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Healing of two and three wall intrabony periodontal defects following treatment with an enamel matrix derivative combined with autogenous bone

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Abstract

Background: There are still limited data on the outcomes of regenerative periodontal surgery using a combination of an enamel matrix protein derivative (EMD) and autogenous bone (AB).

Aim: To evaluate the healing of deep intrabony defects treated with either a combination EMD+AB or EMD alone.

Materials and Methods: Forty patients with advanced chronic periodontitis, with one deep intrabony defect, were randomly treated with either EMD+AB (test) or EMD (control). Clinical assessments were performed at baseline and at 1 year after treatment. The primary outcome variable was relative attachment level (RAL).

Results: Healing was uneventful in all patients. The test sites showed a reduction in the mean probing pocket depth (PPD) of 5.6 ± 0.9 mm ($p < 0.001$), a gain in the mean RAL of 4.2 ± 1.1 mm ($p < 0.001$) and a gain in the mean probing bone level (PBL) of 3.9 ± 1.0 mm ($p < 0.001$). The control group displayed a mean PPD reduction of 4.6 ± 0.4 mm ($p < 0.001$), a mean RAL gain of 3.4 ± 0.8 mm ($p < 0.001$) and a mean PBL gain of 2.8 ± 0.8 mm ($p < 0.001$). RAL gains of $\geqslant 4$ mm were measured in 90% of the test defects and in 55% of the controls. PBL gains of $\geqslant 4$ mm were obtained in 85% of the test defects and in 25% of the control ones. The test treatment resulted in statistically higher PPD reductions, RAL gains and PBL gains compared with the control ($p < 0.01$).

Conclusions: Within their limits, the present results indicate that: (i) at 1 year after surgery, both therapies resulted in statistically significant clinical improvements compared with baseline and (ii) although the combination of EMD+AB resulted in statistically significant higher soft and hard tissue improvements compared with treatment with EMD, the clinical relevance of this finding is unclear.

Key words: autogenous bone; enamel matrix protein derivative; grafting materials; intrabony periodontal defects; regenerative periodontal treatment

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The aims of periodontal therapy are to arrest and control the infection and, ultimately, to regenerate the lost supporting apparatus of the tooth (i.e. root cementum, periodontal ligament and alveolar bone) (Karring et al. 2003).

One well-established method to enhance periodontal regeneration is the use of an enamel matrix derivative (EMD). The rationale for the clinical use of EMD is the observation that enamel matrix proteins (EMPs) are deposited onto the surface of developing tooth roots before cementum formation (Hammarström 1997).

Recent data from a systematic review indicate that EMD affects many different cell types including epithelial cells, periodontal ligament and gingival fibroblasts and osteoblasts (Bosshardt 2008).

Conflict of interest and source of funding statement

The authors declare that they do not have any conflict of interests.

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It has been shown that EMD has effects on cell attachment, spreading, and chemotaxis, cell proliferation and survival, expression of growth factors, cytokines and extracellular matrix molecules, and on the expression of certain molecules that modulate bone remodeling (Bosshardt 2008). The combination of EMD and a demineralized freeze-dried bone allograft (DFDBA) was demonstrated to be osteopromotive, thus resulting in an additional increase in bone formation (Boyan et al. 2000).

Findings from histological studies have provided evidence for periodontal regeneration (i.e. formation of cementum, periodontal ligament and alveolar bone) following regenerative periodontal surgery using EMD (Hammarström et al. 1997, Heijl 1997, Mellonig 1999, Sculean et al. 1999, 2000a, b, Yukna & Mellonig 2000, Majzoub et al. 2005). In intrabony defects, treatment with open flap debridement (OFD) and EMD may lead to significantly higher improvements in terms of clinical attachment gains than treatment with OFD alone (Trombelli & Farina 2008, Esposito et al. 2009). One concern related to the application of EMD is related to its viscous nature, which may not provide sufficient soft tissue/flap support, thus potentially limiting the space available for the regeneration process (Mellonig 1999, Polimeni et al. 2006, Sculean et al. 2008b).

