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ORIGINAL ARTICLE

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Enamel matrix derivative alone or in combination with a bioactive glass in wide intrabony defects

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Abstract This controlled clinical study investigated the clinical and radiographic outcome of wide intrabony periodontal defects treated by enamel matrix derivatives alone or in combination with a bioactive glass over a period of 8 months. Twenty-three chronic periodontitis patients, who received initial therapy and had radiographical interproximal defects with an associated probing depth of 6 mm or more and an intrabony component of at least 4 mm, were included. Each of the patients, contributing at least one intrabony defect, was treated with either enamel matrix derivative alone (group 1, n=10) or the combination (group 2, n=13). In both groups, all clinical and radiographical parameters were improved. Groups 1 and 2 presented a mean pocket reduction of 5.03±0.89 and 5.73±0.80 mm, recession of 0.97±0.24 and 0.56±0.18 mm, relative attachment gain of 4.06±1.06 and 5.17±0.85 mm, and radiographic bone gain of 2.15±0.42 and 2.76±0.69 mm, respectively. An intergroup comparison revealed significant differences for all of the parameters, yielding a more favorable outcome towards the combined approach. Within the limits of the study, both treatments resulted in marked clinical and radiographical improvements, but combined treatment seemed to enhance the results in the treatment of wide intrabony defects.

Keywords Intrabony defects · Periodontal regeneration · Enamel matrix protein derivative · Bone grafts · Bioactive glass

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Introduction

One of the major objectives of periodontal therapy is the morphological and functional regeneration of tooth-supporting tissues, which has been destroyed due to periodontal disease. The biologic principles of periodontal regeneration have been established in the scientific literature for the past 20 years. The magnitude of the evidence firmly establishes the concept of regeneration as an efficacious and valid treatment goal. With various new technologies, biological approaches, and biomaterials, the challenge is now to add to this complex environment and to distill the experience and knowledge contributing to patient outcomes in terms of function, ease of care, esthetics, and long-term maintenance.

Several treatment procedures including bone grafts, guided tissue regeneration, combined approaches, and growth factors have been suggested for regenerative periodontal therapy. To mimic the events that take place during the development of dental root has been recently used as an alternative approach to obtain periodontal regeneration. The discovery of the presence of the enamel matrix layer between the peripheral dentin and the developing periodontal tissues has provided the fundamental concept for enamel matrix protein derivative (EMD)-supported tissue engineering in regenerative periodontal therapy. It has been shown that EMD is a promising and useful tool for periodontal regeneration and the application of EMD on the diseased root surfaces enhances the formation of a new connective tissue attachment and of new alveolar bone [2, 5, 12–15, 20, 30, 31, 48]. Prospective controlled clinical trials have demonstrated that the gains in clinical attachment and bone levels are significantly greater with the use of EMD than open flap surgery alone [8, 15, 24]. Comparable clinical outcomes to bioabsorbable and non-resorbable barriers used for guided tissue regeneration have also been shown [29, 32, 41]. However, it has been suggested that there exist a possible limitation to the regenerative capability of EMD, related to its semi-fluid consistency and lack of space-making effect [20]. Therefore, combining EMD with a

graft material may overcome the problem of flap collapse and space maintenance when using it alone. Thus, more recently, prominence has been given to the use of EMD in combination with graft materials. The positive effects of bone grafts and bone substitutes on the outcome of periodontal regenerative procedures are well documented. Autografts, allografts, xenografts, and synthetic materials have been shown to improve attachment levels and promote osseous defect fill in humans [9]. While materials intended to promote bone formation play an important role in periodontal regeneration, their combination with agents like EMD capable of enhancing cell-mediated phenomena in periodontal wound healing with formation of a functional periodontal ligament and new cementum has the potential to optimize the outcome of periodontal regeneration. To the best of our knowledge, a number of case reports or investigations evaluating the use of EMD combined with various graft materials in the treatment of periodontal osseous defects are available in the literature [1, 4, 18, 26, 27, 32, 36–38, 43, 50]. Recent data from two studies have indicated that the combination of EMD and a cancellous bovine-derived xenograft (BDX) may lead to higher bone fill and less gingival recession compared with treatment with EMD alone [18, 43]. In a controlled clinical study, a combination of EMD + bioactive glass (BG), composed of elements naturally occurring in bone, is also suggested for preventing the collapse of the mucoperiosteal flap, thus minimizing soft tissue recession [34]. Another controlled clinical study comparing EMD alone to BDX + GTR has indicated that both techniques may lead to significant improvements in clinical and radiographic parameters without any significant differences between the treatments [23]. In a very recent study comparing the treatment of wide intrabony defects with a combination of EMD + BG to EMD alone, the clinical results have failed to demonstrate the superiority of the combined approach [37].

In this process, trials evaluating the efficacy of different graft materials and combinations in the treatment of intrabony lesions can add valuable information for the clinician in decision-making regarding effective treatment alternatives for various types of periodontal destruction.

The aim in this study was, therefore, to evaluate the clinical and radiographic outcomes of treatments performed by using EMD alone or combined with BG in the treatment of wide intrabony periodontal defects.

