Implant and Prosthetic Success Following Peri-implant Guided Bone Regeneration in the Esthetic Zone Using an Equine Cortical Bone Membrane and an Equine Enzyme-Treated Bone Graft: A Retrospective Study with 9-year Follow-Up

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Purpose: A barrier membrane consisting of an equine-derived, demineralized cortical bone sheet has been made available, yet evidence of its effectiveness is currently only anecdotal. This study aimed to obtain preliminary evidence concerning the medium-term prosthetic and implant success rates that may be achieved when such a membrane is used in combination with an equine, enzyme-treated bone graft, concomitantly to implant placement in the esthetic zone. Materials and Methods: Records of patients who had one or two implants placed in the anterior sectors of the two arches and had peri-implant bone regeneration carried out using the equine-derived membrane and equine-derived collagen-preserving bone granules were retrospectively collected. Peri-implant marginal bone loss (MBL) was used to assess implant survival. When available, histologic data concerning the equine membrane and cone beam computed tomography (CBCT) scans were analyzed as well. Results: Records of 32 patients (ages 36 to 73 years), corresponding to 44 implants placed, were retrieved and analyzed. The mean follow-up was 113.9 ± 10.2 months. Two implants failed. The implant success rate was 90.9%. Twelve membrane samples could be retrieved and analyzed, showing the membrane was still occlusive at 4.2 ± 1.1 months and only beginning to undergo remodeling. Twelve CBCT scans showed that 65.1 ± 9.8 months after surgery, a newly formed cortical layer could be observed in the zone that had undergone grafting. Conclusion: The equine cortical bone membrane and the enzyme-treated bone graft used in this case series achieved a medium-term implant and prosthetic success rate that was not dissimilar to that of other resorbable membranes and grafts for peri-implant guided bone regeneration augmentation. Preliminary medium-term histologic and CBCT data suggest that the membrane may be occlusive for a period of at least 4 months and may contribute to preserve the ridge thickness over time. Int J Oral Maxillofac Implants 2020;35:824–832. doi: 10.11607/jomi.7906

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Osseointegrated implants should be placed at sites where they best support the prosthesis that provides the most effective and esthetic rehabilitation. Bone at such sites may be lacking, or not be enough to provide the implant with appropriate primary stability, because of congenital defects, traumas, or bone atrophy consequent to edentulism.1–3 These conditions may require bone grafting to restore volume and allow implant insertion. In many cases, bone augmentation is recommended to obtain better and stable esthetic restorative results even when implants can be placed.4 Surgical techniques for bone augmentation include osteodistraction, inlay and onlay grafting, inferior alveolar nerve transposition, and guided bone regeneration (GBR).5–7 The usage of a barrier membrane to prevent the migration of nonosteogenic cells into the regenerating site, thus allowing osteogenic cells to differentiate, proliferate, and regenerate the bone defect is key to GBR.6,8 Barrier membranes for GBR must integrate with the host tissue and be occlusive, space-keeping, and biocompatible.10 The first barriers used were nonresorbable and were removed after healing of the grafted site.11,12 These now include high-density polytetrafluoroethylene (PTFE) barriers13 and nonresorbable titanium meshes.14 Later, resorbable membranes, either synthetic or made of animal-derived collagen, were introduced to avoid removal surgery, reduce exposure, and limit exposure-related complications.15,16 Homologous barriers, made of demineralized human
cortical bone sheets, have also proven to be successful in GBR procedures\(^\text{17-19}\) to manage sinus membrane perforations\(^\text{20}\) and to treat mandibular molar furcations.\(^\text{21}\) However, published evidence about them is limited, possibly because of problems concerning their availability and/or cost arising from their homologous origin. Still, thin bone sheets may display long barrier persistence.\(^\text{19}\) This may be advantageous when regenerating bone defects that might heal slowly because of their size or other anatomical features.\(^\text{4,22}\) For years, authors have been using an equine bone membrane quite similar to homologous laminar bone sheets. It is a thin, flexible lamina made of partially demineralized equine cortical bone. Its manufacturing involves eliminating antigens from equine cortical bone through digestive enzymes, while preserving the original structure of bone collagen. Bone is then made thin by abrasion and flexible by mild acidic treatment. This membrane allowed effective bone regeneration in a patient having horizontal bone augmentation performed in combination with enzyme-deantigenic bone granules.\(^\text{23}\) 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. Another case showed that it was effective in preserving the bone profile in the anterior maxilla when used in combination with an equine enzyme-treated bone graft.\(^\text{24}\) In this patient, a 5-year control CBCT scan showed that peri-implant bone levels and ridge thickness remained unchanged, and the cortical layer in the nonaugmented ridge was also subjected to remodeling. Excluding these two anecdotal cases, no other investigation has been carried out examining the effectiveness and safety of this equine-derived cortical membrane. In light of this consideration, the present study aimed to gather preliminary evidence on the medium-term prosthetic and implant success rates that can be achieved when such a membrane is used in GBR augmentation procedures, concomitantly to implant positioning in the anterior sectors of the two arches.

