ORIGINAL RESEARCH

Treatment of Intrabony Defects Using Equine-derived Bone Granules and Collagen Membranes: A Retrospective Study with a 13-year Follow-up

Giacomo Tarquini

Abstract

Aim: The aim of this study is to investigate the effectiveness of a combination of an equine-derived, enzyme-treated bone graft and an equine collagen membrane to treat intrabony defects caused by periodontitis.

Materials and methods: About 22 patients with a single 1-, 2-, or 3-wall intrabony defect and a probing pocket depth (PPD) of ≥5 mm, who were treated using an enzyme-deantigenated equine bone graft in addition to a collagen membrane and were followed up for at least 10 years, were retrospectively assessed. The plaque index (PI), the sulcus bleeding index (SBI), PPD, and the clinical attachment level (CAL) at each follow-up visit were compared to baseline.

Results: The mean PI, SBI, PPD, and CAL were 0.22 ± 0.41, 1.86 ± 0.78, 7.86 ± 1.39 mm, and 8.84 ± 1.86 mm, respectively, at baseline, and 0.25 ± 0.44, 0.12 ± 0.32, 2.59 ± 0.50, and 4.04 ± 0.77 mm, respectively, at the last follow-up. The difference was significant for all parameters ($p < 0.001$) except PI ($p = 0.83$). The final CAL gain was 4.8 mm (49.8%). The SBI, PPD, and CAL still significantly improved at the 12-month follow-up visit but not at the 24-month follow-up visit. There were no correlations between either the number of defect walls or smoking and outcomes. In one case, a surgical re-entry at 5 years allowed a clinical evaluation, showing that intrabony defect was repaired with the newly formed bone of the patient.

Conclusion: Equine bone granules in addition to an equine collagen membrane effectively and safely treated intrabony defects caused by periodontitis providing long-term results.

Clinical significance: Equine-derived bone grafts have been in the market for more than 20 years. However, to the author's knowledge, no studies have reported long-term results for the use of this type of bone graft in periodontal surgery. The equine-derived bone granules used in the present study appears a promising option for treating intrabony defects due to moderate to severe periodontitis.

Keywords: Barrier membranes, Bone substitutes, Equine bone, Equine collagen, Intrabony defects, Periodontitis.

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Introduction

In predisposed subjects, the formation of a periodontal bacterial biofilm may induce periodontitis, which involves chronic inflammation affecting the hard and soft periodontal tissues. Periodontitis may cause periodontal ligament destruction, alveolar bone resorption and, in the most severe cases, the loss of the affected teeth.[1] For moderate-to-severe cases, when teeth have deep pockets and reduced periodontal support, improvement in short-term and long-term outcomes may be achieved by carrying out concomitant regenerative interventions.[2] Periodontal regenerative procedures involve the use of barrier membranes for guided tissue regeneration (GTR) and grafting bone substitutes, either alone or in a combination with biologically active adjuncts.[2–4] Early studies involved non-resorbable membranes, especially expanded polytetrafluoroethylene (ePTFE) membranes.[5] These membranes need a second surgical procedure for their removal; therefore, newer resorbable membranes, made of collagen of various origins, have been developed and successfully used in humans.[3,6] Xenogeneic collagen-based resorbable membranes may be produced either by processing "native" membranes (i.e., the peritoneum or the pericardium) to make them non-antigenic or by extracting collagen from collagen-rich tissues (such as, the dermis and the tendon) and using it to manufacture a membrane.[6] Processed native membranes usually retain their native structure to some degree and are, therefore, more resistant to suturing and tearing; they also display a longer protection time than those made of extracted collagen.[7] To increase the resistance and protection time of the extracted collagen, a chemical or a physical cross-linking step can be used.[8] Depending on the cross-linking method, inflammatory tissue responses may arise, and the advantage of using cross-linked membranes is still subject to debate.[9–11] Non-cross-linked resorbable membranes of equine origin, produced by extracting collagen from equine tendons using a digestive process followed by lyophilization, compression, and electron-beam sterilization, have been on the market for more than 20 years. These membranes have been used successfully as barriers in interventions aiming to regenerate cartilage in patients with osteoarthritis[12,13] and, in oral surgery, for protecting periodontal, peri-implant, and post-extractive socket grafts and to cover the