Materials and methods

The present clinical study was a controlled trial with a parallel design. Each patient first received non-surgical periodontal therapy consisting of oral hygiene instructions, full-mouth supra-/sub-gingival scaling and root planing under local anesthesia, and occlusal adjustment. Two months after the initial periodontal therapy, the patients underwent a re-evaluation examination. Cases with one-, two-, one- to two-wall intrabony periodontal defects with a probing depth (PD) ≥6, radiographic angle about 45°, and intrabony defect depth ≥4 mm confirmed during surgery were included in chronic periodontitis patients (23 patients with the mean age of 44.7 years). Patients with keratinized gingiva ≤2 mm or with exposed root surfaces were not included. Osseous defects with intrabony component of <4 mm were excluded at the time of surgical exposure of the experimental area. Patients who met the inclusion criteria were systemically healthy and there were no contraindications for periodontal therapy. After an explanation of all aspects of the study as well as alternative treatment regimens, the patients were required to sign an informed consent. The study design and consent were approved by the University Institutional Review Board.

Study groups

Patients were randomly divided into two groups according to the type of the treatment by flip of a coin. In patients contributing more than one defect, all defects were assigned to the same type of treatment modality.

The study groups were designated as follows:

Group 1
: Ten patients treated with EMD alone (Emdogain Gel, BIORA, Malmö, Sweden)

Group 2
: Thirteen patients treated with EMD + BG (Perioglas) (Emdogain Gel TS, BIORA, Malmö, Sweden)

Surgical procedure

The experimental areas selected for surgery were anesthetized. After intracrevicular incisions, aiming to preserve the interdental papilla as much as possible, full-thickness mucoperiosteal buccal and lingual access flaps were reflected. Vertical releasing incisions were performed only if necessary for a better access or to achieve a better closure of the surgical site. All granulation tissues were removed from the defects and the root surfaces were gently scaled and planed using ultrasonic and hand instruments. After defect debridement, the following measurements were made: distance from the edge of the individual occlusal stent to the bottom of the defect (A) and distance from the edge of the stent to the most coronal extension of the alveolar bone crest (B). The intrabony component of the defects was defined as A–B. The horizontal width of the defect at the level of the most coronal extension of the alveolar crest was also evaluated to confirm the wide intrabony defect angle (Fig. 1c). The osseous defects were classified by the number of osseous walls and recorded along with the other surgical notes. The root surfaces adjacent to the defects were then conditioned for 2 min with ethylenediaminetetraacetic acid gel (pH 6.7) (PrefGel, BIORA, Malmö, Sweden). After acid application, the defects and the adjacent mucoperiosteal flaps were then thoroughly rinsed with sterile saline. In all defects, the EMD gel was first applied on the root surfaces and then into the defects. The defects treated with the combined approach were additionally filled up with the mixture of EMD + BG. The

flaps were then replaced and sutured appropriately by interdental sutures. The sutures were free of tension, obtaining a complete coverage of the intrabony defects. A deep horizontal mattress suture was additionally used only if there was any residual tension from the flap margins. After a healing period of 2 weeks, the sutures were removed.

Postoperative care

Postoperative care was directed at the maintenance of wound stability and infection control. The patients received systemic antibiotic therapy for a period of 2 weeks postoperative. The regimen consisted of oral administration of 200 mg of doxycycline on the first day and thereafter at 100 mg daily. In addition, the patients were advised to avoid hard chewing in the surgical areas and to rinse twice daily with a 0.2% solution of chlorhexidine digluconate for 6 weeks. After at least 4 weeks [35], gentle toothbrushing was resumed in the operated areas. Recall appointments were scheduled every second week during the first 2 months after the surgical procedure and the patients were recalled once a month for the remaining observation period. During the 8-month follow-up period, neither sub-gingival instrumentation nor probing of the operated areas was performed.

Clinical assessments

For all patients, the following clinical parameters were recorded preoperatively and at 8 months [15, 47, 49] postoperatively by one trained examiner who was blinded to the treatment assignments. A calibration exercise was carried out to obtain acceptable intra-examiner reproducibility as previously described by Sculean et al. [37].

Plaque index (PI) was measured according to Silness and Löe [39], and sulcular bleeding index (SBI) according to Mühlemann and Son [21]. PD, relative attachment level (RAL), and marginal recession (REC) were measured to the nearest millimeter with a calibrated periodontal probe (PCP 15 UNC, Hu-Friedy, Chicago, IL, USA) using individual occlusal stents as a reference point for probe placement. Occlusal stents for positioning measuring probes were fabricated with cold-cured acrylic resin on a cast model obtained from an alginate impression. It was made to cover the occlusal surface of the tooth being treated and the occlusal surfaces of at least one tooth in the mesial and distal directions. It was also extended apically on the buccal and lingual surfaces to cover the coronal third of the teeth. Grooves were placed so that the postoperative measurements could be at the same position and angulations were as those made before surgery. PD was the distance between the free gingival margin and the probeable bottom of the pocket, RAL was the distance between the probeable bottom of the pocket and the edge of the stent, and REC was the distance between the free gingival margin and the apical edge of the stent. The probe was forced through the soft tissue toward the bone until definite resistance was met. Pl was evaluated at four periodontal sites whereas other measurements were made at six aspects of the selected teeth: mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual. Six grooves were accordingly placed on the stent to standardize the probe positions. Measurements where edge of the stents has taken as the reference points were relative values (RAL and REC) to evaluate the attachment loss/gain and marginal soft tissue level change.