For this purpose, the authors performed a retrospective 9-year follow-up assessment of immediate peri-implant GBR bone augmentation surgeries involving use of this equine cortical bone membrane in combination with an equine-derived, enzyme-treated particulate bone graft.

## MATERIALS AND METHODS

Records of patients treated between January 2007 and December 2009 by the same oral surgeon (D.D.S.) at two private dental clinics in Italy were reviewed to identify subjects who underwent implant placement and concomitant ridge augmentation according to the GBR principles using the cortical membrane under investigation (Osteoplant Cortical Membrane, Biotech) (Fig 1). Patients of these two private dental clinics are routinely followed up, and if standard prosthetic rehabilitation was carried out the same oral surgeon performed the operations; the controls were part of the standard scheduled control routine for all patients of the two dental clinics and included the collection of intraoral radiographs at each visit. No Ethical Committee approval was sought for this study, given that it was retrospective in nature. Records were included if ridge augmentation was carried out in the maxillary or mandibular anterior sectors, including premolars; if patients were concomitantly rehabilitated through one-step implant placement; if they received one or two osseointegrated implants at the healed site and these were left submerged for a minimum 3-month period; and if standard prosthetic rehabilitation was carried out more than 8 weeks after implant placement and the definitive prosthesis, either screw-retained or cemented, was positioned at least 30 days, but not later than 60 days, after the provisional one. All ridge augmentation procedures had to be carried out using the same equine-derived, enzyme-treated particulate bone graft

![Fig 1](image_url)
Sutures were removed after 10 to 14 days. Three to four
using 5-0 nonresorbable sutures (Monomyd, Butterfly).
Full-flap closure was achieved, and flaps were sutured
alloy tacks (Frios, Dentpysl) in the overlapping area.
brane was blocked by inserting one to three titanium
grafted using the equine bone graft, consisting of a 1:1
receiving sites were prepared by drilling holes in the cor-
elevating a full-thickness mucoperiosteal flap. Implant
local anesthesia. Access to the defect was achieved by
prior to surgery and then twice a day for 7 days after
Antibiotic prophylaxis (amoxicillin/clavulanic acid, Aug-
treatment, unless any of the following conditions were present: osteopo-
neoplasia, or psychotic disease; current bisphos-
any of the following conditions were present: osteopo-
tients were eligible for regenerative treatment, unless
and not be affected by any systemic diseases. All pa-
tients were aged between 18 and 70 years
and not be affected by any systemic diseases. All pa-
tients were eligible for regenerative treatment, unless
Data Collection
Data extracted from clinical records included the pa-
ined in ascending concentrations of ethanol and em-
base was achieved. Defects were then
grafted using the equine bone graft, consisting of a 1:1
(w/W) mixture of cortical-cancellous granules sized 0.5
1 mm (Osteoplant Osteoxenon, Bioteck) after hydrat-
ing them using sterile saline. A 25 × 25 × 0.2-mm or 50
× 25 × 0.2-mm cortical membrane (Osteoplant Corti-
cal Membrane, Bioteck) was shaped using sterile scis-
sors, hydrated using sterile saline, and positioned on
the vestibular ridge side, allowing it to overlap the mar-
gins of the defect by approximately 3 mm. The mem-
brane was blocked by inserting one to three titanium
ally tacks (Frios, Dentsply) in the overlapping area.
Full-flap closure was achieved, and flaps were sutured
using 5-0 nonresorbable sutures (Monomyd, Butterfly).
Sutures were removed after 10 to 14 days. Three to four
months later, following antibiotic prophylaxis and anal-
gesic therapy as already described, the implants were
exposed, and healing screws were applied. Whenever
possible, tacks were removed by carrying out a small
soft tissue incision at the tack insertion site. When pos-
sible and ethical, ie, when a portion of the membrane
had to be removed, if still present, to allow implant ex-
posure and correct healing screw positioning, a small
membrane biopsy specimen was collected for further
histologic analysis.
After 8 weeks or more, having achieved proper soft
tissue conditioning, a provisional prosthesis was deliv-
ered to the patients; the definitive prosthesis was deliv-
ered 1 month later. Patients were followed up monthly
for the following 6 months and then every 6 months,
according to the standard control routine of the two
clinics involved in the present study.