Private Practice, Rome, Italy

Corresponding Author: Giacomo Tarquini, Private Practice, Rome, Italy, Phone: +39 06 7800179, e-mail: g_tarquini@libero.it

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access window in sinus augmentation surgeries.[14–18] Equine-derived bone grafts, produced by subjecting equine tissue to an enzymatic antigen-elimination process and partial bone collagen denaturation, have also been on the market for more than 20 years. They have been used for various maxillofacial and oral surgery applications, including the treatment of periapical lesions[19] and grafting in sinus augmentation surgeries.[20–22] However, to the author's knowledge, no studies have reported long-term results for the use of membranes and bone grafts of equine origin in periodontal surgery. The aim of the present study is, therefore, to retrospectively assess the safety and effectiveness of the combined use of an equine bone graft and a collagen membrane to treat intrabony defects due to moderate-to-severe periodontitis.

Materials and Methods

Patients' Selection

Clinical records of patients who underwent regenerative therapy for bone intrabony defects due to periodontitis at the author's private dental clinic (Rome, Italy) between January 2002 and December 2006 were retrospectively selected. Included patients (1) had a single 1-, 2-, or 3-wall intrabony defect; (2) had a probing pocket depth (PPD) of ≥5 mm after initial therapy; (3) had good oral hygiene (with the mean plaque index [PI] ≤1);[23] (4) were treated using an equine bone graft (Bio-Gen, Bioteck, Arcugnano, Italy) and a collagen membrane (Biocollagen, Bioteck, Arcugnano, Italy); (5) were followed up for ≥10 years; (6) were aged 18–70 years; and (7) lacked systemic diseases. All patients who were eligible for regenerative treatment had none of the following: osteoporosis, neoplasia, psychiatric disease, acute oral infections, coagulation disorders, history of chemotherapy or radiotherapy in the head or neck region, immunocompromised status, pregnancy, current bisphosphonate therapy, chronic alcohol or drug abuse, or smoking >10 cigarettes/day. All patients had provided their informed consent. No ethical committee approval was sought for this study given its retrospective nature.

Surgical Procedure

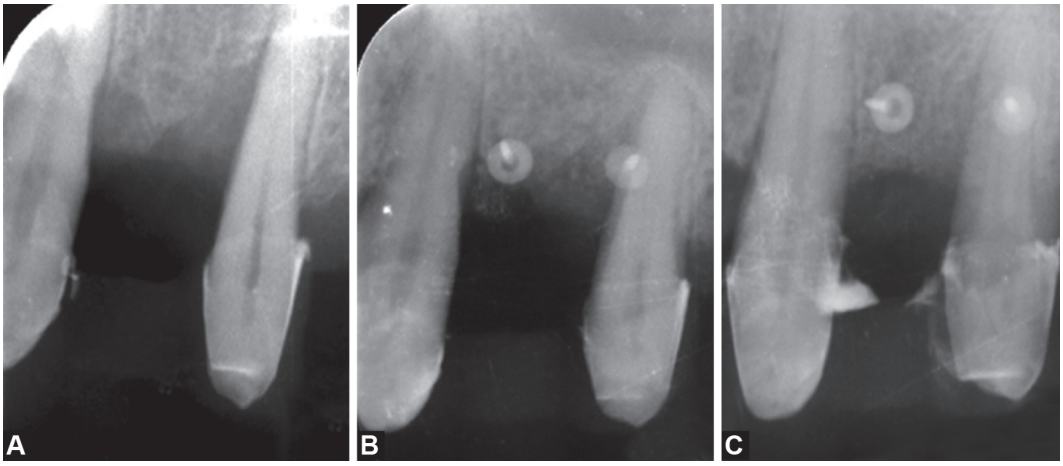
After clinical examination and intraoral radiographic assessment, surgery was performed as follows. Antibiotic prophylaxis (amoxicillin/clavulanic acid, Augmentin, Glaxo-SmithKline, Verona, Italy) (2 g 1 hour before surgery and then every 12 hours for 6 days) was initiated, and patients were subjected to mouth rinses with 0.2% of chlorhexidine (Corsodyl, Glaxo-SmithKline), to be continued for 2 weeks after surgery. In addition, 100 mg of nimesulide (Aulin, Roche, Milano, Italy) was administered 1 hour before the surgery and then twice a day for 3 days. The surgical area was anesthetized using 40 mg/mL of articaine hydrochloride with epinephrine (1:100,000). According to the local anatomy, access to the defect was achieved using either the modified papilla preservation technique[24] or the simplified papilla preservation flap procedure.[25] The reactive, granulomatous tissue of the intrabony defect was debrided using manual instruments (Gracey Curettes, Hu-Friedy, Chicago, IL, USA), and root decontamination was carried out using ultrasonic inserts (Esacrom, Imola, Italy). Root surfaces were not conditioned. The defect was grafted with an equine bone graft, consisting of a 1:1 mixture of 0.5–1 mm equine-derived cortical–cancellous granules (Bio-Gen) after hydrating them using sterile saline. A 25 × 25 × 0.2 mm collagen membrane (Biocollagen) was shaped using sterile scissors, hydrated using sterile saline, and positioned to cover the defect. Titanium pins were used to stabilize the membrane (Citagenix Screw & Tack Kit, Citagenix Inc., Laval, Canada). Full flap closure was achieved, and the flaps were sutured using 5–0 non-resorbable PTFE sutures (Omnia, Fidenza, Italy). Sutures were removed after 14 days, and supragingival professional tooth cleaning was performed every week for 60 days. The patients were then followed up every 3 months. An illustrative case is presented in Figure 1.

