Regeneration of intrabony defects using equine-derived biomaterials: a retrospective study

Rigenerazione di difetti intraossei con biomateriali di origine equina: studio retrospettivo

Received on 8 April 2020
Accepted on 6 July 2020

***Corresponding author**
Giacomo Tarquini
g_tarquini@libero.it

Giacomo Tarquini

Private practice in Rome

ABSTRACT

OBJECTIVES

Periodontitis is an inflammatory disease caused by the formation of a bacterial biofilm that, especially in predisposed subjects, may lead to periodontal ligament destruction and alveolar bone resorption.

The aim of this work was to study the effectiveness of the treatment of intrabony defects due to periodontitis using bone granules and equine-derived pericardium membranes.

MATERIALS AND METHODS

Clinical records of 22 patients who underwent regenerative therapy for intrabony defects following periodontitis at the author's dental center were retrospectively selected. Patients included in this retrospective study a) had a single 1-, 2- or 3-walls intrabony defect; b) had a probing pocket depth (PPD) ≥5 mm after initial therapy; c) had good oral hygiene (mean plaque index - PI ≤1); d) were treated using an equine bone graft and equine pericardium membrane; e) were followed up for ≥12 months.

According to the individual anatomy, access to the defect was achieved using either the modified papilla preservation technique (MPPT) or the simplified papilla preservation flap (SPPF) procedure.

Defects were grafted using an equine bone graft, consisting of a 1:1 mixture of cortical-cancellous granules sized 0.5-1 mm (Osteoxenon, Bioteck, Arcugnano, Vicenza, Italy) after hydrating them using sterile saline solution. A 25 x 25 x 0.2 mm pericardium membrane (Heart, Bioteck, Arcugnano, Italy) was shaped, hydrated, and positioned to protect the graft. Data extracted from clinical records included the patients' demographics (age and sex) and their smoking habits (non-smoker or smoked ≤10 cigarettes/day).

Clinical parameters of interest were the Probing Pocket Depth (PPD), the Clinical Attachment Level (CAL), the Plaque Index (PI), and the Sulcus Bleeding Index (SBI) recorded at baseline (before surgery) and at the 12, 24, and 60-months check-up appointments. Complications and adverse effects were also assessed.

RESULTS

Records were analyzed for 22 non-consecutive patients (12 men and 10 women) with a mean age of 59.0±7.0 years (range from 47 to 77 years). All patients completed the healing period following regenerative surgery with no complications or adverse events.

The mean follow-up period was 60.1±1.13 months. PI, SBI, PPD, and CAL values were significantly lower at all follow-up visits than at baseline ($p < 0.05$ in all cases). At the final follow-up, the CAL gain was 4.8 mm, corresponding to 58% improvement compared to the pre-operative condition, and the PPD was 2.46±0.50 mm. Starting from the 12-month check-up, the mean CAL remained constant. Additionally, starting from the 24-month check-up, the mean PPD did not show significative differences at the subsequent follow-up, indicating

Doi: 10.19256/d.cadmos.06.2021.07

that bone resorption was absent or limited from that time onward.
No correlations were observed at any time point between the measured parameters and the number of walls of the periodontal defect. There were no significant differences between smokers (≤10 cigarettes/day) and non-smokers patients at any time point, except for a difference in the SBI index at the 12-month follow-up visit.

CONCLUSIONS
Equine bone granules plus equine pericardium membranes allowed safe and successful regenerative therapy of intrabony defects caused by periodontitis.

CLINICAL SIGNIFICANCE
The results presented indicate that bone granules and equine-derived pericardium membranes are a safe and effective instrument for the clinician in the treatment of intrabony defects on a medium-term basis.

KEY WORDS
- Periodontitis
- Intrabony defects
- Equine bone
- Barrier membranes
- Equine pericardium

RIASSUNTO

OBIETTIVI
La parodontite è una malattia infiammatoria causata dalla formazione di un biofilm batterico che, specialmente in soggetti predisposti, può portare alla distruzione del legamento parodontale e al riassorbimento osseo alveolare.
Lo scopo di questo lavoro è stato quello di studiare l'efficacia del trattamento dei difetti intraossei dovuti a periodontite utilizzando granuli ossei e membrane in pericardio di origine equina.