Radiographic examination

Preoperative and 8-month postoperative intraoral standardized radiographs (Kodak Ultra Speed, Readymatic, X-omet, Paris Cedex, France) were taken by the paralleling technique using an individual film holder device consisting

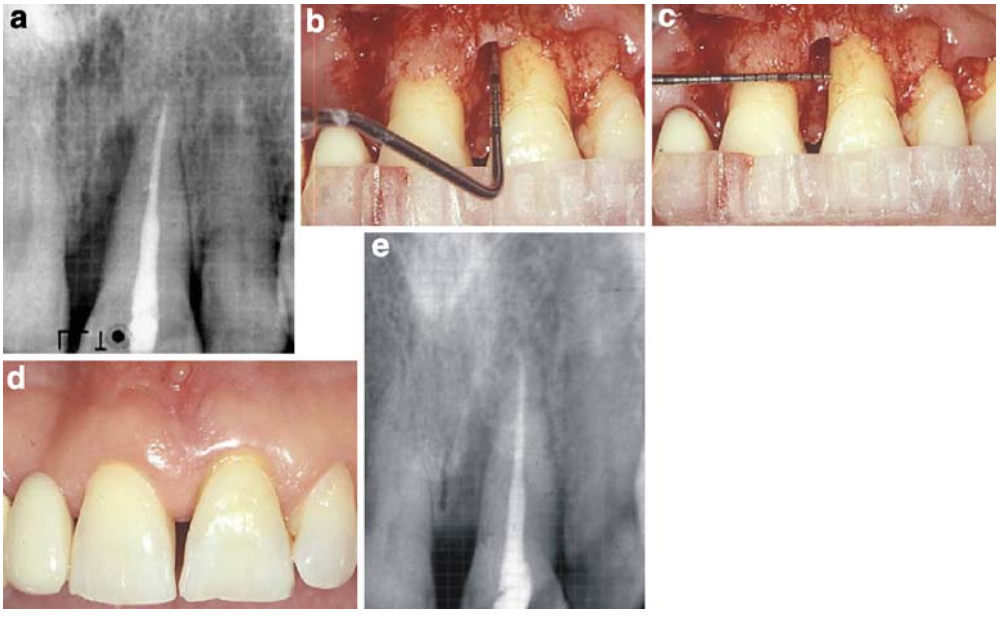

Fig. 1 **a** A preoperative radiograph revealing the presence of an intrabony defect. **b** Intrabony component of the defect. **c** The horizontal width of the defect. **d** The clinical appearance of the defect filled with the combination of enamel matrix derivative and bioactive glass at 8 months. **e** Radiographic appearance at 8 months postoperatively

of a bite block for the evaluation of radiographic bone level (RBL) (roentgenographic system RWT; indicator arm-anterior/57,5026, bite block-anterior/61,5018, Kentzler-Kaschner Dental GmbH, Jagst, Germany) rigidly connected to an acrylic dental splint to achieve identical film placement at each evaluation [47]. The film holder was rigidly coupled to the X-ray tube via an adapter (Aiming Ring-anterior/59, 5123). Pre- and postoperative radiograph pairs were independently assessed on a light box by three experienced clinicians who were not told which radiograph was which. The mode (most frequent) count was accepted [3]. When measuring RBL, the three investigators were blind with respect to the clinical measurements and had to reach agreement in terms of the location of both anatomical and bone loss landmarks. Radiographic measurements were obtained as described elsewhere [17], utilizing an adhesive millimeter grid (X-ray Grid 34 cm, Meyer Haake GmbH, Oberursel, Germany). The differences between pre- and postoperative RBL measurements were considered as the radiographic bone loss/gain.

Statistical analysis

Each parameter was expressed as the mean value±standard deviation. The average results of defects treated in each patient were taken into consideration and the findings were analyzed considering the patient as the unit of evaluation. Statistical analysis was performed, using a commercially available software program (SPSS for Windows, version 13, 2005). The baseline values to assess the homogeneity of the groups (excluding the relative values) and the differences between the groups before and after treatments were compared using the Mann–Whitney *U* test. For the statistical evaluation of the changes from baseline to 8 months within the groups, Wilcoxon matched pairs signed-ranks test was used. In the calculations, the deepest site per defect measurements was included. The value of $p<0.05$ was considered as the level of significance.

The change in RAL was one of the primary outcome variables. A size estimation was carried out based on a previous study by Heijl et al. [15]. Power calculation has demonstrated that a much higher number of patients would have been needed for a power of 0.95; however, after 8 months, the difference in change of RAS was already 1.12 mm (α_1=1.06 mm, α_2=0.85), which resulted in a power of 0.80 and a required sample size of n=10 patients in each group.

Results

All 23 patients returned for clinical and radiographic evaluation at 8 months (Figs. 1a–e and 2a–c, two representative cases for each group). A clinical evaluation of post-surgical healing revealed a good soft tissue response to EMD with no adverse complications.

