Clin Oral Invest (2011) 15:225–232
DOI 10.1007/s00784-009-0369-y

ORIGINAL ARTICLE

Comparative study of DFDBA in combination with enamel matrix derivative versus DFDBA alone for treatment of periodontal intrabony defects at 12 months post-surgery

Simone Domenico Aspriello · Luigi Ferrante · Corrado Rubini · Matteo Piemontese

Received: 10 May 2009 / Accepted: 19 November 2009 / Published online: 7 January 2010

Abstract The aim of this randomized double-blind, clinical trial was to compare the use of enamel matrix derivative (EMD) and demineralised freeze-dried bone allografts (DFDBA) with DFDBA alone for the treatment of human periodontal intrabony defects at 12 months post-surgery. Fifty-six intrabony osseous defects in 56 periodontis patients were randomly assigned to the test group (DFDBA + EMD) or the control group (DFDBA) for periodontal treatment. Clinical and radiographic measurements were made at the baseline and after 12 months. Compared to baseline, the 12-month results indicated that both treatment modalities resulted in significant changes in all clinical parameters (gingival index, bleeding on probing, probing depth (PD), clinical attachment level (CAL), gingival recession; $P<0.05$) and radiographic parameters (hard tissue fill (HTF) and bone depth reduction; $P<0.05$). Furthermore, statistically significant differences were found in the test group compared to the control group in PD reduction (5.0 mm vs. 4.0 mm; $P<0.05$), CAL gain (4.0 mm vs. 3.25 mm), and HTF (4.0 mm vs. 3.5 mm; $P<0.05$). In the test group, 25% of sites gained >4 mm of CAL, while in the control group, 7.1% of sites gained >4 mm of CAL. Both treatments showed a good soft and hard periodontal tissue response. At 12 months post-surgery, the combined use of DFDBA and EMD seemed to produce a statistically significant improvement of PD reduction, CAL gain, and HTF.

Keywords DFDBA · EMD · Bone grafts · Osseous defects · Periodontal regeneration · Periodontitis

S. D. Aspriello · M. Piemontese (✉)
Periodontology—Department of Clinical and Dental Sciences,
Polytechnic University of the Marche,
Via Tronto 10/a,
Torrette di Ancona, Italy
e-mail: m.piemontese@univpm.it

L. Ferrante
Medical Statistics—Department of Clinical Medicine and Applied Biotechnologies, Polytechnic University of the Marche,
Ancona Torrette, Italy

C. Rubini
Periodontology and Oral Pathology—Institute of Harmed Anatomy, Department of Neurosciences,
Polytechnic University of the Marche,
Ancona Torrette, Italy

The ultimate goal of periodontal therapy is not only to prevent periodontal disease progression, but also to regenerate the lost dentition's supporting structures such as cementum, periodontal ligament, and bone to a diseased root surface where appropriate[1, 2]. Various bone materials such as autogenous grafts [3–6], demineralised freeze-dried bone allografts [7], bovine bone xenografts [8–11], or synthetic bone substitutes [12, 13] have demonstrated regenerative potential and have been successfully used in the treatment of intrabony defects. The use of demineralised freeze-dried bone allografts (DFDBA), whether alone or in combination with other treatment modalities for periodontal therapy, has repeatedly demonstrated significant improvements in both soft and hard clinical tissue parameters [14–16].

Recently, the attention of periodontal researchers and clinicians has focused on the use of enamel matrix protein (EMD) [17–21] for periodontal regeneration alone or in combination with graft material. The assumption of combining graft material with EMD is based on the fact that two distinct wound healing processes, osteoinductive and/or osteoconductive, and promoting periodontal regeneration, respectively, may take place together, and this probably results in their synergistic effect. The grafting

material helps to overcome the risk of a flap collapse following application of EMD, especially in deep intrabony defects, enhancing wound stability and providing space for the regeneration process and, at the same time, allows EMD to enhance periodontal regeneration [18]. Observations from human histological case reports support this concept [22]. Boyan et al. [23] found that the addition of 4 mg EMD to active DFDBA increased the amount of bone induction and new bone formation in all examined samples implanted in calf muscle of mice compared to active DFDBA alone. Rosen [24] demonstrated the clinical benefits of using a combined therapeutic approach in which EMD was combined with either DFDBA or FDBA. Gurinsky et al. [25] reported enhancement of hard tissue; parameters when EMD was added to DFDBA compared to EMD alone in the treatment of human intrabony periodontal defects. Harris et al. [26] successfully applied new techniques for treating periodontal defects not involving furcation using EMD, DFDBA combined with EMD, and guided tissue regeneration. Sugai et al. [27] obtained favorable clinical results with re-implantation of a tooth with severe periodontal involvement using EMD in combination with guided tissue regeneration and bone grafting. Harrel et al. succesfully used DFDBA mixed with EMD together with minimally invasive surgery for the treatment of 130 periodontal defects [28]. Recently, Hoidal et al. [29] found that the addition of EMD to DFDBA (reconstituted with EMD) provided no statistically significant improvement in the soft and hard tissue parameters measured, compared to allograft alone 6 months post-surgery. Furthermore, up to now, the data on the various approaches are controversial and, therefore, more studies are needed.

