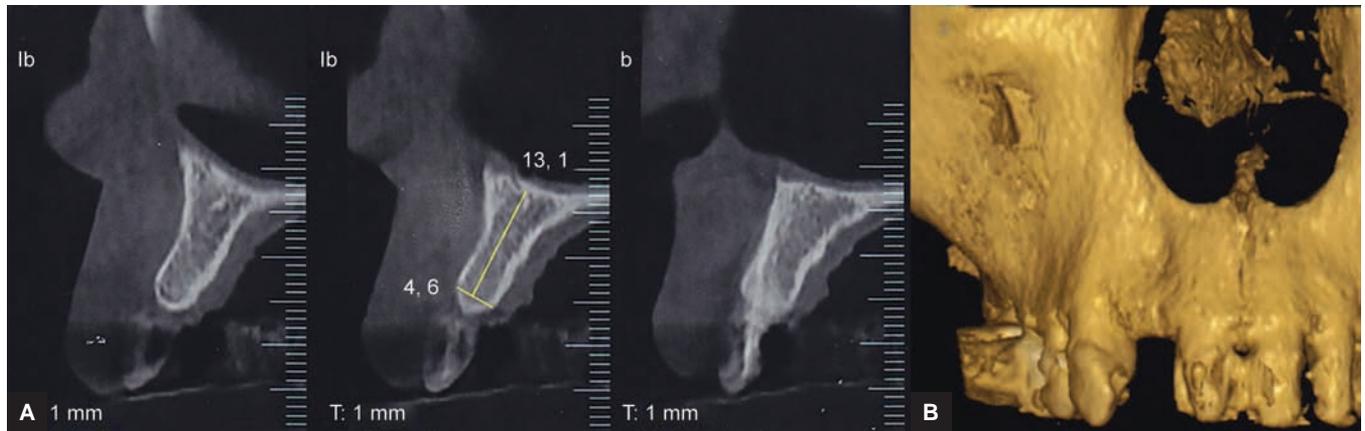
## CASE REPORT

The patient was a 45-year-old female with a noncontributory medical history seeking to improve the esthetics of her anterior upper maxilla. She had lost her right maxillary lateral incisor and had been previously rehabilitated with a four-unit bridge supported by the remaining three incisors. However, the patient was unsatisfied with the appearance of the soft tissue contours around the bridge, the gaps between the soft tissue and the prosthesis, and the unnatural appearance of the ceramics (Fig. 1), which did not match the natural teeth.

Clinical and radiographic examination revealed a horizontal defect of the alveolar ridge at the missing lateral incisor site (Figs 2 and 3). A one-step procedure



**Fig. 2:** Clinical appearance after removal of the bridge. Preparation of the remaining incisors was suboptimal, and a buccal bone deficiency was evident at the site of the missing right lateral incisor



**Figs 3A and B:** Cone beam computed tomographic scan confirmed the presence of buccal bone defect



**Fig. 4:** Remaining incisors were prepared to later support single crowns as part of the rehabilitation plan

that included placement of an implant there along with simultaneous grafting of the buccal side of ridge was developed with the aim of rehabilitating the patient with four single crowns supported by the implant and the three remaining incisors. The patient provided informed consent, and the three incisors were prepared for later crown restoration (Fig. 4).

Based on the cone beam computed tomographic (CBCT) scans and an implant positioning design that was preplanned digitally, a surgical template was fabricated. This was sterilized in an autoclave some days before the surgery. For antibiotic prophylaxis, 2 gm of amoxicillin/clavulanic acid (Augmentin, Glaxo-SmithKline, Verona, Italy) was administered 1 hour before the surgery and then every 12 hours for 8 days. The patient also rinsed for 2 minutes with chlorhexidine 0.20% mouth rinse (Corsodyl, Glaxo-SmithKline) and received 100 mg of a nonsteroidal anti-inflammatory drug (Aulin, Roche, Milano, Italy). Local anesthetic was administered by means of infiltration into the oral mucosa with 1% articaine with epinephrine 1:100,000 (Molteni Dental, Milano, Italy).