An approach to limit soft tissue collapse and maintain the space may be the combination of EMD and grafting materials. It was speculated that such an approach may allow for a combination of the biologic properties of EMD with the tissue-supporting properties of a grafting material (Lekovic et al. 2000, Velasquez-Plata et al. 2002, Sculean et al. 2003, 2008a, d, Zucchelli et al. 2003, Gurinsky et al. 2004, Kuru et al. 2006, Trombelli et al. 2006, Guida et al. 2007). However, the results from controlled clinical studies investigating the possible benefit of a combination of EMD and various types of grafting materials are controversial. While some studies have shown higher clinical improvements following a combination approach (Lekovic et al. 2000, Velasquez-Plata et al. 2002, Zucchelli et al. 2003, Gurinsky et al. 2004, Kuru et al. 2006, Guida et al. 2007), others have failed to show any significant differences (Sculean et al. 2005, 2007, Bokan et al. 2006, Jepsen et al. 2008). Among the grafting materials, autogenous bone (AB) is widely documented and has favourable biological properties: it bears osteogenetic and osteoinductive potential, and it is bioresorbable and easily available (Dragoo & Sullivan 1973, Hiatt et al. 1978, Stahl et al. 1983). Histological observations from animals have demonstrated periodontal regeneration following the treatment of intrabony defects with EMD+AB, while data from a controlled clinical study have indicated higher improvements in terms of defect fill following this combination when compared with treatment with EMD alone (Cochran et al. 2003, Guida et al. 2007). The data from controlled clinical studies evaluating the potential benefit of treating intrabony defects with a combination of EMD+AB are, however, still limited.

The aim of the present controlled clinical study was therefore to evaluate the healing of advanced intrabony defects treated with either a combination of EMD+AB or with EMD alone.

Materials and Methods

Forty patients (16 females and 24 males) (aged 30–50 years), suffering from advanced chronic periodontitis, were included in this parallel-design study (i.e. 20 patients in each group) after having signed an informed consent. The study was performed in accordance with the Helsinki Declaration of 1975, as revised in 2000. The study protocol has been reviewed and approved by the ethical committee of the Yeditepe University Istanbul, Turkey (number: YTU/2007-336). The study design and patient flow are depicted in Fig. 1. The patients were consecutively enrolled in the study when the following inclusion criteria were fulfilled: (1) no systemic diseases such as diabetes mellitus or cardiovascular diseases that could influence the outcome of the therapy, (2) no smokers, (3) a good level of oral hygiene [plaque index (PI) < 1] (Löe 1967), (4) compliance with the maintenance programme and (5) presence of one intrabony defect with a probing depth of at least 6 mm and an intrabony component of at least 3 mm as detected on the radiographs. The following clinical parameters were assessed without local anaesthesia before and 1 year after the surgical procedure using the same type of periodontal probe (UNC 15, Hu-Friedy, Chicago, IL, USA): PI (Löe 1967), gingival index (GI) (Löe 1967), bleeding on probing (BOP), probing pocket depth (PPD) and relative attachment level (RAL). The measurements were made at six sites per tooth, mesiovestibular (mv), midvestibular (v), distovestibular (dv), mesiolingual (ml), midlingual (l) and distolingual (dl), using a customized acrylic stent with markings by the same calibrated examiner (G. C.). Following local anaesthesia, probing bone-level (PBL) measurements were made at the same six sites.

The examiner was not aware, in any of the cases, of the type of treatment provided. Pre- and postoperative radiographs were taken using the long cone paralleling technique. For screening, the depth of the intrabony component was estimated before surgery on radio-

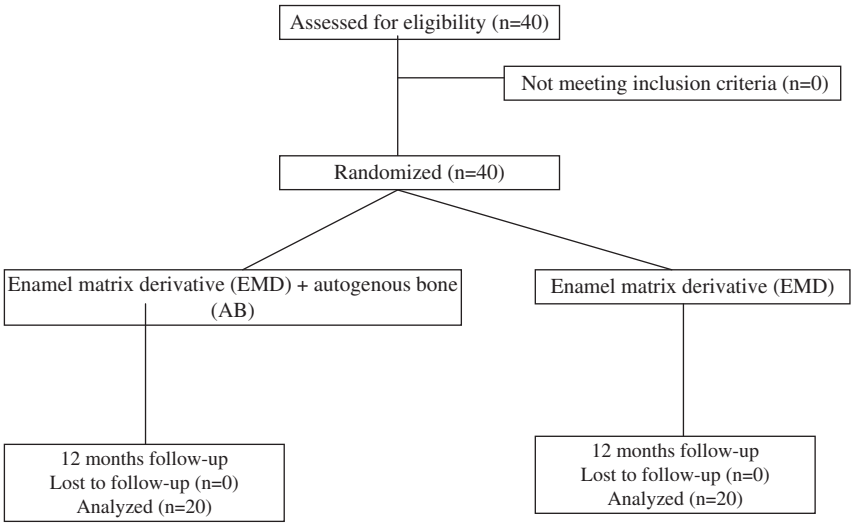

Fig. 1. Patient flow chart (flow of participants through each stage).

graphs. During surgery, the depth on the intrabony component (INTRA) was directly measured and was defined as the distance from the coronal extension of the alveolar crest to the bottom of the defect. Defect configuration was also recorded during surgery.