MATERIALI E METODI
Le cartelle cliniche di 22 pazienti che avevano avuto accesso allo studio dentistico dell'autore ed erano stati sottoposti a terapia rigenerativa per difetti intraossei a seguito di parodontite sono state selezionate retrospettivamente. I pazienti inclusi in questo studio retrospettivo: a) avevano un singolo difetto intraosseo a 1, 2 o 3 pareti; b) avevano una profondità di sondaggio (PPD) ≥5 mm dopo la terapia iniziale; c) avevano una buona igiene orale (indice di placca medio - PI ≤1); d) erano stati trattati utilizzando un innesto osseo equino e una membrana in pericardio equino; e) sono stati seguiti per ≥12 mesi.
In base all'anatomia individuale, l'accesso al difetto è stato ottenuto utilizzando la tecnica modificata di preservazione della papilla (MPPT) o la procedura semplificata di preservazione della papilla (SPPF). I difetti sono stati colmati usando un innesto osseo equino, costituito da una miscela 1:1 di granuli cortico-spongiosi di 0,5-1 mm (Osteoxenon, Bioteck, Arcugnano, Vicenza, Italia) previa idratazione con soluzione salina sterile.
Una membrana in pericardio da 25 x 25 x 0,2 mm (Heart, Bioteck, Arcugnano, Italia) è stata modellata, idratata e posizionata a protezione dell'innesto.
I dati estratti dalle cartelle cliniche comprendevano età e sesso dei pazienti nonché le loro abitudini al fumo (non fumatori o fumatori di ≤10 sigarette/giorno). I parametri clinici di interesse comprendevano il PPD, il livello di attacco clinico (CAL), il PI e l'indice di sanguinamento (SBI) registrati prima dell'intervento chirurgico e agli appuntamenti di controllo a 12, 24 e 60 mesi.
Sono state inoltre valutate complicanze ed eventi avversi.

RISULTATI
I risultati di 22 pazienti non consecutivi (12 uomini e 10 donne) con un'età media di 59,0±7,0 anni (intervallo da 47 a 77 anni) sono stati analizzati. Tutti i pazienti hanno completato il periodo di guarigione dopo l'intervento chirurgico rigenerativo senza complicazioni o eventi avversi.
Il periodo di follow-up medio è stato di 60,1±1,13 mesi. I valori di PI, SBI, PPD e CAL erano significativamente più bassi in tutte le visite di follow-up rispetto al valore pre-operatorio ($p < 0,05$ in tutti i casi).
Al follow-up finale, il valore CAL era diminuito di 4,8 mm, corrispondente al 58% di decremento dal valore pre-operatorio, e il PPD residuo era 2,46±0,50 mm. A partire dai 12 mesi il CAL medio rimaneva costante. Allo stesso modo, a partire dai 24 mesi il PPD medio non si modificava significativamente, dimostrando assente o limitato riassorbimento osseo.
Non sono state osservate correlazioni tra i parametri misurati e il numero di pareti del difetto parodontale in nessun controllo. Non ci sono state differenze significative tra i pazienti fumatori (≤10 sigarette/giorno) e i non fumatori, a eccezione di una differenza nell'indice SBI alla visita di follow-up di 12 mesi.

CONCLUSIONI
Il biomateriale in granuli associato a una membrana in pericardio equino ha permesso una terapia rigenerativa sicura ed efficace dei difetti intraossei causati da parodontite.

IMPLICAZIONI CLINICHE
I risultati di questo studio indicano che i granuli ossei e le membrane pericardiche di derivazione equina sono uno strumento sicuro ed efficace per il clinico nel trattamento dei difetti intraossei a medio termine.

PAROLE CHIAVE
- Periodontite
- Difetti intraossei
- Osso equino
- Pericardio equino
- Membrana protettiva

1. INTRODUCTION

Periodontitis is an inflammatory disease caused by the formation of a bacterial biofilm that, especially in predisposed subjects, may lead to periodontal ligament destruction and alveolar bone resorption. In severe cases, teeth affected by periodontitis become mobile and eventually fall out[1].

Treatment of periodontitis involves plaque control, scaling and root planing; in moderate to severe cases additional surgical procedures are required to improve the short- and long-term outcomes of teeth that present deep pockets and reduced periodontal support[2].

Periodontal regeneration aims to obtain an increase in the periodontal attachment level of a severely compromised tooth, a complete resolution of intrabony defects, a decrease in pocket depth and, finally, no or minimal decrease in gingival recession[3]. The clinical effectiveness of periodontal regenerative procedures has been extensively studied. These procedures involve the use of barrier membranes, grafting of bone substitutes, and employment of biologically active regenerating materials (alone or in combination). It is agreed that regenerative techniques allow better short- and long-term clinical outcomes compared to open flap debridement alone[2,3].