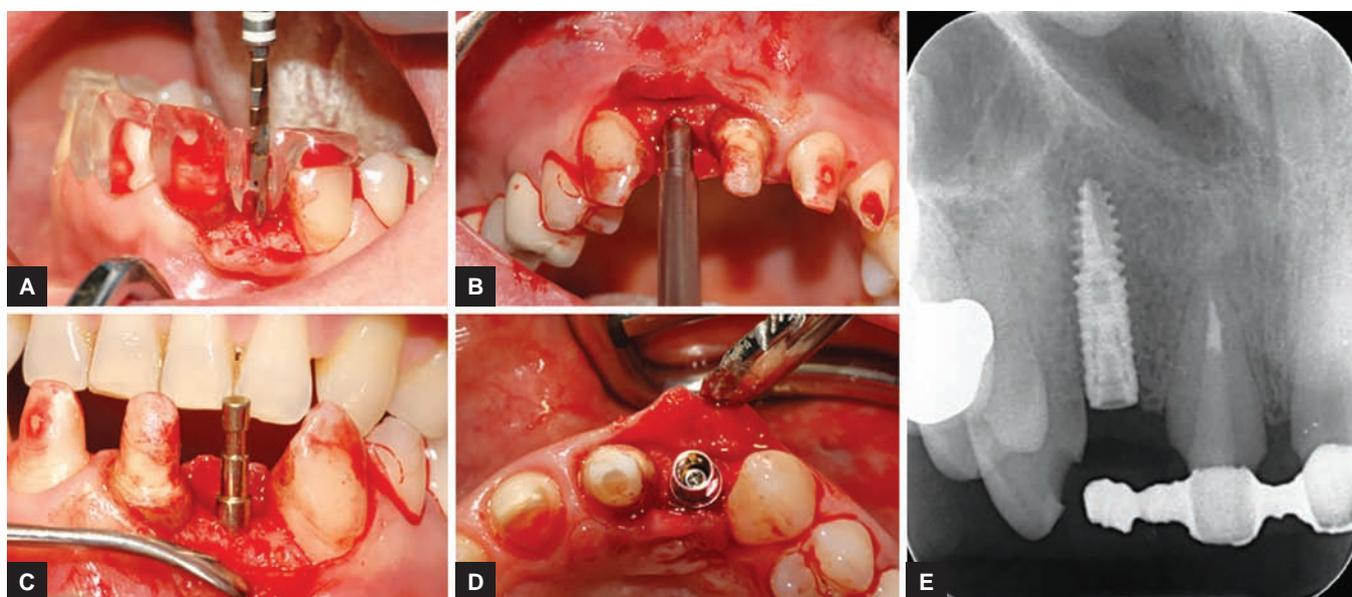
A mid-crestal full-thickness incision was created within the keratinized mucosa of the edentulous ridge,

extending it partially to the right central incisor and cuspid through an intrasulcular incision; an envelope flap was elevated. The implant site was prepared by drilling with the surgical guide and then using an osteotome. Then, a cylindrical 3.3 mm × 13 mm implant (Xive, Dentsply, York, PA, USA) was placed (Fig. 5). Its alignment was verified using a paralleling pin.

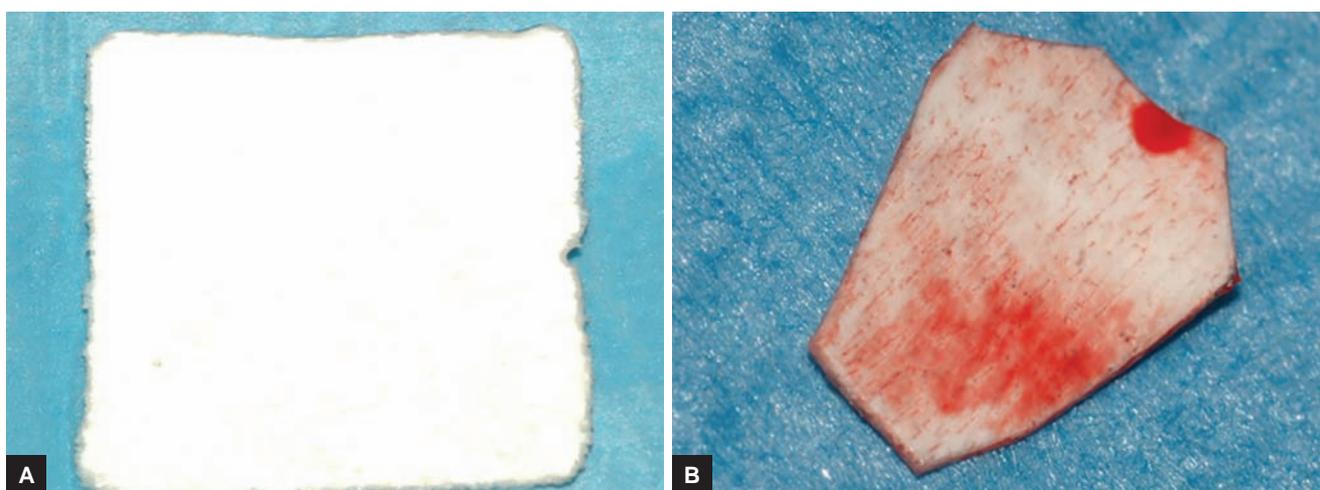
An equine cortical bone membrane (Osteoxenon<sup>®</sup> Cortical membrane, 25 mm × 25 mm × 0.2 mm, Bioteck, Arcugnano, Italy, Fig. 6A) was cut to the desired shape (Fig. 6B), hydrated with sterile saline, and inserted between the flap and the ridge. The gap between the membrane and the ridge was filled with equine enzyme-processed 0.5 to 1.0 mm bone granules (Osteoxenon<sup>®</sup> Mix Bone Granules, Bioteck, Arcugnano, Italy) (Figs 7A and B). As the membrane was already stabilized by the flap, no pins or screws were used to secure it to the alveolar ridge.