Data Collection

Data extracted from clinical records comprised the patients' demographics (age and sex) and their smoking habits (non-smoker or smoked <10 cigarettes/day). Clinical parameters of interest comprised the PPD, the clinical attachment level (CAL), the PI, and the sulcus bleeding index (SBI) (Mühlemann, 1971) recorded at baseline (before surgery) and at the 12, 24, and last (13 years) follow-up visits. The incidence of complications and adverse effects was also assessed.

Statistical Analysis

Patient's characteristics at baseline and clinical parameters (PI, SBI, PPD, and CAL) at each follow-up visit were analyzed using



Figs 1A to C: Intraoral radiograph of an intrabony defect between 2.3 and 2.5 with PPD in mm: (A) Preoperative: PPD of 2.3: B 3-2-7/P 3-2-6 and 2.5: B 8-2-2/P 7-2-3; (B) 1 year follow-up: PPD of 2.3: B 2-2-3/P 2-3-2; 2.5: B 3-2-2/P 3-2-2; (C) 13 years follow-up: PPD of 2.3: B 2-2-2/P 2-3-2 and 2.5: B 2-1-2/P 2-1-2. (B: buccal; P: palatal)

descriptive statistics. All four variables were observed not to have a normal distribution (Shapiro–Wilk test used for normality). Accordingly, they were compared using the non-parametric Friedman test followed by *post hoc* Wilcoxon signed-rank tests. Spearman's correlation coefficients were calculated for the correlations between the number of walls of the defect and the changes (compared to baseline) in PI, SBI, PPD, and CAL at the follow-up time points. Mann–Whitney *U* tests were used to assess whether smoking (non-smokers vs patients who smoked ≤10 cigarettes/day) was associated with changes in the PI, SBI, PPD, and CAL at the follow-up time points.

The significance level for all tests was 0.05. A dedicated software program (Origin 9.0, OriginLab, Northampton, MA, USA) was used for all statistical analyses. All values are presented as mean ± standard deviation.

Results

Records were analyzed for 22 non-consecutive patients (12 men and 10 women) with a mean age of 58.9 ± 7.5 (range 42–75). All patients completed the healing period following the regenerative surgery with no complications or adverse events. The mean follow-up duration was 166.0 ± 13.01 months (range 131.0–182.3; median 167.0). The patient's characteristics at baseline are shown in Table 1. The distribution of the types of teeth being treated according to the number of defect walls is shown in Table 2.