A wide range of barrier membranes are available for guided tissue regeneration (GTR). Initial studies involved non-resorbable membranes, especially expanded-polytetrafluorethylene (ePTFE) membranes, that were specifically designed for the GTR procedure and have been shown to be effective in the clinical setting[4]. As removal of these membranes requires a second surgery, different resorbable membranes, made of collagen of various origins, have been successfully used in humans[3,5].

A resorbable membrane of equine origin, produced by applying an enzymatic antigen-elimination system to equine pericardium, has been introduced into the market; it has been successfully used as a dural replacement[6] and, in oral surgery, for protecting against bone defects following removal of an apical cyst[7]. At present, no other use of this membrane in the clinical setting has been reported. Equine-derived bone grafts, also produced by subjecting tissue of equine origin to an enzymatic antigen-elimination process, have been successfully used in orthopedic settings[8], and in various maxillo-facial and oral surgery applications, comprising Le Fort I and III osteotomies[9], sinus augmentation[10,11] and horizontal and/or vertical ridge augmentation[12].

The enzymatic process applied to manufacture this bone graft allows preservation of unaltered bone collagen; this could account for the greater osteoclast adhesion and activity that were observed with this graft[13] compared to inorganic bovine bone[14]. The latter, in fact, being produced by a high-temperature treatment, does not contain organic molecules[15]. This may explain why the equine bone graft allowed faster bone formation than anorganic bovine bone in socket preservation[16], sinus augmentation procedures and early implant placement.

To the author's knowledge, no studies have reported the use of this bone graft in periodontal surgery, as is true for the equine pericardium membrane mentioned above.

The aim of the present study was, therefore, to retrospectively assess the safety and effectiveness of the combined use of an equine bone substitute and a pericardium membrane to treat intrabony defects due to moderate to severe periodontitis.

2. MATERIALS AND METHODS

Patients selection

Clinical records of patients who underwent regenerative therapy for bone intrabony defects following periodontitis at the author's dental center between January 2010 and December 2016 were retrospectively selected. Patients included in this retrospective study a) had a single 1-, 2- or 3-walled intrabony defect; b) had a probing pocket depth (PPD) ≥5 mm after initial therapy; c) had good oral hygiene (mean plaque index - PI ≤1); d) were treated using an equine bone graft (Osteoxenon, Bioteck, Arcugnano, Italy) and equine pericardium membrane (Heart, Bioteck, Arcugnano, Italy); e) were followed up for ≥12 months. Other inclusion criteria were age between 18 and 80 years and the lack of any systemic diseases.

All patients were eligible for regenerative treatment – that is, they had none of the following: pregnancy; osteoporosis, neoplasia, or psychiatric disease; acute oral infections; coagulation disorders; history of chemotherapy or radiotherapy in the head or neck region; immunocompromised status; current bisphosphonate therapy; chronic alcohol or drug abuse; or smoking ≥10 cigarettes/day.

All patients provided their informed consent. Given the retrospective nature of the present study, the approval to the conduction of the study was released by the Ethical Committee of Università Campus Bio-Medico of Rome (Prot. N.: 55/20 OSS.NOT ComEt CBM).

Surgical procedure

After clinical examination and intraoral radiographic assessment, surgery was performed as follows. Antibiotic prophylaxis (amoxicillin/clavulanic acid, Aug-

mentin, Glaxo-SmithKline, Verona, Italy) (2 g at 1 hour before surgery and then every 12 hours for 6 days) was initiated, and patients were subjected to mouth rinses with 0.2% chlorhexidine (Corsodyl, Glaxo-SmithKline, Verona, Italy), which continued for 2 weeks after surgery. Nimesulide 100 mg (Aulin, Roche, Milan, Italy) was also administered 1 hour before surgery and then twice a day for 3 days. The surgical area was anesthetized using 40 mg/ml articaine hydrochloride with epinephrine (1:100,000).

According to the individual anatomy, access to the defect was achieved using either the modified papilla preservation technique (MPPT)[17] or the simplified papilla preservation flap (SPPF) procedure[18]. Intrabony defects were debrided of reactive, granulomatous tissue using manual instruments (Gracey Curettes, Hu-Friedy, Chicago, Illinois), and root decontamination was carried out by means of ultrasonic inserts (Esacrom, Imola, Italy). Root surfaces were not conditioned.