Marginal Bone Loss
Intraoral radiographs were collected, digitalized, con-
verted to 600-dpi resolution TIFF images, stored in
a computer, and analyzed with dedicated software
(Image), National Institutes of Health) to measure the
peri-implant MBL. In particular, once all images were
loaded, the software’s distance measuring tool was
calibrated using the implant diameter (known) at the
most coronal portion of the implant neck. Then, the
distance between the implant-abutment interface and the
crestal bone apex closest to the implant was measured
to the nearest 0.01 mm on both the mesial and distal
sides. The two measurements were averaged to obtain
a single peri-implant marginal bone level. The MBL for
a given implant at a given time point was obtained by
subtracting the peri-implant bone level at follow-up to
that at implant insertion (baseline).

Histologic Processing and Analysis
Each biopsy specimen was placed in a test tube con-
taining buffered 10% formalin. The tube was marked
with a unique alphanumeric code. Bone biopsy speci-
mens were placed for 21 days in a 0.76 M sodium for-
matate and 1.6 M formic acid solution (Panreac Quimica)
for decalcification. Samples were subsequently dehy-
drated in ascending concentrations of ethanol and em-
bedded in paraffin.
A representative clinical case. A patient presented with an old, unesthetic prosthesis asking for a valid functional and esthetic solution. After prosthesis removal, a rehabilitation plan was devised that involved placing an implant at position 22 to deliver an implant-supported single crown and rehabilitating teeth 11 and 21 by means of single veneered ceramic tooth-supported crowns. The bone ridge showed marked horizontal atrophy; while placing the implant was still possible, adequate bone profiles had to be restored by grafting the equine particulate that was protected using the equine cortical membrane.
RESULTS

Records of 32 patients (12 men and 20 women) matching the study criteria were identified; they received a total of 44 implants. Their ages ranged from 36 to 73 years (mean: 52.2 ± 9.8 years); 9 of the subjects were smokers (< 10 cigarettes/day) (28.1%).

Twenty-one implants (47.7%) were inserted in the maxilla and 23 (52.3%) in the mandible. Twenty patients (62.5%) received one implant, and 12 (37.5%) received two implants. Implant diameters varied from 3.8 to 4.5 mm, and lengths varied from 9.5 to 13 mm. After surgery, all patients healed uneventfully, and no subjective complaints were reported. Patients were rehabilitated with 30 (93.7%) screw-retained and 2 (6.3%) cemented prostheses. Average loading time at the 108-month follow-up was 102.6 ± 6.3 months (103.5 ± 5.4 months for screw-retained and 103.4 ± 12.7 months for cemented prostheses, respectively; the difference was not statistically significant, \( P = .63 \)). The average follow-up was 113.9 ± 10.2 months. A total of 338 intraoral radiographs were analyzed.

Two cases of peri-implantitis affecting two implants in one female smoker were observed during the follow-up period, both showing a significant MBL that extended to approximately half the implant length. The patient was lost at the following controls. These two implants were deemed as lost and excluded from MBL calculation concerning the whole dataset. The rate of adverse events was therefore 2 out of 44 (4.54%) concerning implants and 1 out of 32 (3.12%) concerning patients.