Table 1: The characteristics of patients at baseline. Smokers were patients who smoked ≤10 cigarettes/day

	No. of patients
Gender	
M	12
F	10
No. of defect walls	
1	2
2	15
3	5
Smoker	
N	16
Y	6

Clinical Parameter Analysis

Clinical parameters at baseline and at follow-up visits are summarized in Table 3. The SBI, PPD, and CAL were significantly lower at all follow-up visits compared to baseline ($p < 0.05$ in all cases), but the differences in PIs were not significant. At the last follow-up, the CAL gain was 4.8 mm, corresponding to 49.8%, and the residual PPD was 2.59 ± 0.50 mm. Starting from the 12-month follow-up visit, the mean CAL was not significantly different from the mean CAL at the previous visit. In addition, starting from the 24-month follow-up visit, the mean PPD was not significantly different from the mean PPD at the previous visit, indicating that bone resorption was absent or limited from that time onward (an illustrative case is shown in Fig. 1).

The results of the correlation tests between the number of walls and the CAL gain and PPD reduction at each time point are summarized in Supplementary Table 1. No correlation was observed at any time point, indicating that PPD and CAL variations were not dependent on the number of walls of the periodontal defect. There were also no significant differences between patients who smoked ≤10 cigarettes/day and non-smokers at any time point (Supplementary Table 2).

In one case, a surgical re-entry was necessary at 5 years from the surgery, due to a second guided bone regeneration procedure next to the intrabony defect reported here. Such a condition required a flap opening involving the intrabony defect and thus allowing its clinical evaluation. Indeed, the defect was completely repaired, and there were no residuals of grafting material visible (Fig. 2C).

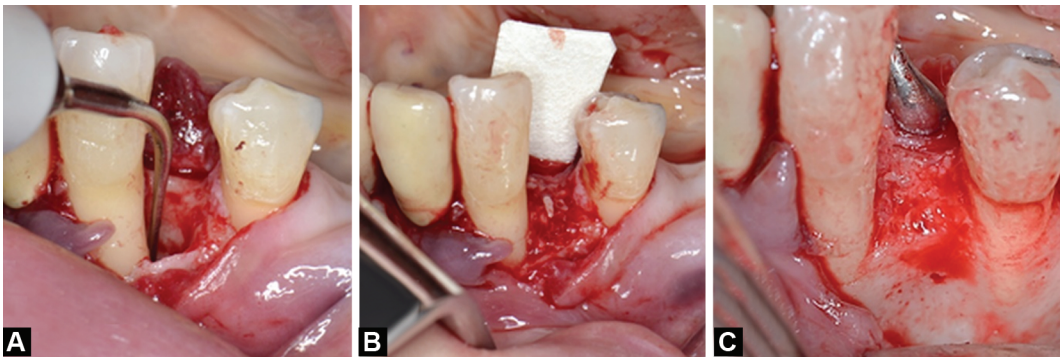
Table 2: Distribution of teeth that were treated according to their type (incisor, canine, premolar, or molar) and the number of defect walls (1–3)

		No. of defect walls			
		1	*2*	*3*	*Total*
Tooth	Incisor	2	2	1	5
	Canine	0	3	0	3
	Premolar	1	5	2	8
	Molar	1	3	2	6
Total		4	13	5	22

Table 3: The mean values of clinical parameters at baseline and at each follow-up visit

Time point	*PI*	*SBI*	*PPD*	*CAL*
Baseline	0.22 ± 0.42	1.86 ± 0.78	7.86 ± 1.39	8.84 ± 1.86
12 months (12.5 ± 1.7 months)	0.20 ± 0.40 (1.000)	0.53 ± 0.64 (<0.001)[a]	2.76 ± 0.51 (<0.001)[a]	4.14 ± 0.92 (<0.001)[a]
24 months (24.7 ± 1.7 months)	0.24 ± 0.43 (1.000)	0.10 ± 0.30 (<0.001)[a]	2.59 ± 0.50 (<0.001)[a]	3.92 ± 0.87 (<0.001)[a]
13 years (166.0 ± 13.1 months)	0.25 ± 0.44 (0.832)	0.12 ± 0.32 (<0.001)[a]	2.59 ± 0.50 (<0.001)[a]	4.04 ± 0.77 (<0.001)[a]
Friedman test *p* value comparing all time points	0.972	<0.001[a]	<0.001[a]	<0.001[a]
Friedman test *p* value comparing 12 months, 24 months, 13 years	0.902	0.003[a]	0.345	0.412
Post hoc Wilcoxon signed-rank test *p* value comparing 12 months, 24 months	0.687	0.006[a]	0.012[a]	0.023[a]
Post hoc Wilcoxon signed-rank test *p* value comparing 24 months, 13 years	1.000	1.000	1.000	0.307