Defects were grafted using an equine bone graft, consisting of a 1:1 mixture of cortical-cancellous granules sized 0.5-1 mm (Osteoxenon, Bioteck, Arcugnano, Italy) after hydrating them using sterile saline solution. A 25 x 25 x 0.2 mm pericardium membrane (Heart, Bioteck, Arcugnano, Italy) was shaped using sterile scissors, hydrated using sterile saline, and positioned to cover the defect. Titanium pins were used to stabilize the membrane (Allmed, Lissone, Italy). Full flap closure was achieved, and the flaps were sutured using 6-0 resorbable polyglycolic acid (PGA) sutures (Omnia, Fidenza, Italy). Sutures were removed after 14 days.

Supragingival professional tooth cleaning was performed every week for 60 days. Patients were then followed up every 3 months.

An illustrative case is presented in **figg. 1a-l**.

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 were the PPD, the Clinical Attachment Level (CAL), the Plaque Index (PI), and the Sulcus Bleeding Index (SBI) recorded at baseline (before surgery) and at the 12, 24, and 60-month check-up appointments. Complications and adverse effects were also assessed.

Statistical analysis

Patients' characteristics at baseline, and outcome variables at each check-up, were analyzed by means of descriptive statistics. To study the effectiveness of the treatment, clinical parameters at each check-up were compared with those at baseline. All four variables – that is, PI, SBI, PPD, and CAL – did not have a normal distribution when normality was checked using the Shapiro-Wilk test.

Accordingly, they were compared using the non-parametric Friedman test followed by the post-hoc application of the Wilcoxon signed-rank test. To investigate if the number of walls of the defect correlated with the decrease in PI, SBI, PPD, and CAL following surgery, the decrease in PI, SBI, PPD, and CAL was calculated, for each time point, as the difference between the PI, SBI, PPD, or CAL value measured at that time point and the corresponding value at baseline; a correlation analysis was then carried out by calculating the Spearman correlation coefficients between the number of walls and the PPD and CAL decrease at that time point.

To investigate whether smoking was correlated with the PI, SBI, PPD, and CAL variations over time, PI, SBI, PPD, and CAL values of the two groups, smokers (≤10 cigarettes/day) and non-smokers, were compared using Mann-Whitney U test.

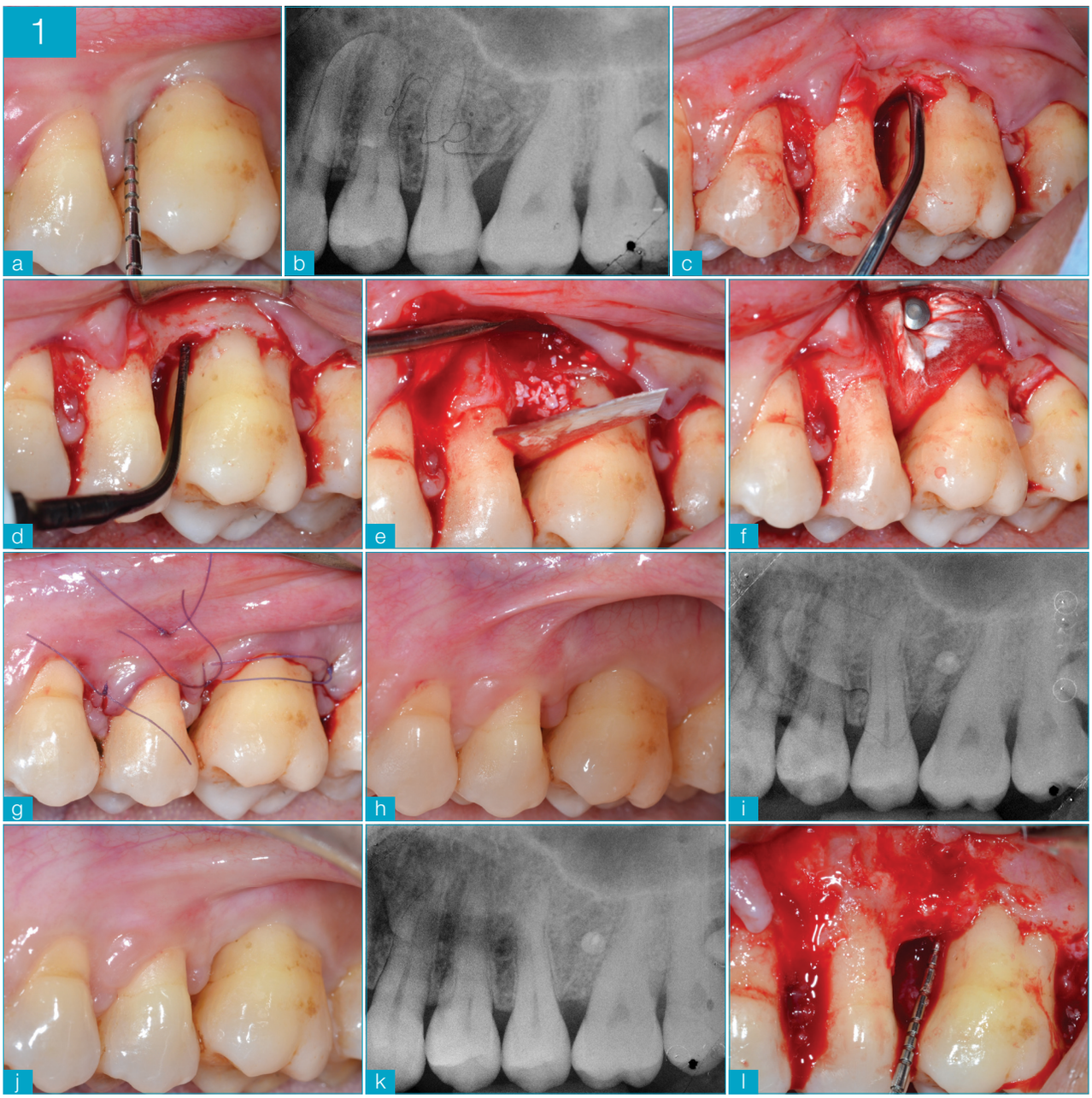
Results were considered statistically significant with P values ≤0.05. A dedicated software program (Origin 9.0, OriginLab Northampton, MA, USA) was used for all statistical analyses. All values are presented as mean±standard deviation (SD).