Intra-examiner reproducibility

Five patients, each displaying 10 teeth (single and multi rooted) with probing depths >6 mm on at least one aspect of each tooth, were used to calibrate the examiner. The examiner evaluated the patients on two separate occasions, 48 h apart. Calibration was accepted if measurements at baseline and at 48 h were similar to the millimetre at >90% level. The examiner was not aware of the surgical procedure to be performed.

Surgical procedure

All patients were treated at the Department of Periodontology of Yeditepe University, Istanbul, by the same experienced surgeon (S. Y.). The surgeries were performed between April 2007 and January 2008. The operative procedure for the application of EMD+AB was performed as follows: following local anaesthesia, intracrevicular incisions were placed and full-thickness flaps were raised vestibularly and orally. If necessary, vertical releasing incisions were performed for a better access or to achieve a better closure of the surgical site. Following removal of granulation tissue from the defects, the roots were thoroughly scaled and planed using hand and ultrasonic instruments.

After the defects were completely debrided, they were randomly assigned by a toss of coin to one of the two treatment groups. First, flipping was performed for assigning the treatment groups. The side of the coin (heads = EMD, tails = EMD+AB) determined the assignment of each treatment. Subsequently, another flipping was performed for the distribution of the patients to the groups. In this way, the patients were consecutively treated by random allocation to the two treatment groups until 20 patients belonging to either of the groups were operated. Then, the rest of the patients were allocated to the other group in order to achieve groups of equal size. This occurred in consecutively treated patient number 36, belonging to EMD+AB (i.e. patients number 37–40 were treated with EMD alone).

In both groups, the root surfaces adjacent to the defects were conditioned for 2 min. with an ETDA gel (pH 6.7) (Straumann PrefGel™, Straumann, Basel, Switzerland) in order to remove the smear layer (Blomlöf et al. 1996). The defects and the adjacent mucoperiosteal flaps were then thoroughly rinsed with sterile saline in order to remove all EDTA residues.

Following root conditioning, EMD (Straumann Emdogain®, Straumann) was applied onto the root surfaces and into the defects with a sterile syringe.

Cortico-cancellous AB was harvested from the retromolar area using a trephine bur with a diameter of 3 mm (KLS Martin Group, Gebrüder Martin GmbH & Co. KG, Tuttlingen, Germany). The remaining EMD was then mixed with AB and the defects were completely filled with the mixture of EMD+AB. Care was exercised not to overfill the defects. Finally, the flaps were advanced coronally and closed with vertical or horizontal mattress sutures (4-0 silk, Doğsan A. Ş. Trabzon, Turkey). The sites treated with EMD received exactly the same treatment including root conditioning with EDTA, but without the application of AB.

Postoperative care

The postoperative care consisted of administration of antibiotics for 1 week (3 × 500 mg amoxicillin/day) and of painkillers for 2–3 days postoperatively (2 × 275 mg non-steroidal antiinflammatory drug i.e. NSAID/day). All patients used 0.2% chlorhexidine rinses twice a day for 4 weeks. Only after this period was toothbrushing resumed in the operated areas. The sutures were removed 14 days after the surgery. Recall appointments were scheduled every second week during the first 2 months after surgery and once per month following the rest of the observation period of 1 year. Neither probing nor subgingival instrumentation was performed during the first 6 months after surgery.

Statistical analysis

Because the defects showed a normal distribution, parametric tests were used.

The statistical analysis was performed using the package for social sciences 12.0 for Windows (SPSS®, Chicago, IL, USA). The primary outcome variable was the change in RAL. The secondary variables were PPD reduction and PBL change. Only one measurement per tooth, the deepest site of the selected defect at baseline, was included in the calculations. For the statistical evaluation of the changes from baseline to 1 year, the paired *t*-test was used. For the comparisons between the groups, the unpaired *t*-test was used. The α-error was set at 0.05. The power of the study, given 1 mm as a significant difference between the groups, was calculated to be 0.80.