No other implants were lost during the follow-up period, and no other cases of peri-implant infection, implant mobility, or radiolucency around the implant were observed, corresponding to a survival rate of 95.5%. The number of successful implants, according to the Albrektsson and Zarb criteria, was 40 out of 44, corresponding to a success rate of 90.9%. No prosthetic failure was observed during the observation period.

Average MBL values at the follow-up control visits at both the implant and patient levels are shown in Table 1. At the implant level, a marginal bone resorption of 0.32 ± 0.25 mm was observed at the last follow-up (Figs 3a and 4). No significant differences were observed among marginal bone resorption values collected from the 48-month control visit onward (ANOVA; \( P = .35 \)). At the patient level, average MBL at the last follow-up was 0.34 ± 0.22 mm (Fig 3b). Again, no significant differences were observed among average MBL values collected from the 48-month control visit onward (ANOVA, \( P = .52 \)). Distribution of successful implants among nonsmokers and smokers was as follows: among nonsmokers, 14 implants were successful and 1 failed; among smokers, 27 were successful and 2 failed.
The difference between the two groups was not significant \((P = .98)\). Average MBL at the last follow-up visit according to the number of implants placed is shown in Table 2; the \(t\) test showed that the two values were not significantly different \((P = .91)\). Histologic assessment involved 12 samples of membranes, collected at \(4.2 \pm 1.1\) months after surgery; for all samples, no signs of inflammation could be detected, and no perforations were observed; few zones of scarce remodeling could be observed (Fig 5). CBCT control scans were available in 12 out of 32 cases; the average time of the last CBCT scan acquisition was \(65.1 \pm 9.8\) months after surgery. In all samples, a cortical layer, variable in thickness, could be observed where a cortical membrane was placed to protect the bone graft (Fig 6).

**DISCUSSION**

Factors that contribute to a successful outcome of a GBR procedure, in addition to the surgical technique and those related to the patient, include membrane features such as the persistence of the barrier effect, the stability, and the space-keeping properties. Resorbable membranes may help reduce the risk of morbidity, as they do not call for a removal surgery; to be effective, they should feature a certain stiffness together with enough malleability to finally meet the specific geometry needed for each reconstruction.\(^{10,28}\)

The barrier porosity also has a key role in preventing cell invasion and allowing the diffusion of fluids, oxygen, nutrients, and growth factors.\(^{29,30}\) Finally, the membrane borders must create a seal capable of preventing the connective tissue from invading the defect site.\(^{31}\)

Resorbable membranes often display an unpredictable resorption time. This implies that matching the membrane type to the defect characteristics is often challenging. If the resorption time is too short, in fact, one might observe a defective bone formation.\(^{32}\) If resorption is too fast, the membrane also may lose rigidity and consequently have diminished space-keeping capability.

Membranes used in the present study may display optimal porosity, as they are made of cortical bone. Indeed, natural cortical bone porosity is pivotal to allow the exchange of nutrients and fluids at the peristem-bone interface.\(^{33}\) These membranes, therefore, should display appropriate exchange of fluid, oxygen, and nutrients with the graft.

When they were implanted, these membranes were easily moldable but stiff enough to prevent collapse;
they were stiffer than resorbable membranes made of collagen, but less stiff and more moldable than titanium-reinforced e-PTFE ones. Cortical membranes would collapse if not supported by the graft; accordingly, they should not be used without concomitant grafting, as already observed by Majzoub et al regarding the comparable homologous bone sheet. Unlike observations by Majzoub et al, the mechanical properties of equine cortical membranes used in the present study allowed the defect margins to seal easily; given the fact that both the cortical equine bone and the laminar bone sheet used in the past have the same thickness (200 µm), this difference in malleability may indicate that the two membranes have been subjected to a different degree of demineralization. Equine cortical membranes were well-tolerated by the overlying tissues, as no inflammation (either redness or swelling), infection, or tissue dehiscence were ever observed. This was also consistent with previous observations concerning the homologous bone lamina. Correct soft tissue closure still remains mandatory to ensure appropriate tissue healing and low complication incidence.