The baseline defect characteristics and distribution are presented in Table 1. Both groups presented similar baseline conditions in terms of oral hygiene levels, bleeding scores, probing, and intrabony component depths. The full-mouth PI and SBI scores for each of the two groups, at baseline and after 8 months, are summarized in Table 2. Although PI and SBI values were low before the initiation of surgical therapy, both PI and SBI improved significantly compared to baseline values.

Recession as negative changes in REC values before and after treatments was observed in both groups and found to be statistically significant (Table 3). The average amount of recession was 0.56±0.18 mm ($p<0.001$) in the EMD + BG group while it was 0.97±0.24 mm ($p<0.01$) for the EMD gel group. Intergroup differences were found to be significant ($p<0.001$) (Table 3). Greater recession occurred with EMD gel application.

At 8 months, the EMD + BG group showed a reduction of 5.73±0.80 mm in PD ($p<0.001$) and a change of 5.17±0.85 mm ($p<0.001$) in RAL (Table 3). In the EMD gel group, the mean reduction of PD was 5.03±0.89 mm ($p<0.01$) while the mean RAL change was 4.06±1.06 mm ($p<0.01$) (Table 3). The intergroup comparison revealed a statistically significant difference both for PD ($p<0.05$) and RAL measurements ($p<0.05$) (Table 3). Greater PD reduction and attachment gain occurred with EMD + BG application.

An evaluation of the hard tissue findings indicated that both treatment modalities result in bone gain at 8 months. The EMD + BG group showed 2.76±0.69 mm ($p<0.001$) of RBL change considered as the radiographic bone gain, while this was 2.15±0.42 mm ($p<0.01$) for the EMD group (Table 3). The difference between the groups was in favor of the combined group ($p<0.05$) (Table 3).

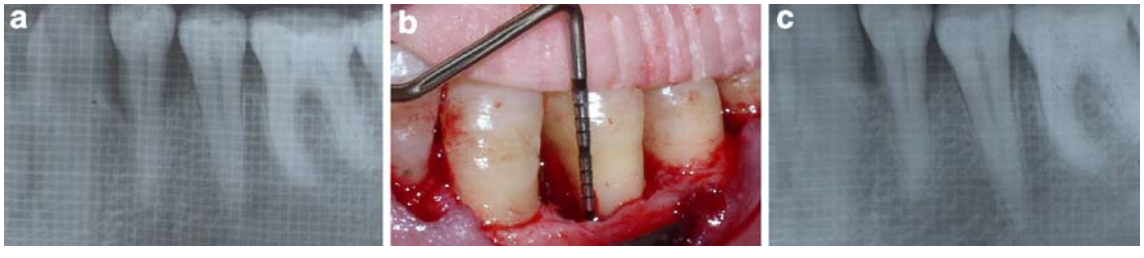

Fig. 2 **a** A preoperative radiographic appearance. **b** Intrabony component of the defect. **c** At 8 months after treatment with enamel matrix derivative

Table 1 Baseline defect characteristics and distribution

Variable	Group 1 (10 patients)	Group 2 (13 patients)
Total defect number	20	20
1-walled defect number	4	5
2-walled defect number	9	6
1- to 2-walled defect number	7	9
Patient number with 1 defect	1	6
Patient number with 2 defects	8	7
Patient number with 3 defects	1	–
Intrabony component (mm)	5.68±0.59	5.48±0.62
Probing depth (mm)	9.47±0.81	9.77±1.01
RBL (mm)	6.38±0.62	6.24±0.78

Discussion

This study was undertaken to evaluate the clinical and radiographic effectiveness of EMD alone or combined with a BG in the treatment of wide intrabony periodontal defects. Both groups presented similar baseline conditions. There was similar distribution in anatomical location of the defects (tooth type). Defect configurations (one, two, one- to two-wall), probing depths as well as the depth of the intrabony component were comparable among the groups. The number of patients contributing only one defect was one patient in group 1 vs six patients in group 2. However, the group distribution of two or three defects is comparable and the measurements of defects treated in each patient were averaged as they received the same treatment. The findings were analyzed considering the patient as the unit of evaluation.

The clinical outcomes of any regenerative procedure are positively correlated with the morphology of the osseous defect [9]. Based on clinical evidence, predominantly three-wall defects have been associated with greater regenerative potential in conventional or bone grafting procedures [25]. Tonetti et al. [42] have reported that the number of bone walls affects the clinical outcomes of the EMD therapy. Presuming a positive correlation between the number of osseous walls forming the intrabony defect and degree of regeneration, three-wall intrabony defects were deliberately excluded in our study to evaluate the type of bone defects, which are not as predictable as three-wall defects in regenerative treatment.