In any case, the purpose of this study was to investigate the possible additional effects of EMD in the treatment of periodontal intrabony defects by comparing clinical and radiographic outcomes obtained by the use of DFDBA plus EMD to DFDBA alone.

Material and methods

Study patients

Fifty-six patients (34 females and 22 males) diagnosed with generalized chronic periodontitis [30] participated in the study. Their ages ranged from 48 to 62 years, and they had more than eight diseased areas with clinical attachment level (CAL) ≥5 mm and together with radiographic evidence of bone loss. Candidates were selected from the patient pool at the Polytechnic University of the Marche in Ancona Torrette. All patients were informed of the purpose of the study and signed an informative consent form approved by the committee for the Protection of Human Subjects at the abovementioned University.

Study design

The study was designed as a randomized, double-blind clinical trial comparing the periodontal outcomes of the use of DFDBA[1] alone (controlled) or combined with EMD[2] (test) in the treatment of intrabony defects. Twenty-eight patients were assigned to either test or control group. The criteria for inclusion of patients and areas in this study were individuals who were non-smokers, free from systemic complications, and with no history of allergies; they had not used antibiotics in the 6 months prior to treatment nor had they been treated for periodontitis during the previous 2 years; they had radiographic and clinical evidence of one defect with a probing depth (PD) ≥6 mm, CAL loss ≥6 mm, osseous defect depth estimated from radiographic evaluation as ≥3 mm, and two or three osseous walls without extending into a furcation area.

All patients received initial therapy including oral hygiene instruction, full-mouth scaling, and root planing utilizing 40 mg/ml of articainhydroclorid with 1:100,000 epinephrine and occlusal adjustment when indicated. Re-evaluation examinations were accomplished 2 months after initial therapy to determine patient response to the therapy and to confirm the need for periodontal surgery. Surgical therapy was initiated on patients when adequate plaque control judged by <10 using the O'Leary plaque index [31] was demonstrated.

Clinical measurements

On the day of the surgical procedure, baseline clinical measurements were recorded by the same examiner (SDA) blind to the treatment and were repeated after 12 months using the same type of probe[3] with manual pressure of approximately 0.3 N. Measurements were collected at the deepest point of the selected defect and were recorded and rounded up to the nearest millimeter. The reference point was the cemento-enamel junction (CEJ) or, if the CEJ was not visible, a restoration margin. The outcome variables included: PD, CAL, gingival recession (REC) considered as the position of the CEJ.

Defects were randomly assigned to one of the following treatments at the time of surgery: DFDBA (control) or EMD in combination with DFDBA (reconstituted with saline solution). Randomisation was performed by the toss of a coin immediately following periodontal intrabony defect debridement.

[1] MTF, Musculoskeletal Transplant Foundation, Edison, NJ.
[2] Emdogain, Straumann Biologics Division, Waltham, MA; previously, Biora, Inc., Malmo, Sweden
[3] UNC-15 periodontal probe, Hu-Friedy, Chicago, IL.

Radiographic examinations

Radiographs were taken at the initial examination and 12 months post-surgery by the same investigator (SDA) who performed all measurements without knowing the procedure category. Radiographs were performed using the long-cone paralleling technique. All X-rays were obtained using the same radiographic equipment[4], film[5], exposure, and development conditions. A template (stent) was fabricated for each patient to allow reproducible positioning during subsequent radiographs. A caliper was used for these measurements, which were expressed in millimeters and corrected to account for the magnification factor of the equipment used. The following linear distances were measured in mm: (1) the distance from the CEJ to the base of the defect (BD), (2) the distance from the alveolar crest (AC) to the BD, (3) the distance from the CEJ to the AC. When an interproximal restoration was present, its most apical extension was used instead of the CEJ. The most coronal area where the periodontal ligament maintained an even width was identified to measure the most apical extension of the intrabony defect. The crossing of the silhouette of the alveolar crest with the root surface was defined as alveolar crest. The differences between 12 month and baseline values of the CEJ-BD indicated the amount of the hard tissue fill (HTF) within the osseous defect. The differences between the AC-BD and CEJ-AC recorded at baseline and at an examination 1 year later were identified as the bone defect resolution (BDR) and the amount of crestal bone resorption, respectively.