The flap was closed using nonresorbable suture material (Monomyd 4-0/5-0 Polyamide Monofilament Suture, Butterfly, Cavenago, Italy) (Fig. 7C), leaving the implant submerged. The patient wore a provisional restoration that was supported by the incisors for the next 4 months. Then, under the same antibiotic prophylaxis previously described, the implant was uncovered, and a healing screw was placed to allow for proper soft tissue conditioning. Nonresorbable sutures (Monomyd 4-0/5-0 Polyamide Monofilament Suture, Butterfly, Cavenago, Italy) (Fig. 8) were placed and removed 6 days later. Soft tissue conditioning occurred over the ensuing 4 months (Fig. 9), at which point the patient was rehabilitated definitively (Fig. 10). She returned for follow-up assessment every 6 months for the next 5 years. At that point, she needed replacement of an old posterior maxillary bridge, and another CBCT scan was required. This enabled assessment of the bone volume that corresponded to the earlier augmentation procedure.

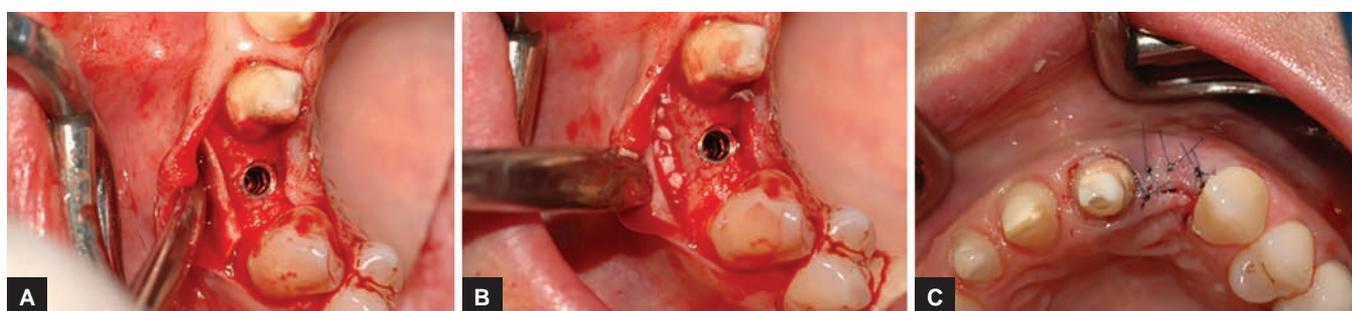
The appearance of the rehabilitation was still quite satisfactory (Figs 11A and B), with no gingival recession



**Figs 5A to E:** Implant placement. Site was prepared with the aid of a surgical guide, drills and an osteotome



**Figs 6A and B:** The equine cortical bone membrane. The membrane is a 25 mm × 25 mm × 0.2 mm cortical flexible bone sheet: (A) before cutting and hydration; and (B) after cutting but before hydration and placement