Table 2 Full-mouth PI and SBI values at baseline and 8 months after treatment

		Group 1	Group 2	*z*
PI	Before treatment	0.29±0.06	0.30±0.05	−0.62, NS
	After treatment	0.18±0.05	0.19±0.05	
	Difference	0.11±0.05[a]*	0.11±0.03[b]**	−0.22, NS
SBI	Before treatment	0.27±0.04	0.30±0.06	−1.56, NS
	After treatment	0.19±0.02	0.17±0.05	
	Difference	0.08±0.03[a]*	0.13±0.05[b]**	−2.59[c]*

NS Not significant
*$p \leq 0.01$; **$p \leq 0.001$
[a]Intragroup difference (group 1)
[b]Intragroup difference (group 2)
[c]Intergroup difference (group 1 vs group 2)

Table 3 Clinical and radiographic outcomes at 8 months

	Group 1	Group 2	*z*
Increase in REC	−0.97±0.24[a]*	−0.56±0.18[b]**	−3.57[c]****
Decrease in PD	5.03±0.89[a]*	5.73±0.80[b]**	−1.98[c]***
Gain in RAL	4.06±1.06[a]*	5.17±0.85[b]**	−2.53[c]***
Gain in RBL	2.15±0.42[a]*	2.76±0.69[b]**	−2.08[c]***

*$p<0.01$; **$p<0.001$**; ***$p \leq 0.05$; ****$p \leq 0.001$
[a]Intragroup difference (group 1)
[b]Intragroup difference (group 2)
[c]Intergroup difference (group 1 vs group 2)

Establishing nontension primary wound closure of various soft tissue flaps is paramount for optimal postsurgical wound healing. Regenerative surgical procedures that require clinical flap manipulation also require excellence in suturing. The primary objective of dental suturing is to position and secure surgical flaps. Surgical sutures should hold flap edges in apposition until the wound has healed enough to withstand normal functional stresses. When the proper suture technique is used, primary intention healing occurs. Accurate apposition of surgical flaps is significant to patient comfort, blood clot stabilization, and prevention of unnecessary bone destruction. If surgical wound edges are not properly approximated and are therefore inadequate, blood and serum may accumulate under the flap, delaying the healing process by separating the flap from the underlying bone [40]. Interrupted suture techniques achieve excellent clinical results when used for wound closure with tension-free flaps. Another suturing technique, which is a variation of the interrupted suture, is the mattress technique. This technique usually is used in areas where tension-free flap closure cannot be accomplished. Mattress suturing techniques generally are used to resist muscle pull, evert the wound edges (this keeps epithelium away from underlying structures), and adapt the tissue flaps tightly to the underlying structures (e.g., bone graft, tissue graft, alveolar ridge, regenerative membrane, or dental implant). When performing a mattress suture, the needle penetration through the surgical flap should be 8 to 10 mm away from the flap edge or just above the mucogingival junction in keratinized tissue. In this study, interrupted sutures were used and flaps were placed back to their original places. The sutures were free of tension, obtaining a complete coverage of the intrabony defects. A deep horizontal mattress suture was additionally used, only if there was any residual tension from the flap margins (2 defects out of 40).

In clinical studies, the control of plaque and gingivitis is important because both vary in association with periodon-

titis and affect measured healing response to therapy [19]. Periodontitis is usually associated with gingivitis, and if one is to test a treatment modality for periodontitis, it becomes necessary to attempt to separate the effects of that therapy on periodontitis from the effects on gingivitis. In this study, plaque accumulation was controlled by home care instructions and chlorhexidine application at 6 weeks postoperatively and gingivitis was minimized by initial periodontal treatment including supra- and sub-gingival scaling and root planing before the start of the experimental period. Frequent recalls (every second week during the first 2 months postoperatively and once a month for the remaining observation period) prevented accumulation of plaque, and all patients maintained a good level of oral hygiene and gingival status throughout the study consistent with that achieved before the initiation of surgical therapy.

Our results showed that both treatment approaches provide significant improvements in soft and hard tissue measurements compared to baseline values as confirmed by previous studies which demonstrated that clinical improvement of presurgical clinical parameters in intrabony defects can be achieved with both EMD alone [8, 13–15, 20, 24, 30, 31, 41, 42] and in combination with graft materials [1, 4, 18, 26–34, 32, 36–38, 43, 50]. EMD has been investigated in recent years as a tool to enhance periodontal tissue regeneration, according to the principles of biomimicry. It has been reported to be safe and efficient for periodontal regeneration [2, 4–6, 11, 12, 14, 20, 31, 48]. Most of the above studies also report histological evidence to the actual regenerative potential of EMD in periodontal tissues. However, it has been suggested recently that, due to its limited space-making potential, the gel form could be mixed with bone replacement grafting materials both to take the advantage of their synergistic effects in healing and to prevent flap collapse by solid graft particles placed in the defect [20]. It has also stated that, when adding EMD to graft materials, the handling properties of both materials were improved and the viscosity of EMD helped in the delivery of graft particles by maintaining them together and application into the defect was easier [43]. When comparing two treatment modalities in this study, significant differences were found for pocket reduction, recession, attachment gain, and radiographic bone gain, yielding a more favorable outcome towards the combined approach of EMD + BG. The result of our study corroborate the findings from two clinical studies in which the effectiveness of EMD used alone was compared to the combination with BDX in the treatment of intrabony lesions, most of which are two, two- to three-, and three-wall defects [18, 43]. In the study by Lekovic et al. [18], postoperative measurements taken at 6 months revealed a significantly greater reduction in PD in the combined group (3.43±1.32 mm on buccal sites and 3.36±1.35 mm on lingual sites) when compared to the EMD group (1.91±1.42 mm on buccal sites and 1.85±1.38 mm on lingual sites). The combined group also presented with significantly more attachment gain (3.13±1.41 mm on buccal sites and 3.11±1.39 mm on lingual sites) than the EMD group (1.72±1.33 mm on buccal sites and 1.75±1.37 mm on lingual sites). A surgical re-entry of the treated defects revealed a significantly greater amount of defect fill in favor of the combined group (3.82±1.43 mm on buccal sites and 3.74±1.38 mm on lingual sites) as compared to the EMD group (1.33±1.17 mm on buccal sites and 1.41±1.19 mm on lingual sites). The results of this study indicated that combined treatment was superior in reducing PD to the maintainable levels, improving attachment levels, and promoting defect fill when compared to presurgical levels. In the study by Velasquez-Plata et al. [43], the most significant results were that recession was greater for the group treated with EMD alone (0.8±0.8 mm) compared to the EMD + BDX (0.3±0.6 mm) and bone fill was greater for EMD + BDX (4.0±0.8 mm) compared to EMD alone (3.1±1.0 mm). Intergroup comparison revealed a significant difference for recession and bone fill, yielding a more favorable outcome towards the combined approach. In a very recent study, Sculean et al. [37] treated deep intrabony defects with EMD alone or in combination with a BG. At 1 year after therapy, the combined group showed a PD reduction of 4.2±1.4 mm and a clinical attachment gain of 3.2±1.7 mm. In the EMD group, the mean PD reduction was 4.5±2.0 whereas the clinical attachment gain was 3.9±1.8. There were no significant differences between the groups. For a direct comparison, this study is the only study evaluating EMD + BG and EMD alone in the treatment of intrabony defects. In our study, the combined group showed a mean reduction of 5.73±0.80 mm in PD and the mean change for RAL as the attachment gain was 5.17±0.85 mm. In the EMD group, the mean PD reduction was 5.03±0.89 mm while the mean RAL change was 4.06±1.06 mm. Based on the aforementioned data, we found the intergroup differences significant (Table 3). However, the data of PD and RAL change for the EMD gel group in our study compare well with this and other similar studies in the literature [24, 28, 33, 41].