Surgical procedures

One surgeon performed all the operations (MP). The patient was instructed to rinse with 0.2% chlorhexidine gluconate for 2 min prior to the surgical procedure. The surgical procedures were performed using routine local anesthetics (2% arthycaine with epinephrine 1:100,000). Sulcular incisions were made for all teeth. Mucoperiosteal flaps were then raised on the buccal and lingual/palatal parts of the teeth. The flaps included all affected teeth in the quadrant, taking care to preserve as much of the gingival connective tissue as possible. A vertical releasing incision extending into the alveolar mucosa was placed only when necessary for proper access to the defect. Epithelium and granulation tissues from the inner surface of the flaps were carefully removed. Thorough soft tissue debridement and root planing were accomplished with hand instruments, ultrasonic instruments, rotating diamond stones, and finishing burs.

The surgical area was rinsed with copious amounts of 0.9% NaCl irrigation; then, once hemostasis had been achieved, 24% ethylenediaminetetraacetic acid pH 6.7[6] was applied to the root surface with a cotton pellet for 2 min to remove the smear layer, detoxify the root surface, and expose collagen fibrils [32–34]. The surgical area was then thoroughly irrigated with sterile saline solution.

Patients were randomly treated by either DFDBA alone (control) or EMD in combination with the DFDBA (test). In the control group, defects were grafted with DFDBA which had been previously reconstituted with sterile saline solution in a sterile dappen dish. The graft material was gently packed in the defects to the most coronal level of the surrounding bony walls using amalgam condenser. In the test group, EMD gel in 0.3 ml sterile syringe was applied onto the root surfaces and into the defects in the apico-coronal direction and left in place for 3 min during which time the bleeding from the adjacent areas was controlled with the use of gauzes. The graft (DFDBA previously reconstituted with sterile saline solution in a sterile dappen dish) was gently packed into the defects to fill the most coronal levels of the defect walls using an amalgam condenser.

After grafting, great care was taken to obtain complete closure of the interdental soft tissues above the treated defects in both patient groups: flaps were repositioned slightly coronal to obtain as complete a primary interproximal closure as possible. The flaps were stitched with a 6-0 polyammide[7] material using modified vertical mattress and single detached suturing as necessary and damp gauze pressure was applied for 3 min. Patients were placed on Ceftibuten 400 mg/daily for 6 days and Piroxicam[8] 20 mg/daily for 10 days. Patients rinsed with 0.20% chlorhexidine gluconate mouthrinse[9] twice daily for the first 2 weeks following the surgery and were instructed not to brush or floss in the areas where surgery had been performed for 10 days. Sutures were removed when the flap and root soft tissue interface was stable, usually after 10 days. Thereafter, gentle brushing on buccal and lingual surfaces with a soft toothbrush was recommended. Supragingival professional tooth cleaning was performed weekly for the first 6 weeks post-surgery. Thereafter, each patient was reinstructed in proper oral hygiene measures including sulcular and interproximal brushing and was recalled once a month up to 12 months post-surgery for oral hygiene reinforcement and prophylaxis.

[4] Trophy Radiologie, 708 G, 70 kV, Vincennes, France.
[5] Kodak Ultraspeed DF58, Eastman Kodak, Rochester NY.
[6] Prefgel, Straumann Biologic Division
[7] MONOMYD MM-2620 Butterfly Italia, Cavenago B.za (MI), Italy, www.butterflyitalia.com
[8] CICLADOL Rottapharm SpA, Monza, Italy, www.rottapharm.it
[9] Corsodyl, GlaxoSmithKline

Statistical analysis

Results were summarized using median and interquartile ranges (IQR) for the clinical and radiographic parameters. This data was statistically evaluated by a non-parametric test using the statistical program R (2.7.1 version). Taking into account the paired nature of the changes from baseline to 12 months in each group, the Wilcoxon signed-rank matched pair test was performed for the pairwise statistical analysis of these data. The Mann-Whitney *U* test was applied to compare clinical and radiographic outcomes between test and control groups at baseline and 12 months post-surgery. A prospective power analysis was conducted before data were collected. A sample size of at least 56 patients (28 DFDBA and 28 DFDBA + EMD) was established to detect a clinically significant mean difference of at least one standard deviation at the 0.05 level with power of 98%. This was determined using a population standard deviation of HTF ≤1.0 mm, allowing for the detection of a clinically significant mean HTF difference between treatment groups ≥1.0 mm.

The null hypothesis was rejected when the risk percentage was below 5% ($P<0.05$).

Results

All enrolled subjects completed the study. All sites healed uneventfully. Table 1 show the distribution of all subjects regarding age and gender, number of osseous walls in the treated defects, and treated teeth. The medians of clinical and radiographical outcomes at baseline and after 12 months are shown in Table 2. Box and Whisker plots of clinical and radiograpocal Δ (baseline to 12 months) values are shown in Fig. 1.