[a]$p < 0.05$; *p* values in brackets: significance compared to baseline (Wilcoxon signed-rank test for paired data). PI, plaque index; SBI, sulcus bleeding index; PPD, probing pocket depth; CAL, clinical attachment level



Figs 2A to C: An illustrative case: (A) Debridement of defect; (B) Grafting with equine bone graft; (C) Surgical re-entry procedure after 5 years

Discussion

Many clinical studies and systematic reviews (with meta-analyses) have shown that the treatment of periodontal defects by grafting bone substitutes both alone or in combination with GTR membranes reduces PPD and improves CAL.[26] With respect to natural grafts derived from mammals, such as, the grafts used in the present study, there is evidence indicating the effectiveness of allografts (tissues derived from human donors) and inorganic bovine bone obtained through thermal treatment (to remove the organic components of the bone). Allografts have been used successfully both alone and in conjunction with a barrier membrane, the latter not always showing a clear clinical advantage.[27] The combined application of anorganic bovine bone graft together with barrier membranes was shown to provide a significant improvement in the defect fill, PPD reduction, and CAL gain (ranging from 1.0 to 5.5 mm, over a mean follow-up ranging from 9 months to 1 year) than implantation alone or flap surgery alone.[28,29] The results of the present study are consistent with these observations. Furthermore, they show that the CAL gain was maintained over time, which concurs with the results of other studies either using bone grafts, barrier membranes, open flap techniques, and biological adjuncts[30,31] or specifically using anorganic bovine bone with resorbable barrier membranes.[32–34]

The results of this study are consistent with those already present in the published literature. In a prospective study with 4 years follow-up, Górsky et al. treated 15 intrabony defects based on GTR principles using anorganic bovine bone and a membrane. At 4 years follow-up, PPD was reduced by 4 mm and CAL was reduced by 5.7 mm.[35] In another study with 5 years follow-up, Döri et al. treated 12 intrabony defects using the Enamel Matrix Derivative combined with anorganic bovine bone. On average, at 5 years follow-up, the value of PPD decreased to 4.9 mm, whereas CAL was reduced to 4.3 mm.[36] Furthermore, a systematic review on the treatment of intrabony defects due to periodontitis by Kao et al. reported the value of reduction in PPD and CAL for several studies showing an overall improvement in both parameters.[30] Other long-term follow-ups of 10 years by Pretzl et al., Nickles et al., and Nygaard-Østby et al. assessed the use of GTR and a resorbable membrane and/or a bone graft, observing an average value of reduction in PPD = 4 mm and an average CAL reduction of 3.1 mm.[37–40] Thus, the values of reduction in PPD (5.3 mm) and CAL (4.8 mm) reported here, at 13 years of follow-up, are in agreement with those in the literature.

However, there is a consensus that positive short-term and long-term outcomes of the intrabony defect treatment are probably more dependent on appropriate patient's behaviors and surgical approaches than the tooth and defect characteristics or the specific combination of biomaterials used.[3,30,41]

The xenograft rather used in the present study was obtained through a manufacturing process that uses a specific enzyme mixture at low temperature, which allows complete deantigenation of the equine bone while preserving its natural mineral structure as well as retaining fragmented type I collagen of the equine bone. Such an enzyme-treated equine bone has shown to be an optimal scaffold for mesenchymal stem cells differentiation *in vitro*.[42] Moreover, there are *in vivo* clinical evidences in maxillary sinus surgery, showing the remodelling of the grafting material with the patient's newly formed bone.[22] It is noteworthy that the results are consistent with those of studies using partially non-resorbable anorganic bovine bone obtained by thermal treatment,[15,43–46] which was considered to be more effective for long-term preservation of volume. The results of the present study showed how, using enzyme-treated equine xenografts, volume preservation was possible during 13 years of follow-up, even though the grafting material was not obtained using mineral-modifying thermal treatment. The advantage of the enzyme-treated equine xenograft is that volume is retained with the patient's newly formed bone, which progressively replaces the equine xenograft, allowing a physiological restoration of the intrabony defect.[22] Such findings were confirmed by a direct clinical evaluation for one patient in this study (Fig. 2). In this case, a surgical re-entry independent of the intrabony defect previously treated allowed a clinical evaluation of the previous surgical site. This clinical inspection revealed that the intrabony defect was repaired and the aspect was that of the newly formed mature bone of the patient (Fig. 2C). Similar clinical results are comparatively rare in the literature,[47] and they represent an important clinical evidence in the success of intrabony defect healing. These results call for confirmation using well-designed controlled prospective studies involving challenging intrabony defects, e.g., defects with only one wall and/or defects with a wide angle between the wall and the long axis of the tooth, which consistently lead to less attachment than defects with smaller angles.[48]