Both treatment modalities used in this study also improved RBL values. The combined group showed 2.76 mm of RBL difference between pre- and postoperative values as the radiographic bone gain, while this was 2.15 mm for the EMD gel group. Sculean et al. [37] gave no information about the hard tissue measurements; therefore, it is not possible to compare our results of the EMD + BG group directly with any published data. However, the results of our combined grafting group confirm previous findings of Lekovic et al. [18] and Velasquez-Plata et al. [43] who demonstrated superior improvements achieved with EMD + BDX when compared to EMD alone. In our study, the combination of EMD with a graft material showed superior clinical improvements and bone gain over EMD alone ($p<0.05$). A possible explanation for the superiority may be related to the enhancement of blood clot stabilization [44]. Another possibility which should be kept in mind is that the regenerated tissues in areas treated with grafting materials are denser and resistant to the penetration of the probe. Wound stability and defect space maintenance are desirable qualities for periodontal regeneration. In addition, the properties of the graft material should also be taken into consideration. The

treatment of intrabony defects with various grafting materials has provided a baseline for what can be achieved in reference to regenerative efforts to create bone fill. BG has the property to promote adsorbtion and concentration of proteins utilized by osteoblasts to form a mineralized extracellular matrix and, thus, promote osteogenesis by allowing rapid formation of bone. There are references in the literature stating the positive properties and effects of BG as allowing the formation of bone–tissue bond [45, 46] and retarding the down-growth of epithelial tissue [7]. Recent histological findings from a study on monkeys have shown that treatment of intrabony defects with BG may lead to greater amounts of new connective tissue attachment and alveolar bone than conventional flap surgery alone [16]. However, two recent human histologic studies have shown conflicting results about the regenerative potential of BG in periodontal treatment [22, 38]. According to these two studies, although the clinical results are encouraging and radiographs evidenced radiopacities within the defects, in histological analysis, BG as a periodontal grafting material has only limited regenerative properties [22, 38]. Further studies are needed with the similar methodology and with the use of various graft materials whose properties other than their space-making effects are clinically and histologically proven. With a greater knowledge and understanding of the interaction and biologic effects that EMD and bone grafts can produce, future outcomes in periodontal regeneration may reach high levels of success and predictability. The reasons for the discrepancies between similar trials in the literature remain speculative but may be attributed to various factors including disease/patient/population characteristics, the number/type/depth of the defects, the baseline clinical conditions, the use of relative values, or the evaluation methods as well as the graft material itself.

In our study, recession was seen in both groups but was greater for EMD alone (0.97±0.24) than that of the combined grafting therapy (0.56±0.18) (Table 3). According to the literature, recession was seen between 0.4 and 1.3 mm after surgical application of EMD [8, 24, 27, 31, 33, 41–43, 47]. The recession data from these studies also compare well with our results. Scheyer et al. [27] and Velasquez-Plata et al. [43], on the other hand, reported minimal amounts of gingival recession when combining EMD + BDX. Sculean et al. [32, 37], in their studies, mentioned about the prevention of flap collapse and, thus, minimization of soft tissue recession in their combined groups. The collapse of the flap might have been prevented in the combined approach by the space-maintaining effect attributed to solid graft particles present in the defect as well as its bony walls.