For between-group comparisons at baseline, PD, CAL, REC, CEJ-BD, AC-BD, and CEJ-AC demonstrated no statistically significant differences between the study groups. After 12 months, the DFDBA + EMD sites compared to the DFDBA alone ones did not have lower median PD, CAL, and REC and there were no significant differences between the study groups for median radiographic CEJ–BD, AC–BD, and CEJ–AC. However, within-group comparisons showed that both treatments of the intrabony defects (DFDBA + EMD or DFDBA alone) led to an overall clinical improvement in PD, CAL and REC at 12 months compared to baseline.

At 12 months post-surgery, PD and CAL decreased in both DFDBA + EMD and DFDBA + saline groups (Table 2).

Table 1 Patient age, gender, number of osseous walls, and tooth location

Characteristics	DFDBA + EMD (n=28)	DFDBA + Saline (n=28)
Age (mean ± SD)	55.4±4.6	56.6±6.4
Females	16	18
Males	12	10
Osseous walls		
2-wall defect	14	13
3-wall defect	14	15
Teeth treated		
Maxillary incisors	4	5
Mandibular incisors	4	5
Mandibular canines	2	1
Maxillary canines	2	1
Maxillary premolars	7	6
Mandibular premolars	4	5
Maxillary molars	3	2
Mandibular molars	2	3

Table 2 Medians (IQ) of clinical and radiographic measurements at the baseline and after 12 months (n=28 each treatment group)

Index/treatment	Baseline	12 months	P value
PD (mm)			
DFDBA + EMD	9.0 (2.125)	4.0 (1.5)	<0.001
DFDBA + saline	8.5 (1.625)	4.75 (1.375)	<0.001
P value	NS	NS	
CAL (mm)			
DFDBA + EMD	8.5 (1.75)	4.5 (2.125)	<0.001
DFDBA + saline	8.0 (2.5)	4.5 (3.25)	<0.001
P value	NS	NS	
REC (mm)			
DFDBA + EMD	0.5 (0.5)	−0.5 (1.0)	<0.01
DFDBA + saline	0.5 (1.5)	−0.25 (1.125)	<0.01
P value	NS	NS	
CEJ-BD (mm)			
DFDBA + EMD	9.0 (2.5)	6.0 (2.0)	<0.001
DFDBA + saline	9.0 (2.625)	5.5 (3.0)	<0.001
P value	NS	NS	
AC-BD (mm)			
DFDBA + EMD	5.5 (1.375)	1.5 (1.875)	<0.001
DFDBA + saline	5.0 (2.25)	1.5 (1.25)	<0.001
P value	NS	NS	
CEJ-AC			
DFDBA + EMD	3.5 (0.5)	3.75 (1.5)	NS
DFDBA + saline	4.0 (0.0)	4.0 (0.625)	NS
P value	NS	NS	

Statistically significant difference($P<0.05$)