3. RESULTS

Records were analyzed for 22 non-consecutive patients (12 men and 10 women) with a mean age of 59.0±7.0 (range 47 to 77). All patients completed the healing period following regenerative surgery with no complications or adverse events. The mean follow-up period was 60.1±1.13 months (range 51.4 to 73.4, median 62.3). Characteristics of patients at baseline are shown in **tab. I**. The distribution of the types of teeth being treated according to the number of defect walls is shown in **tab. II**.

Clinical parameter analysis

Clinical parameters at baseline and at the subsequent check-up visits are shown in **tab. III**. PI, SBI, PPD, and CAL values were significantly lower at all follow-up visits than at baseline (P <0.05 in all cases). At the final follow-up, the CAL gain was 4.8 mm, corresponding to 58%, and the residual PPD was 2.46±0.50 mm. Starting from the 12-month check-up, the mean CAL was not significantly different from that at the previous visit. Additionally, starting from the 24-month check-up, the mean PPD was not significantly different from that at the previous visit, indicating that bone resorption was absent or limited from that time onward.



Figg. 1a-l An illustrative case: pre-operative PPD of 6 mm at the mesio-buccal aspect of a first maxillary molar (a); pre-operative radiograph (b). After flap preparation, debridement followed using manual (c) and piezoelectric (d) instruments. Grafting using equine granules was performed (e). The membrane was reflexed and fixed using a pin (f); suturing followed (g). After 1 year, soft tissues appear healthy (h) and bone volumes are preserved (i). After 5 years, both soft (j) and hard (k) tissue appear unchanged. A clinical view of the surgery site after 5 years (l). No graft granules are visible and bone volumes are preserved

Results of correlation tests between the number of walls and the CAL gain and PPD reduction at each time point are shown in **tab. IV**. No correlations were observed at any time point, indicating that variations in PPD and CAL were not dependent on the number of walls of the periodontal defect. There were no significant differences between patients who smoked ≤10 cigarettes/day and non-smokers at any time point, except for a difference in the SBI index at the 12-month follow-up visit (**tab. V**).

In one case, a second GBR procedure next to the intrabony defect presented here was

Tab. I 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	3
2	18
3	6
Smoker	
N	16
Y	6

Tab. II Distribution of teeth that were treated according to their type (incisor, canine, premolar, or molar) and the number of defect walls that they presented (1 to 3)

		Number of defect walls			
		1	**2**	**3**	*Total*
Tooth	Incisor	1	7	1	*9*
	Canine	0	3	2	*5*
	Premolar	1	6	1	*8*
	Molar	1	2	2	*5*
Total		*3*	*18*	*6*	*27*

Tab. III Mean values of clinical parameters at baseline and at each subsequent check-up