Qualitative histologic assessment provided in the present study indicated that, within a time frame of 4 to 6 months, the equine cortical membrane was still occlusive and had not been subjected to significant remodeling, in line with observations by Majzoub et al, who showed that the homologous laminar bone sheet maintained its integrity and remained nonvital up to 8 months postoperatively. This observation provides a first confirmation of the hypothesis, based on histologic evidence from a single case, that these
equine cortical membranes may undergo slow resorption and remain occlusive for a significantly longer time than other resorbable membranes. This matter should be the subject of systematic histologic investigations. Further histologic studies should also be carried out to assess if these bone membranes integrate to the peripheral host bone, as was observed in the canine study by Smukler et al concerning homologous laminar bone sheets. Qualitative analysis of the few control CBCT scans collected in the present studies showed that a cortical layer was always present in the area corresponding to implant placement. When an equine cortical membrane, like that used in the present study, was used to perform a ridge augmentation according to the GBR procedure, CBCT scans taken 5 years later allowed observation of a cortical bone layer, in the regenerated area, whose radiographic appearance was not different from that observed in other areas of the ridge. These observations suggested that the formation of a novel cortical bone layer occurred, and the membrane underwent complete remodeling and replacement with a physiologic cortical bone layer. Indeed, cortical bone grafts displayed certain properties when both the graft and recipient site underwent osteoclastic remodeling and subsequent replacement with newly formed bone; in this case, a continuum was created between the two. The cortical membrane used in the present study underwent a specific enzymatic treatment to render it nonantigenic. This process preserved the bone collagen in its native state, which should allow for an improved bone-regeneration process, given the well-known biologic properties of collagen. Indeed, osteoclasts cultured over such equine, enzyme-treated, and collagen-preserving bone substitutes have significantly higher adhesion and activity than that found for osteoclasts grown over collagen-free, heat-treated, deproteinized bovine bone. A randomized controlled clinical trial comparing histomorphometric measurements of bone samples collected from sinuses augmented using the two materials showed that they were subjected to different remodeling kinetics, with the equine-derived substitute being remodeled significantly faster. Further studies should therefore also investigate how these collagen-preserving membranes remodel and if their presence may modulate the formation of a novel cortical layer in the regenerated area. Finally, results concerning implant success and survival observed in the present study do not differ significantly from those observed in other studies concerning implant rehabilitation following GBR augmentations, showing that—as far as prosthetic and implant success and survival are concerned—the combination of the equine membrane and the equine graft used in the present study is safe both in the early postsurgical period and over a medium-term (> 5 years) basis.

The limitations of the present study were its retrospective design and the small number of patients and implants involved. Patients undergoing treatment did not present any relative or absolute contraindication to implant and regenerative surgery; yet, they were heterogeneous. Further prospective studies should therefore focus on more homogenous patient groups. Moreover, the present study design did not involve any control group; the membrane and graft used in the present study should therefore be compared to other membranes and biomaterials within the context of appropriately designed comparative investigations. Overall, the results of the present study, concerning the implant and prosthetic success that can be achieved by performing immediate GBR augmentations concomitantly to implant placement using equine cortical membrane and bone graft, should be confirmed by prospective investigations on a larger number of subjects. Evidence about the preservation of ridge thickness following augmentation, the formation of a novel buccal bone plate, and data about the persistence of the membrane barrier effect presented in this study should be regarded as altogether preliminary. Available CBCT scans and histologic data were, in fact, too few to draw any definitive conclusion on these two points. Accordingly, further prospective, controlled studies on a greater number of subjects should be carried out to better investigate the clinical, histologic, and radiographic outcome of GBR augmentation procedures carried out using this equine cortical enzyme-treated barrier membrane either in combination with the equine bone graft used in the present study or others.

CONCLUSIONS

The equine cortical bone membrane used in this case series, in combination with an enzyme-treated equine bone graft, allowed for a medium-term implant and prosthetic success rate that was not dissimilar to that of other resorbable membranes and grafts for peri-implant GBR augmentation. Preliminary data from CBCT scans and histologic assessment suggest that this membrane may be occlusive for a period of at least 4 months and, in combination with an enzyme-treated, collagen-preserving equine bone graft, may contribute to preserve the ridge thickness over time. Both its effectiveness and its remodeling properties should be the subject of further investigations.

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