NS not significant

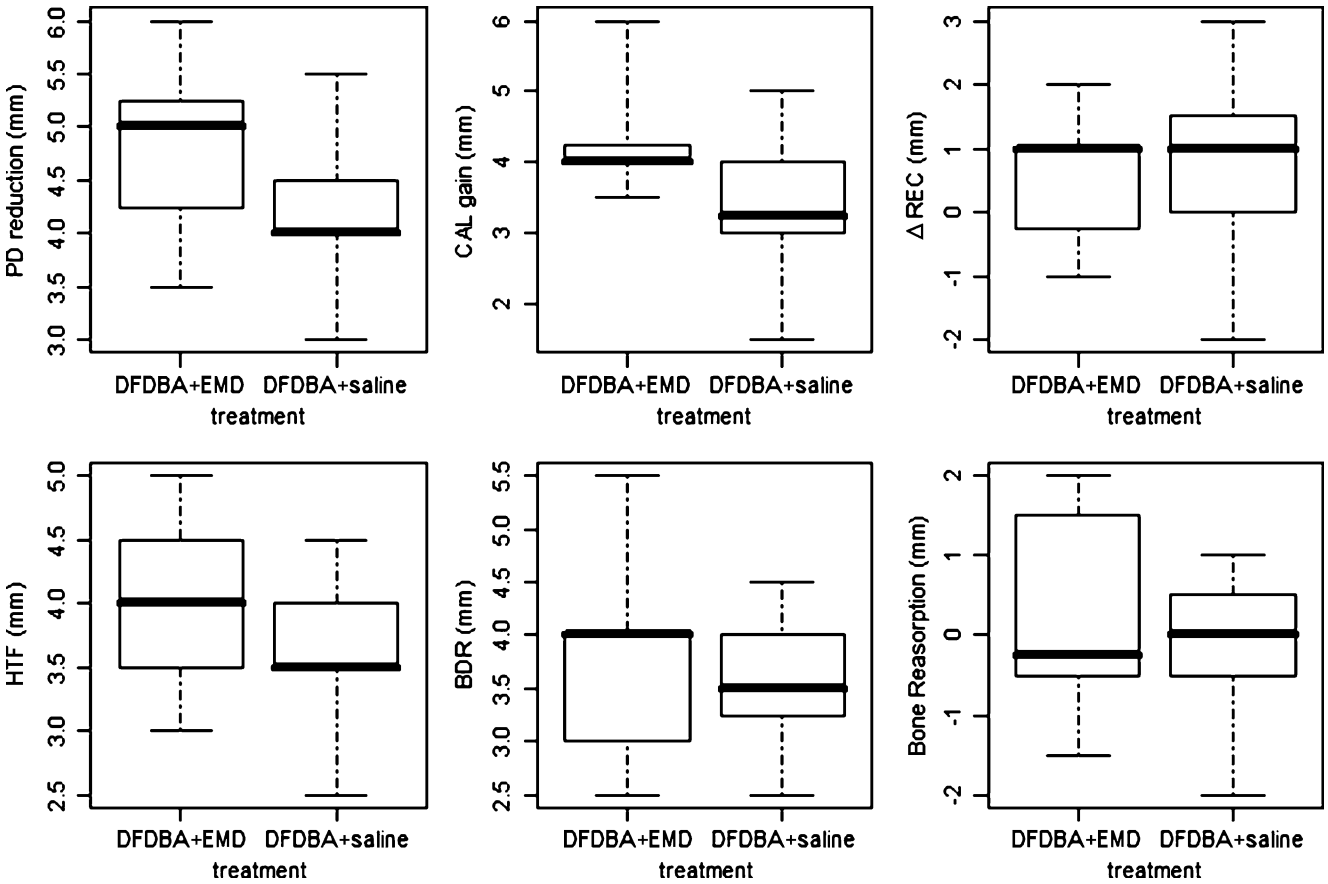

Fig. 1 Box and Whisker plots of clinical and radiographic Δ (baseline to 12 months) values

There was a significant decrease in PD at 12 months compared to baseline in both study groups. The reduction in PD was 5.0 mm with an IQR equal to 0.75 for DFDBA + EMD-treated sites and 4.0 mm with an IQR equal to 0.5 for areas treated with DFDBA alone (Fig. 1). There was a significantly greater reduction in PD in the DFDBA + EMD treated sites compared to the DFDBA alone treated areas $P=0.002$).

However, the CAL gain in DFDBA + EMD-treated areas compared to the DFDBA alone-treated ones was significantly greater (4.0 mm with IQR of 0.125 vs. 3.25 mm with IQR of 1.0, respectively; $P<0.001$; Fig. 1).

In both study groups, the median REC increased significantly at 12 months compared to baseline, but there were no significant differences in the ΔREC of the two groups (1.0 mm with IQR of 1.125 vs. 1.0 mm with IQR of 1.5; $P>0.05$; Fig. 1).

At 12 months post-surgery, the radiographic CEJ-BD and AC-BD decreased in both DFDBA + EMD and DFDBA + saline groups (Table 2). Table 3 illustrates the frequency distribution of CAL gain for the two treatment groups. In the DFDBA + EMD test group, 25% of site gained >4 mm of CAL.

Furthermore, there was a significant difference in HTF achieved at 12 months in the DFDBA + EMD compared to the DFDBA alone study groups (4.0 mm with IQR of 1.0 vs. 3.5 mm with IQR of 0.5, respectively; $P=0.0092$), whereas no significant differences were found in BDR (4.0 mm with IQR of 1.0 vs. 3.5 mm with IQR of 0.625, respectively; $P=0.583$; Fig. 1). The crestal bone resorption was similar in both groups (−0.25 mm with IQR of 2.0 vs. 0.0 mm with IQR of 1.0; $P=0.425$; Fig. 1).

Table 3 Frequency distribution of CAL gain in control and test groups ($n=28$ for each group)

CAL gain (mm)	Frequency			
	Test		Control	
	n	Percentage	*n*	Percentage
≤2	0	0	2	7.1
3	0	0	12	42.9
4	21	75	12	42.9
>4	7	25	2	7.1

Discussion

The use of DFDBA in combination with a biological mediator for treatment of periodontal intrabony defects could produce a synergistic interaction [15]. However, it is unclear whether the addition of EMD to a human bone allografts imparts any additional benefits beyond those of graft alone. Subject age, gender, and teeth with treated osseous defects were similar in both groups at baseline. Each subject demonstrated excellent oral hygiene and a generally healthy condition throughout this study.