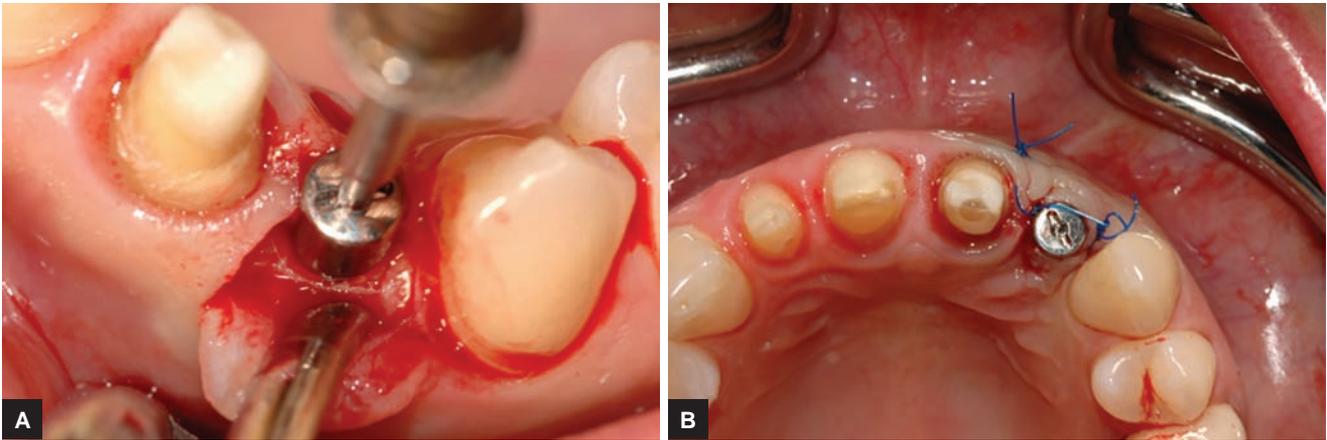


**Figs 7A to C:** Membrane was placed under the flap, and gap between membrane and ridge was filled with equine enzyme-deantigenic bone granules

evident. The peri-implant bone levels also had been maintained (Fig. 11C). The CBCT scan showed that no ridge thickness had been lost at the recipient site and that a new cortical layer could be observed, while the cortical layer in the pristine ridge had undergone remodeling (Fig. 12).

**DISCUSSION**

The basic principle of GBR involves using membranes to isolate the blood clot and biomaterial and protect them from connective tissue. The isolated secluded space can then be invaded by bone-forming cells, and bone



**Figs 8A and B:** Uncovering of the implant. Healing screw was placed to allow proper soft tissue conditioning



**Fig. 9:** Soft tissue conditioning

formation can occur. In addition, the barrier membrane helps to stabilize the graft and prevent micromovement that could hinder the bone regeneration process.

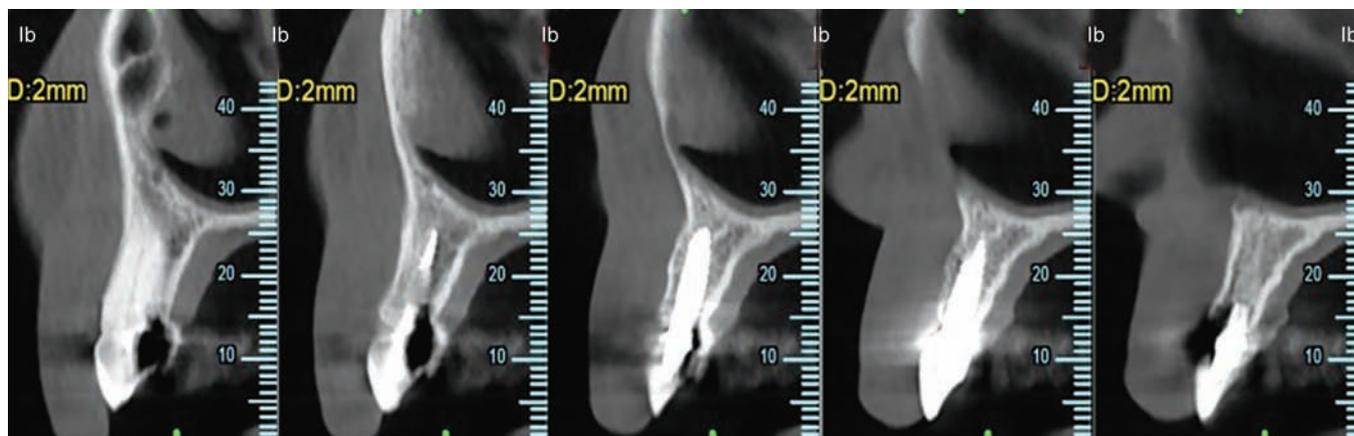
To work properly, the barrier must meet some basic requirements.<sup>12,14</sup> First, it must be biocompatible, i.e., completely tolerated by the host tissue. Second, it should maintain a suitable space for tissue ingrowth while providing adequate support to the covering tissue. This requires a certain stiffness, which may be achieved by modulating the membrane thickness and/or using materials with proper physical properties. However, the membrane also should have enough malleability so that it can be shaped to the specific geometry needed for each