Time point	PI	SBI	PPD	CAL
Baseline	0.39±0.55	1.79±0.83	7.45±1.28	8.31±1.56
12 months (12.28±0.15)	0.24±0.43 (0.004)*	0.54±0.61 (<0.001)*	2.61±0.55 (<0.001)*	3.52±0.96 (<0.001)*
24 months (24.24±1.40)	0.22±0.42 (0.005)*	0.09±0.29 (<0.001)*	2.48±0.53 (<0.001)*	3.42±0.82 (<0.001)*
60 months (60.13±1.13)	0.22±0.42 (0.012)*	0.10±0.31 (<0.001)*	2.46±0.50 (<0.001)*	3.49±0.82 (<0.001)*
Friedman test P value comparing all time points	*0.379*	*<0.001**	*<0.001**	*<0.001**
Friedman test P value comparing 12, 24, 60 months	*1.000*	*<0.001**	*0.361*	*0.661*
Post-hoc Wilcoxon signed-rank test P value comparing 12 months, 24 months	*0.766*	*<0.001**	*0.004**	*0.095*
Post-hoc Wilcoxon signed-rank test P value comparing 24 months, 60 months	*1.000*	*1.000*	*0.812*	*0.263*

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; *P <0.05

required after 5 years from the surgery. The flap opening involved the intrabony defect, allowing its clinical evaluation. Indeed, as shown in **fig. 1l**, the bone volume was completely restored. Moreover, no grafting material residues were visible.

4. DISCUSSION

The efficacy of bone grafting in treating intrabony defects of periodontal origin has been the subject of multiple studies and meta-analysis. While initial concerns were expressed about a lack of sufficient evidence to support the clinical use of bone grafts, subsequent pre-clinical and clinical studies have shown that using bone grafts alone, or in combination with GTR membranes, can provide additional benefits in reducing the pocket depth and improving CALs[19].

Concerning allografts (tissues derived from human donors), positive results were observed in different studies in conjunction with barrier membranes, even though some studies failed to show an improvement with combining the graft with the barrier, compared to treatment with the barrier alone[20].

Studies on anorganic bovine bone showed that its combination with barrier membranes provided greater pocket depth reduction, CAL gain, and defect fill, compared with its implantation alone or with flap surgery alone[21]. In these studies, CAL gain at 1 year post surgery ranged from 1.0 to 5.5 mm, which is consistent with the results of the present investigation.

Outcomes of the present study also show that periodontal probing depth and CAL improvement were maintained over time; these results are consistent with previous long-term outcomes concerning the use of bone grafts, barrier membranes, and open flap techniques[22] as well as those specifically involving the use of anorganic bovine bone in conjunction with resorbable barrier membranes[23]. However, positive long-term results following treatment of intrabony defects could be more ascribable to good oral hygiene than to the specific regenerative therapy used to treat the intrabony defects[3]. Outcomes of the present study do not differ significantly from published evidence on the effectiveness of organic-free xenografts, but the quality of the regenerated bone tissue was not evaluated. Considering that the new bone was observed to form faster with the equine-derived, collagen-preserving grafts than with the anorganic bovine grafts, it is reasonable to suppose that the defects treated in this study present a larger amount of vital autologous bone than do defects treated with anorganic bovine bone. It is thus noteworthy that the reduction of probing depth was maintained for the entire follow-up period with no significant losses.

These results call for further studies aimed to better investigate this issue, and whether or not the equine-derived, collagen-preserving grafts can provide an advantage in treating particularly challenging intrabony defects. Indeed, as showed in **fig. 1l**, after 5 years from the surgery, the bone volume was maintained and no residual granules were visible, suggesting that the grafted biomaterial was replaced with patient's newly formed bone. Although, this should be confirmed with a histological examination, as several histomorphometric studies already showed the fast remodeling time of such xenografts[16]. Bone defects that could benefit from the higher remodeling ratio of these xenografts comprise those featuring one wall only and/or those having a large width – that is, a wide angle between the bony wall and the long axis of the tooth; wide-angle defects, in fact, were shown to consistently lead to less attachment than narrower ones[24].

Tab. IV Results of correlation analysis (Spearman's r coefficients, and significance) between the number of defect walls and corresponding PI, SBI, and PPD reduction, and CAL gain, at all check-up visits. No correlations were observed in any analysis

Time point	PI	SBI	PPD	CAL
12 months	0.1457	0.0334	0.0628	0.1355
	0.239	*0.788*	*0.613*	*0.274*
24 months	0.0227	0.2082	0.1035	0.1146
	0.855	*0.091*	*0.405*	*0.356*
60 months	0.0733	0.2071	0.1024	0.0996
	0.556	*0.093*	*0.410*	*0.422*

PI, Plaque Index; SBI, Sulcus Bleeding Index; PPD, Probing Pocket Depth; CAL, Clinical Attachment Level