This investigation showed that both treatment modalities significantly improved clinical and radiographic parameters between baseline and 12 months. However, the DFDBA + EMD group compared to the DFDBA alone group showed statistically significant differences in PD reduction (5.0 vs. 4.0 mm), CAL gain (4.0 vs. 3.25 mm) and HTF (4.0 vs. 3.5 mm). The clinical superiority of the test compared to the control treatment can also be confirmed by the frequency distribution data of CALgain supporting an additional significant benefit in terms of periodontal regeneration.

The observed CAL gain and HTF in our control group compares well with other studies treating human intrabony defects with DFDBA alone [35–38]. The results of our test group also compare similarly to other studies evaluating the use of DFDBA in combination with EMD for the treatment of periodontal intrabony defects [25, 28].

The statistically significant differences in PD reduction, CAL gain, and HTF observed in the DFDBA + EMD-treatment group respect to DFDBA treatment group in the present study could be explained by the additional biologic effects of EMD on periodontal tissues supporting/enhancing wound healing and new periodontal tissue formation [39].

Combining osseous grafting (DFDBA) with EMD has a potentially synergistic effect since the former may act osteoinductively and/or osteoconductively in conjunction with a defect space maintenance for the newly forming tissue promoted specifically by the EMD in terms of new cementum and new attachment apparatus formation.

Recently, Hoidal et al. [29] comparing EMD with DFDBA versus DFDBA alone in the treatment of periodontal osseous defects 6 months post surgery, found no statistically significant differences in PD reduction (2.56 vs. 2.45 mm), CAL gain (1.47 vs. 1.63 mm), and HTF (1.91 vs. 2.33 mm).

The differences observed in the present study compared to the one by Hoidal et al. [29] could be explained by the different power of statistics analysis, methods, and follow-up.

Another combination of periodontal therapy using EMD with bone-derived xenograft (BDX) for treating intrabony defects resulted in significantly better clinical and radiographic periodontal parameters than the ones achived with EMD alone[40–42] and this improvement was attributed, at least in part, to the space-maintenance properties of bone graft.

Conversely, Scheyer et al. [43] evaluating the adjunctive use of EMD to BDX, found no statistically significant differences for any of the measured soft and hard tissue parameters. EMD possesses an osteo-promotive effect on bone and medullary regeneration during wound healing of injured long rat bones [44, 45], and its application to a rat skull defect accelerated new bone formation [46]. However, other in vivo studies found that EMD failed to exhibit extraskeletal, bone-inductive properties in the muscle of rats [47] and it was ineffective in regeneration of rat calvaria critical-size bone defects [48].

Hence, futher studies are needed to clarify if the combination of bone grafts and EMD for treatment of periodontal intrabony defects is useful compared to the use of bone grafts alone.

Conclusion

In this study, both treatment groups showed a good soft and hard periodontal tissue response. However, at 12 months after surgery, the combined use of EMD and DFDBA for treating periodontal intrabony defects seemed to result in a statistically significant PD reduction, CAL gain, and HTF compared to DFDBA alone. Further studies are required to confirm these results in longitudinal investigations.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. American Academy of Periodontology (2001) Glossary of periodontal terms, 4th edn. American Academy of Periodontology, Chicago, p 44
2. American Academy of Periodontology (2005) Periodontal regeneration (position paper). J Periodontol 76:1601–1622
3. Stahl SS, Froum S, Kushner L (1983) Healing responses of human intraosseous lesions following use of debridement, grafting and citric acid root treatment. II. Clinical and histologic observations: one year postsurgery. J Periodontol 54:325–338
4. Dragoo MR, Sullivan HC (1973) A clinical and histologic evaluation of autologous bone grafts in humans. II. External root resorption. J Periodontol 44:614–625
5. Hiatt W, Schallhorn R, Aaronian A (1978) The induction of new bone and cementum formation. IV. Microscopic examination of the periodontium following human bone and marrow allograft, autograft and non-graft periodontal regenerative procedures. J Periodontol 49:495–512
6. Shirmohammadi A, Chitsazi MT, Lafzi A (2009) A clinical comparison of autogenous bone graft with and without autoge-