**Figs 10A to C:** Final restoration



**Figs 11A to C:** Esthetic appearance of the prosthesis was still optimal at the 5-year follow-up visit. The intraoral radiograph showed no signs of peri-implant bone resorption



**Fig. 12:** Five-year follow-up CBCT scan. Bone volume at the implant and graft site was preserved. While the graft had undergone remodeling, a new cortical layer could be observed where the cortical membrane was placed (sections 25 and 26)

reconstruction. In addition, the barrier should guarantee occlusivity to avoid fibrous tissue formation. The main factor conditioning the degree of membrane occlusivity is porosity, a feature that may not strictly depend on the membrane material. Porosity has been shown to play a key role in preventing cell invasion and allowing the diffusion of fluids, oxygen, nutrients, and growth factors.<sup>15,16</sup> The membrane also must integrate with the host tissue, i.e., its borders must adapt to the original bone adjacent to the defect(s) and create a seal capable of preventing fibrous connective tissue entry into the defect site.<sup>17</sup>

The membrane used in the present study is made of cortical bone, i.e., the same type of tissue that constitutes the natural outer layer of the alveolar ridge. Its stiffness and malleability give it excellent handling properties. In the present case, it provided sufficient rigidity to prevent collapsing, but it was easily adapted to make its borders stick to the bone adjacent to the defect. Cortical bone porosity is determined also by the structural and functional connections it has with the periosteum; it also modulates the exchange of nutrients and fluids at the periosteum–bone interface.<sup>18</sup> It is, therefore, reasonable to assume that a thin cortical bone sheet might display optimal porosity for guaranteeing both fluid and nutrient with the underlying graft exchange while preventing soft tissue cells from invading the grafted site.

Cortical bone grafts display certain properties when both the graft and recipient sites may undergo osteoclastic remodeling and consequent replacement with newly formed bone; in this case, a continuum is created between the two.<sup>19,20</sup> This supports the hypothesis that, being made of cortical bone and being susceptible to osteoclastic remodeling, the membrane used in the present study could have incorporated at the bone borders adjacent to the graft site, creating an effective seal and preventing soft tissue invasion. Indeed, the results of the present study show that the cortical bone membrane allowed

bone regeneration and remodeling in the graft volume to occur. This is consistent with previous studies by Lopez et al<sup>21</sup> and Wachtel et al,<sup>22</sup> who observed that a xenogeneic cortical lamina, even if thicker (0.5–3 mm), was capable of providing optimal support to a granular bone graft in a technique mimicking the Khoury bone block approach for lateral augmentation.<sup>23</sup>

Remodeling properties of the cortical membrane used in the present study might be ascribed to the specific enzymatic treatment it undergoes to make it nonantigenic. That process preserves bone collagen in its native state, which should allow for an improved bone regeneration process, given collagen's well-known biological properties.<sup>24–31</sup> Indeed, osteoclasts cultured over such equine, enzymatically deantigenated, and collagen-preserving bone substitutes have significantly higher adhesion and activity than that found for osteoclasts grown over collagen-free, totally deproteinized bovine bone.<sup>32,33</sup> When sites augmented with equine bone alone were compared with others augmented with the same material added to autogenous bone, immunohistochemical tests showed no differences between the two regarding the expression of some biochemical markers of bone regeneration.<sup>34</sup>

Partially demineralized equine cancellous blocks also have been performed successfully in lateral ridge augmentation without any bone loss detectable on CT scans with respect to the grafted volume.<sup>35</sup> Yet such remodeling properties do not explain completely the other noteworthy observations of the present study, i.e., that the patient's pristine cortical bone layer underwent remodeling and a new cortical bone layer could be observed on the regenerated buccal ridge side. Similar behavior was observed by the author when a rigid equine bone block, again made nonantigenic using enzymes and preserving the native bone collagen, was grafted as an onlay below an expanded PTFE membrane to perform

a horizontal augmentation of an atrophic ridge.<sup>36</sup> Both the effectiveness of the cortical membrane used in the present study and the pristine cortical layer remodeling and substitution described should be the object of further clinical studies.

## CONCLUSION

The equine cortical bone membrane used in the present case allowed for successful GBR in the horizontal augmentation of a partially atrophic ridge. Both its effectiveness and its remodeling properties should be subjected to further investigations.

## CLINICAL SIGNIFICANCE

With the increasing esthetic and functional demands of the patient, buccal bone augmentation has become a common technique for implant placement and survival, while presenting a challenge to oral surgeon. The equine enzyme-deantigenic membrane placed with bone granules in accordance with GBR principle appears as a promising option for effective implant-supported prosthetic rehabilitation in the esthetic zone.

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