Tab. V 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 test comparing the two groups is provided in italics. No significant difference could be observed at any time point, except for the SBI values at the 12-month follow-up

Time point	Smoker (Y/N)	PI	SBI	PPD	CAL
Baseline	Y	0.37±0.53	1.78±0.81	7.50±1.22	8.33±1.63
	N	0.43±0.60	1.81±0.87	7.33±1.42	8.29±1.42
		0.752	*0.965*	*0.479*	*0.912*
12 months	Y	0.24±0.43	0.65±0.64	2.59±0.58	3.46±1.00
	N	0.24±0.44	0.28±0.46	2.67±0.48	3.67±0.86
		0.999	*0.025**	*0.485*	*0.334*
24 months	Y	0.22±0.42	0.13±0.34	2.48±0.55	3.41±0.88
	N	0.24±0.44	0.00±0.00	2.48±0.51	3.43±0.68
		0.859	*0.088*	*0.950*	*0.872*
60 months	Y	0.24±0.43	0.13±0.34	2.39±0.49	3.44±0.81
	N	0.19±0.40	0.05±0.22	2.62±0.50	3.62±0.86
		0.667	*0.313*	*0.086*	*0.450*

PI, Plaque Index; SBI, Sulcus Bleeding Index; PPD, Probing Pocket Depth; CAL, Clinical Attachment Level; *P <0.05

Results of the present study concerning the effect of smoking on the outcome of the periodontal treatment are inconsistent with those of previous reviews and meta-analysis[25], which indicated that smoking has a detrimental effect on periodontal regeneration. However, these studies have the limitation of having considered "smokers" as patients smoking a different number of cigarettes/day. In the present study, the absence of any effect of smoking on periodontal regeneration could be due to the fact that the subjects who smoked were light smokers (≤10 cigarettes/day); in addition, the number of smokers may have been too small (n = 9) to detect a significant difference when comparing with non-smoking patients. Further studies should be carried out to investigate this matter.

In summary, the outcomes of the present study concerning equine, collagen-preserving bone graft and pericardium membrane are consistent with previous investigations of the combined use of xenografts and barrier membranes, and show that their combined use is safe and effective on a medium-term basis.

Limitations of the present study include its retrospective nature and the limited number of patients. Additional controlled, prospective, and possibly comparative investigations should be carried out to compare the performance of the combination of an equine bone graft and an equine pericardium membrane with that of other bone substitutes and barrier membranes.

5. CONCLUSIONS

The combination of equine bone granules and equine pericardium membrane allowed successful and safe regenerative therapy for intrabony defects caused by periodontitis, and provided medium-term results that are consistent with those already published concerning the use of xenografts and resorbable barriers.

Randomized, controlled prospective clinical trials should be carried out to investigate this subject further. ■

CONFLICT OF INTEREST

The author confirm that are no known conflicts of interest associated with this publication.

FUNDING FOR THE STUDY

The author has not have significant financial support for this work that could have influenced its outcome.

INFORMED CONSENT

For the publication of the case, including photos, the consent of the patient was obtained.

BIBLIOGRAFIA

1. **Kinane DF, Stathopoulou PG, Papapanou PN.** Periodontal diseases. Nat Rev Dis Primers 2017 Jun 22;3:17038.

2. **Graziani F, Karapetsa D, Alonso B, Herrera D.** Nonsurgical and surgical treatment of periodontitis: how many options for one disease? Periodontol 2000 2017 Oct;75(1):152-88.

3. **Cortellini P, Tonetti MS.** Clinical concepts for regenerative therapy in intrabony defects. Periodontol 2000 2015 Jun;68(1):282-307.

4. **Needleman IG, Worthington HV, Giedrys-Leeper E, Tucker RJ.** Guided tissue regeneration for periodontal infra-bony defects. Cochrane Database Syst Rev 2006 Apr 19;(2):CD001724.

5. **Bottino MC, Thomas V.** Membranes for periodontal regeneration - A materials perspective. Front Oral Biol 2015;17:90-100.

6. **Centonze R, Agostini E, Massaccesi S, Toninelli S, Morabito L.** A novel equine-derived pericardium membrane for dural repair: a preliminary, short-term investigation. Asian J Neurosurg 2016 Jul-Sep;11(3):201-5.

7. **Di Stefano DA, Andreasi Bassi M, Cinci L, Pieri L, Ammirabile G.** Treatment of a bone defect consequent to the removal of a periapical cyst with equine bone and equine membranes: clinical and histological outcome. Minerva Stomatol 2012 Nov-Dec;61(11-12):477-90.