nous periodontal ligament graft in the treatment of periodontal intrabony defects. Clin Oral Investig 13:279–286
7. Rummelhart JM, Mellonig JT, Gray JL, Towle HJ (1989) A comparison of freeze-dried bone allograft and demineralized freeze-dried bone allograft in human periodontal osseous defects. J Periodontol 60:655–663
8. Stavropoulos A, Karring (2005) Five-year results of guided tissue regeneration in combination with deproteinized bovine bone (Bio-Oss) in the treatment of intrabony periodontal defects: a case series report. Clin Oral Invest 9:271–277
9. Hanna R, Trejo PM, Weltman RL (2004) Treatment of intrabony defects with bovine-derived xenograft alone and in combination with platelet-rich plasma: a randomized clinical trial. J Periodontol 75:1668–1677
10. Windisch P, Szendroi-Kiss D, Horváth A, Suba Z, Gera I, Sculean A (2008) Reconstructive periodontal therapy with simultaneous ridge augmentation. A clinical and histological case series report. Clin Oral Investig 12:257–264
11. Sculean A, Stavropoulos A, Windisch P, Karring T, Gera I (2004) Healing of human intrabony defects following regenerative periodontal therapy with a bovine-derived xenograft and guided tissue regeneration. Clin Oral Investig 8:70–74
12. Heinz B, Kasaj A, Teich M, Jepsen S (2009) Clinical effects of nanocrystalline hydroxyapatite paste in the treatment of intrabony periodontal defects: a randomized controlled clinical study. Clin Oral Investig. doi:10.1007/s00784-009-0325-x
13. Stratul SI, Schwarz F, Becker J, Willershausen B, Sculean A (2006) Healing of intrabony defects following treatment with an oily calcium hydroxide suspension (osteoinductal). A controlled clinical study. Clin Oral Invest 10:55–60
14. De Leonardis D, Garg AK, Pedrazzoli V, Pecora GE (1999) Clinical evaluation of the treatment of class ii furcation involvements with bioabsorbable barriers alone or associated with demineralized freeze-dried bone allografts. J Periodontol 70:8–12
15. Piemontese M, Aspriello SD, Rubini C, Ferrante L, Procaccini M (2008) Treatment of periodontal intrabony defects with demineralized freeze-dried bone allograft in combination with platelet-rich plasma: a comparative clinical trial. J Periodontol 79:802–810
16. Ilgenli T, Dündar N, Kal BI (2007) Demineralized freeze-dried bone allograft and platelet-rich plasma vs. platelet-rich plasma alone in infrabony defects: a clinical and radiographic evaluation. Clin Oral Investig 11:51–59
17. Venezia E, Goldstein M, Boyan BD, Schwartz Z (2004) The use of enamel matrix derivative in the treatment of periodontal defects: a literature review and meta-analysis. Crit Rev Oral Biol Med 15:382–402
18. Döri F, Arweiler N, Gera I, Sculean A (2005) Clinical evaluation of an enamel matrix protein derivative combined with either a natural bone mineral or beta-tricalcium phosphate. J Periodontol 76:2236–2243
19. Chitsazi MT, Mostofi Zadeh Farahani R, Pourabbas M, Bahaeddin N (2007) Efficacy of open flap debridement with and without enamel matrix derivatives in the treatment of mandibular degree II furcation involvement. Clin Oral Investig 11:385–389
20. Sculean A, Donos N, Brecx M, Karring T, Reich E (2000) Healing of fenestration-type defects following treatment with guided tissue regeneration or enamel matrix proteins. An experimental study in monkeys. Clin Oral Investig 4:50–56
21. Sculean A, Windisch P, Keglevich T, Fabi B, Lundgren E, Lyngstadaas PS (2002) Presence of an enamel matrix protein derivative on human teeth following periodontal surgery. Clin Oral Investig 6:183–187, Epub 2002 Aug 13
22. Sculean A, Windisch P, Keglevich T et al (2003) Clinical and histological evaluation of human intrabony defects treated with enamel matrix protein derivative combined with a bovine-derived xenograft. Int J Periodontics Restor Dent 23:47–55
23. Boyan BD, Weesner TC, Lohmann CH et al (2000) Porcine fetal enamel matrix derivative enhances bone formation induced by demineralised freeze dried bone allograft in vivo. J Periodontol 71:1278–1286
24. Rosen PS, Reynolds MA (2002) A retrospective case series comparing the use of demineralized freeze-dried bone allograft and freeze-dried bone allograft combined with enamel matrix derivative for the treatment of advanced osseous lesions. J Periodontol 73:942–949
25. Gurinsky BS, Mills MP, Mellonig JT (2004) Clinical evaluation of demineralized freeze-dried bone allograft and enamel matrix derivative versus enamel matrix derivative alone for the treatment of periodontal osseous defects in humans. J Periodontol 75:1309–1318
26. Harris RJ, Harris LE, Harris CR, Harris AJ (2007) Clinical evaluation of a combined regenerative technique with enamel matrix derivative, bone grafts, and guided tissue regeneration. Int J Periodontics Restorative Dent 27:171–179
27. Sugai K, Sato S, Suzuki K, Ito K (2008) Intentional reimplantation of a tooth with severe periodontal involvement using enamel matrix derivative in combination with guided tissue regeneration and bone grafting: a case report. Int J Periodontics Restorative Dent 28:89–94
28. Harrel SK, TGJr W, Nunn ME (2005) Prospective assessment of the use of enamel matrix proteins with minimally invasive surgery. J Periodontol 76:380–384
29. Hoidal MJ, Grimard BA, Mills MP, Schoolfield JD, Mellonig JT, Mealey BL (2008) Clinical evaluation of demineralised freeze-dried bone allograft with and without enamel matrix derivative for the treatment of periodontal osseous defects in humans. J Periodontol 79:2273–2280
30. Armitage GC (1999) Development of a classification system for periodontal diseases and conditions. Ann Periodontol 4:1–6
31. O'Leary TJ, Drake RB, Naylor JE (1972) The plaque control record. J Periodontol 43:38
32. Gamal AY, Mailhot JM (2003) The effects of EDTA gel conditioning exposure time on periodontitis-affected human root surfaces: surface topography and PDL cell adhesion. J Int Acad Periodontol 5:11–22
33. Putzer P, Hoy L, Günay H (2008) Highly concentrated EDTA gel improves cleaning efficiency of root canal preparation in vitro. Clin Oral Investig 12:319–324
34. Sauro S, Mannocci F, Toledano M, Osorio R, Pashley DH, Watson TF (2009) EDTA or H3PO4/NaOCl dentine treatments may increase hybrid layers' resistance to degradation: a microtensile bond strength and confocal-micropermeability study. J Dent 37:279–288, Epub 2009 Jan 19
35. Bender SA, Rogalski JB, Mills MP, Arnold RM, Cochran DL, Mellonig JT (2005) Evaluation of demineralized bone matrix paste and putty in periodontal intraosseous defects. J Periodontol 76:768–777
36. Parashis A, Andronikaki-Faldami TK (2004) Clinical and radiographic comparison of three regenerative procedures in the treatment of intrabony defects. Int J Periodontics Restorative Dent 24:81–90
37. Quintero G, Mellonig JT, Qambill VM, Pelleu QB (1982) A six-month clinical evaluation of decalcified freeze-dried bone allografts in periodontal osseous defects. J Periodontol 53:726–730
38. Mellonig JT (1984) Decalcified freeze-dried bone allograft as an implant material in human periodontal defects. Int J Periodontics Restorative Dent 4:40–55
39. Bosshardt DD (2008) Biological mediators and periodontal regeneration: a review of enamel matrix proteins at the cellular and molecular levels. J Clin Periodontol 35:87–105
40. Lekovic V, Camargo PM, Weinlaender M, Nedic M, Aleksic Z, Kennedy EB (2000) A comparison between enamel matrix