In the present study, smoking seemed to have no detrimental effect on the outcome of the periodontal treatment. This finding is not consistent with those of systematic reviews on the matter.[49] However, the absence of an effect of smoking might be due to the fact that the subjects who smoked were light smokers (≤10 cigarettes/day) and, also, there may have been too few smokers ($n = 6$) to detect a significant difference.

In summary, the outcomes of the present study using partially collagen-preserving equine bone grafts and collagen membranes

are consistent with published evidence on the combined use of xenografts and barrier membranes and show that their combined use is safe and effective in the long-term. Noteworthy, the enzyme-treated xenograft used in this study seems to allow the maintenance of bone volumes over time with the patient's newly formed bone. Limitations of the present study include its retrospective nature and the limited number of patients. Moreover, histological evaluation should be necessary to evaluate the nature of the regenerated bone tissue. Further prospective studies should be carried out to confirm the findings of the present study and compare the performance of the combination of an equine bone graft and an equine collagen membrane with that of other bone substitutes and barrier membranes.

Conclusion

The equine-derived bone granules used in the present long-term study appears to be a promising and safe option for treating intrabony defects due to moderate-to-severe periodontitis. The intrinsic limits of this retrospective case series and the lack of published evidence on direct comparative evaluation with different bone grafts in periodontal surgery, call for randomized, controlled prospective clinical trials to further investigate this subject.

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Supplementary Materials

Supplementary Table 1: The results of correlation analysis (Spearman's correlation coefficients, and significance) between the number of defect walls and the corresponding PI, SBI, and PPD reduction, and CAL gain, at follow-up visits. No correlation was observed in any analysis

Time point	*PI*	*SBI*	*PPD*	*CAL*
12 months	0.1190	0.0229	0.0761	0.1389
	0.406	*0.873*	*0.595*	*0.331*
24 months	0.0873	0.0336	0.0384	0.1031
	0.543	*0.815*	*0.789*	*0.471*
13 years	0.1863	0.0218	0.0328	0.1145
	0.191	*0.879*	*0.843*	*0.424*

Italic values are significant

Supplementary Table 2: The mean PI, SBI, PPD, and CAL values at the different time points for smokers (≤10 cigarettes/day) and non-smokers. The significance of Mann–Whitney *U* tests comparing the two groups is provided in italics. There were no significant differences at any time point

Time point	*Smoker*	*PI*	*SBI*	*PPD*	*CAL*
Baseline	Y	0.07 ± 0.27	1.57 ± 0.65	7.64 ± 1.65	8.86 ± 2.25
	N	0.27 ± 0.45	1.97 ± 0.80	7.95 ± 1.29	8.84 ± 1.72
		0.131	*0.106*	*0.388*	*0.889*
12 months	Y	0.21 ± 0.43	0.64 ± 0.74	2.79 ± 0.58	4.21 ± 0.97
	N	0.19 ± 0.40	0.49 ± 0.61	2.76 ± 0.49	4.11 ± 0.91
		0.854	*0.535*	*0.917*	*0.781*
24 months	Y	0.36 ± 0.50	0.14 ± 0.35	2.57 ± 0.51	3.93 ± 0.83
	N	0.19 ± 0.40	0.00 ± 0.00	2.59 ± 0.50	3.92 ± 0.89
		0.217	*0.158*	*0.892*	*0.956*
13 years	Y	0.29 ± 0.47	0.07 ± 0.27	2.50 ± 0.52	4.00 ± 0.78
	N	0.24 ± 0.43	0.14 ± 0.35	2.62 ± 0.49	4.05 ± 0.81
		0.769	*0.545*	*0.443*	*0.813*