This study evaluated the performance of EMD and BG, used one by itself (EMD) and in combination. Both treatment modalities achieved improvements clinically and radiographically compared to baseline values. A much larger sample size than ours is necessary to definitely show a statistically significant difference between the two therapies [10]; however, when comparing the results of both modalities in this study within its limits, a difference was found for pocket reduction, recession, attachment gain, and radiographic bone gain, yielding a more favorable outcome towards the combined approach.

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References

1. Camargo PM, Lekovic V, Weinlaender M, Vasilic N, Kenney EB, Madzarevic M (2001) The effectiveness of enamel matrix proteins used in combination with bovine porous bone mineral in the treatment of intrabony defects in humans. J Clin Periodontol 28:1016–1022
2. Cattaneo V, Rota C, Silvestri M, Piacentini C, Forlino A, Gallanti A, Rasperini G, Cetta G (2003) Effect of enamel matrix derivative on human periodontal fibroblasts: proliferation, morphology and root surface colonization. An in vitro study. J Periodontal Res 38:568–574
3. Christgau M, Schmalz G, Reich E, Wenzel A (1995) Clinical and radiographical split-mouth-study on resorbable versus non-resorbable GTR-membranes. J Clin Periodontol 22:306–315
4. Cochran DL, Jones A, Heijl L, Mellonig JT, Schoolfield J, King GN (2003) Periodontal regeneration with a combination of enamel matrix proteins and autogenous bone grafting. J Clin Periodontol 74:1269–1281
5. Cochran DL, King GN, Schoolfield J, Velasquez-Plata D, Mellonig JT, Jones A (2003) The effect of enamel matrix proteins on periodontal regeneration as determined by histological analyses. J Clin Periodontol 74:1043–1055
6. Donos N, Sculean A, Glavind L, Reich E, Karring T (2003) Wound healing of degree III furcation involvements following guided tissue regeneration and/or Emdogain. A histologic study. J Clin Periodontol 30:1061–1068
7. Fetner A, Hartigan M, Low S (1994) Periodontal repair using Perioglass in non-human primates: clinical and histological observations. Compendium (Newt. Pa.) 7:932–938
8. Froum SJ, Weinberg MA, Rosenberg E, Tarnow D (2001) A comparative study utilizing open flap debridement with and without enamel matrix derivative in the treatment of periodontal intrabony defects: a 12-month re-entry. J Clin Periodontol 72:25–34
9. Garrett S (1996) Periodontal regeneration around natural teeth. In: Newman MG (ed) Annals of periodontology. The American Academy of Periodontology, Chicago, pp 621–666
10. Gunsolley J, Elswick RK, Davenport JM (1998) Equivalence and superiority testing in regeneration clinical trials. J Clin Periodontol 69:521–527
11. Haase H, Bartold PM (2001) Enamel matrix derivative induces matrix synthesis by cultured human periodontal fibroblast cells. J Clin Periodontol 72:341–348
12. Hammarstrom L (1997) Enamel matrix, cementum development and regeneration. J Clin Periodontol 24:658–668
13. Heden G (2000) A case report study of 72 consecutive Emdogain-treated intrabony defects: clinical and radiographic findings after 1 year. Int J Periodontics Restor Dent 20:127–139
14. Heijl L (1997) Periodontal regeneration with enamel matrix derivative in one human experimental defect. A case report. J Clin Periodontol 24:693–696
15. Heijl L, Heden G, Svardström G, Östgren A (1997) Enamel matrix derivative (Emdogain) in the treatment of intrabony periodontal defects. J Clin Periodontol 24:705–714
16. Karatzas S, Zavras A, Greenspan D, Amar S (1999) Histologic observations of periodontal wound healing after treatment with PerioGlas in nonhuman primates. Int J Periodontics Restor Dent 19:489–499