proteins used alone or in combination with bovine porous bone mineral in the treatment of intrabony periodontal defects in humans. J Periodontol 71:1110–1116
41. Zucchelli G, Amore C, Montebugnoli L, De Sanctis M (2003) Enamel matrix proteins and bovine porous bone mineral in the treatment of intrabony defects: a comparative controlled clinical trial. J Periodontol 74:1725–1735
42. Velasquez-Plata D, Scheyer ET, Mellonig JT (2002) Clinical comparison of an enamel matrix derivative used alone or in combination with a bovine-derived xenograft for the treatment of periodontal osseous defects in humans. J Periodontol 73:433–440, Erratum in: J Periodontol 73:684
43. Scheyer ET, Velasquez-Plata D, Brunsvold MA, Lasho DJ, Mellonig JT (2002) A clinical comparison of a bovine-derived xenograft used alone and in combination with enamel matrix derivative for the treatment of periodontal osseous defects in humans. J Periodontol 73:423–432
44. Schwartz Z, Carnes DL Jr, Pulliam R et al (2000) Porcine fetal enamel matrix derivative stimulates proliferation but not differentiation of pre-osteoblastic 2T9 cells, inhibits proliferation and stimulates differentiation of osteoblast-like MG63 cells, and increases proliferation and differentiation of normal human osteoblast NHOst cells. J Periodontol 71:1287–1296
45. Kawana F, Sawae Y, Sasaki T et al (2001) Porcine enamel matrix derivative enhances trabecular bone regeneration during wound healing of injured rat femur. Anat Rec 264:438–446
46. Yoneda S, Itoh D, Kuroda S et al (2003) The effects of enamel matrix derivative (EMD) on osteoblastic cells in culture and bone regeneration in a rat skull defect. J Periodontal Res 38: 333–342
47. Donos N, Kostopoulos L, Tonetti M, Karring T, Lang NP (2006) The effect of enamel matrix proteins and deproteinised bovine bone mineral on heterotopic bone formation. Clin Oral Implants Res 17:434–438
48. Intini G, Andreana S, Buhite RJ, Bobek LA (2008) A comparative analysis of bone formation induced by human demineralised freeze-dried bone allograft and enamel matrix derivative in rat calvaria critical-size defects. J Periodontol 79:1217–1224