8. **Sonmez MM, Armagan R, Ugurlar M, Eren T.** Allografts *versus* equine xenografts in calcaneal fracture repair. J Foot Ankle Surg 2017 May-Jun;56(3):510-3.

9. **Mattioli B, Iacoviello P, Aldiano C, Verrina G.** Subcranial Le Fort III advancement with equine-derived bone grafts to correct syndromic midfacial hypoplasia: a case report. J Maxillofac Oral Surg 2018;17(3):296-300.

10. **Arosio P, Greco GB, Zaniol T, Iezzi G, Perrotti V, Di Stefano DA.** Sinus augmentation and concomitant implant placement in low bone-density sites. A retrospective study on an undersized drilling protocol and primary stability. Clin Implant Dent Relat Res 2018 Apr;20(2):151-9.

11. **Zaniol T, Zaniol A, Tedesco A, Ravazzolo S.** The low window sinus lift: a CAD-CAM-Guided surgical technique for lateral sinus augmentation: a retrospective case series. Implant Dent 2018 Aug;27(4):512-20.

12. Pistilli R, Signorini L, Pisacane A, Lizio G, Felice P. Case of severe bone atrophy of the posterior maxilla rehabilitated with blocks of equine origin bone: histological results. Implant Dent 2013 Feb;22(1):8-15.
13. Perrotti V, Nicholls BM, Piattelli A. Human osteoclast formation and activity on an equine spongy bone substitute. Clin Oral Implants Res 2009 Jan;20(1):17-23.
14. Perrotti V, Nicholls BM, Horton MA, Piattelli A. Human osteoclast formation and activity on a xenogenous bone mineral. J Biomed Mater Res A 2009 Jul;90(1):238-46.
15. Benke D, Olah A, Möhler H. Protein-chemical analysis of Bio-Oss bone substitute and evidence on its carbonate content. Biomaterials 2001 May;22(9):1005-12.
16. Di Stefano DA, Zaniol T, Cinci L, Pieri L. Chemical, clinical and histomorphometric comparison between equine bone manufactured through enzymatic antigen-elimination and bovine bone made non-antigenic using a high-temperature process in post-extractive socket grafting. A comparative retrospective clinical study. Dent J (Basel) 2019 Jul 1;7(3):70.
17. Cortellini P, Prato GP, Tonetti MS. The modified papilla preservation technique. A new surgical approach for interproximal regenerative procedures. J Periodontol 1995 Apr;66(4):261-6.
18. Cortellini P, Prato GP et al. The simplified papilla preservation flap. A novel surgical approach for the management of soft tissues in regenerative procedures. Int J Periodontics Restorative Dent 1999 Dec;19(6):589-99.
19. Döri F, Arweiler NB, Szántó E, Agics A, Gera I, Sculean A. Ten-year results following treatment of intrabony defects with an enamel matrix protein derivative combined with either a natural bone mineral or a β-tricalcium phosphate. J Periodontol 2013 Jun;84(6):749-57.
20. Trejo PM, Weltman R, Caffesse R. Treatment of intraosseous defects with bioabsorbable barriers alone or in combination with decalcified freeze-dried bone allograft: a randomized clinical trial. J Periodontol 2000 Dec;71(12):1852-61.
21. Tonetti MS, Cortellini P, Lang NP, Suvan JE, Adriaens P et al. Clinical outcomes following treatment of human intrabony defects with GTR/bone replacement material or access flap alone. A multicenter randomized controlled clinical trial. J Clin Periodontol 2004 Sep;31(9):770-6.
22. Gottlow J, Nyman S, Karring T. Maintenance of new attachment gained through guided tissue regeneration. J Clin Periodontol 1992 May;19(5):315-7.
23. Stavropoulos A, Karring T. Guided tissue regeneration combined with a deproteinized bovine bone mineral (Bio-Oss) in the treatment of intrabony periodontal defects: 6-year results from a randomized-controlled clinical trial. J Clin Periodontol 2010 Feb;37(2):200-10.
24. Tsitoura E, Tucker R, Suvan J, Laurell L, Cortellini P, Tonetti M. Baseline radiographic defect angle of the intrabony defect as a prognostic indicator in regenerative periodontal surgery with enamel matrix derivative. J Clin Periodontol 2004 Aug;31(8):643-7.
25. Patel RA, Wilson RF, Palmer RM. The effect of smoking on periodontal bone regeneration: a systematic review and meta-analysis. J Periodontol 2012 Feb;83(2):143-55.