17. Kılıç AR, Efeoğlu E, Yılmaz S (1997) Guided tissue regeneration in conjunction with hydroxyapatite-collagen grafts for intrabony defects. A clinical and radiological evaluation. J Clin Periodontol 24:372–383
18. Lekovic V, Camargo PM, Weinlaender M, Nedic M, Aleksic Z, Kenney 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 Clin Periodontol 71:1110–1116
19. Lindhe J, Westfelt E, Nyman S, Socransky SS, Heijl L, Bratthall G (1982) Healing following surgical/non-surgical treatment of periodontal disease. A clinical study. J Clin Periodontol 9:115–128
20. Mellonig JT (1999) Enamel matrix derivative for periodontal reconstructive surgery: technique and clinical and histologic case report. Int J Periodontics Restor Dent 19:9–19
21. Mühlemann HR, Son S (1971) Gingival sulcus bleeding: a leading symptom in initial gingivitis. Helv Odontol Acta 15:107–112
22. Nevins ML, Camelo M, Nevins M, King CJ, Oringer RJ, Schenk RK, Fiorellini JP (2000) Human histologic evaluation of bioactive ceramic in the treatment of periodontal osseous defects. Int J Periodontics Restor Dent 20:458–467
23. Pietruska MD (2001) A comparative study on the use of Bio-Oss and enamel matrix derivative (Emdogain) in the treatment of periodontal bone defects. Eur J Oral Sci 109:178–181
24. Pontoriero R, Wennström J, Lindhe J (1999) The use of barrier membranes and enamel matrix proteins in the treatment of angular bone defects. A prospective controlled clinical study. J Clin Periodontol 26:833–840
25. Quintero G, Mellonig JT, Gambill VM, Pelleu GB Jr (1982) A six-month clinical evaluation of decalcified freeze-dried bone allografts in periodontal osseous defects. J Clin Periodontol 53:726–730
26. Rosen P, 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 Clin Periodontol 73:942–949
27. 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. J Clin Periodontol 73:423–432
28. Sculean A, Donos N, Blaes A, Lauermann M, Reich E, Brecx M (1999) Comparison of enamel matrix proteins an bioabsorbable membranes in the treatment of intrabony defects. A split-mouth study. J Clin Periodontol 70:255–262
29. Sculean A, Donos N, Windisch P, Gera I, Brecx M, Reich E, Karring T (1999) Healing of human intrabony defects following treatment with enamel matrix proteins or guided tissue regeneration. J Periodontal Res 34:310–322
30. Sculean A, Reich E, Chiantella CG, Brecx M (1999) Treatment of intrabony periodontal defects with an enamel matrix protein derivative (Emdogain): a report of 32 cases. Int J Periodontics Restor Dent 19:157–163
31. Sculean A, Chiantella GC, Windisch P, Donos N (2000) Clinical and histologic evaluation of treatment of intrabony defects with an enamel matrix protein derivative (Emdogain). Int J Periodontics Restor Dent 20:375–381
32. Sculean A, Donos N, Brecx M, Reich E, Karring T (2000) Treatment of intrabony defects with enamel matrix proteins and guided tissue regeneration. An experimental study in monkeys. J Clin Periodontol 27:466–472
33. Sculean A, Windisch P, Chiantella GC, Donos N, Brecx M, Reich E (2001) Treatment of intrabony defects with enamel matrix proteins and guided tissue regeneration. A prospective controlled clinical study. J Clin Periodontol 28:397–403
34. Sculean A, Barbe G, Chiantella GC, Arweiler NB, Berakdar M, Brecx M (2002) Clinical evaluation of an enamel matrix derivative combined with a bioactive glass for the treatment of intrabony periodontal defects in humans. J Clin Periodontol 73:401–408
35. 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
36. Sculean A, Windisch P, Keglevich T, Chiantella CG, Gera I, Donos N (2003) Clinical and histologic evaluation of human intrabony defects treated with an enamel matrix protein derivative combined with a bovine derived xenograft. Int J Periodontics Restor Dent 23:47–55
37. Sculean A, Pietruska M, Schwartz F, Willershausen B, Arweiler NB, Auschill TM (2005) Healing of human intrabony defects following regenerative periodontal therapy with an enemal matrix protein derivative alone and combinated with a bioactive glass. J Clin Periodontol 32:111–117
38. Sculean A, Windisch P, Keglevich Gera I (2005) Clinical and histologic evaluation of an enamel matrix protein derivative combined with a bioactive glass for the treatment of intrabony periodontal defects in humans. Int J Periodontics Restor Dent 25:139–147
39. Silness J, Löe H (1964) Periodontal disease in pregnancy (II). Correlation between oral hygiene and periodontal conditioning. Acta Odontol Scand 22:121–135
40. Silverstein LH (2005) Suture selection for optimal flap closure and tissue healing. Perio-implant showcase. Pract Periodontics Aesthet Dent 16:2–3
41. Silvestri M, Ricci G, Rasperini G, Sartori S, Cattaneo V (2000) Comparison of treatments of intrabony defects with enamel matrix derivative, guided tissue regeneration with a nonresorbable membrane and Widman modified flap. A pilot study. J Clin Periodontol 27:603–610
42. Tonetti MS, Lang NP, Cortellini P, Suvan JE, Adrian P, Dubravec D, Fonzar A, Fourmousis I, Mayfield L, Rossi R, Silvestri M, Tiedman C, Topoll H, Vangsted T, Walkamm B (2002) Enamel matrix proteins in the regenerative therapy of deep intrabony defects. A multicenter in the regenerative therapy of deep intrabony defects. A multicenter randomized controlled clinical trial. J Clin Periodontol 9:317–325
43. Velasquez-Plata D, Scheyer TE, 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 Clin Periodontol 73:433–440
44. Wikesjö UME, Selvig KA (1999) Periodontal wound healing and regeneration. Periodontol 2000 19:21–39
45. Wilson J, Low (1992) Bioactive ceramics for periodontal treatment: comparative study in the patus monkey. J Appl Biomater 3:123–129
46. Wilson J, Clark AE, Hall M, Hench LL (1993) Tissue response to Bioglass endosseous ridge maintenance implants. J Oral Implantol 19:295–302
47. Yılmaz S, Kuru B, Altuna-Kırac E (2003) Enamel matrix proteins in the treatment of periodontal sites with horizontal type of bone loss. J Clin Periodontol 30:197–206
48. Yukna RA, Mellonig J (2000) Histologic evaluation of periodontal healing in humans following regenerative therapy with enamel matrix derivative. A 10-case series. J Periodontol 71:752–759
49. Zetterström O, Andersson C, Eriksson C (1997) Clinical safety of enamel matrix derivative (EMDOGAIN) in the treatment of periodontal defects. J Clin Periodontol 24:697–704
50. 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