Results

All patients completed the study. No dropouts occurred. Patient-centred parameters were not recorded separately, but the postoperative healing was uneventful in all cases. Minor complications were related to usual postoperative swelling and occurred within the first days after surgery. No adverse reactions related to the materials used were reported.

There were no differences in the gender distribution between the groups (i.e. there were nine females and 11 males in the test group and seven females and 13 males in the control one).

Table 1 illustrates the mean PI, GI and BOP for both groups. GI and BOP improved statistically significantly compared with baseline, but no statistically significant differences were found between the two groups.

The defects displayed a comparable distribution and configuration in the two groups (Table 2). The depth of the

Table 1. Mean (± SD) plaque, gingival and bleeding scores at the treated sites at baseline and the 1-year examination

	EMD+AB ($N = 20$)	EMD ($N = 20$)	*p* value
Plaque index scores			
Baseline	0.5 ± 0.1	0.4 ± 0.1	0.48
12 months	0.3 ± 0.1	0.3 ± 0.3	0.84
p value	0.33	0.54	
Gingival index scores			
Baseline	1.2 ± 0.2	1.3 ± 0.3	0.74
12 months	0.6 ± 0.3	0.7 ± 0.2	0.80
p value	<0.01	<0.01	
Bleeding scores			
Baseline	50%	49%	0.78
12 months	15%	16%	0.84
p value	<0.001	<0.001	

AB, autogenous bone; EMD, enamel matrix protein derivative.

Table 2. Distribution and configuration of treated defects

	EMD+AB ($N = 20$)	EMD ($N = 20$)
Maxilla	8	10
Mandible	12	10
Anterior teeth	6	6
Pre-molars	8	6
Molars	6	8
2 wall	7	8
2–3 wall	13	12

AB, autogenous bone; EMD, enamel matrix protein derivative.

Table 3. Clinical parameters at baseline and 1 year expressed in mm ($N = 20$ for each group)

	Baseline	1 year	Difference	Significance
Probing pocket depth				
EMD+AB	8.4 ± 1.2	2.8 ± 1.1	5.6 ± 0.9	<0.001
EMD	8.2 ± 0.7	3.5 ± 0.6	4.6 ± 0.4	<0.001
p value			<0.01	
Gingival recession				
EMD+AB	3.3 ± 1.5	4.7 ± 1.0	1.4 ± 0.9	<0.001
EMD	3.1 ± 1.1	4.3 ± 1.2	1.2 ± 0.8	<0.001
p value			0.42	
Relative attachment level				
EMD+AB	11.7 ± 1.0	7.5 ± 0.7	4.2 ± 1.1	<0.001
EMD	11.3 ± 0.9	7.8 ± 1.1	3.4 ± 0.8	<0.001
p value			<0.01	
Probing bone level				
EMD+AB	12.3 ± 1.0	8.3 ± 0.6	3.9 ± 1.0	<0.001
EMD	12.1 ± 0.9	9.2 ± 1.0	2.8 ± 0.8	<0.001
p value			<0.01	

AB, autogenous bone; EMD, enamel matrix protein derivative.

intrabony component (INTRA) as measured during surgery was 5.4 ± 1.0 mm in the test group and 5.2 ± 0.7 mm in the control one. There were no statistically significant differences in the depth of the intrabony component between the two groups ($p = 0.74$).

At 1 year after therapy, the test sites showed a reduction in the mean PPD of 5.6 ± 0.9 mm ($p < 0.001$), a gain in the mean RAL of 4.2 ± 1.1 mm ($p < 0.001$) and a gain in the mean PBL of 3.9 ± 1.0 mm ($p < 0.001$) (Table 3). The control group showed a mean PPD reduction of 4.6 ± 0.4 mm ($p < 0.001$), a mean RAL gain of 3.4 ± 0.8 mm ($p < 0.001$) and a mean PBL gain of 2.8 ± 0.8 mm ($p < 0.001$) (Table 3). The test treatment resulted in statistically higher PPD reductions, RAL gains and PBL gains compared with the control one ($p < 0.01$).

GR increased statistically significantly in both groups compared with baseline ($p < 0.001$), but without a significant difference between the groups ($p = 0.42$) (Table 3).

RAL gains of ⩾4 mm were measured in 90% of the test defects and in 55% of the controls (Table 4). PBL gains of ⩾4 mm were obtained in 85% of the test defects and in 25% of the control ones (Table 5).

Table 4. Frequency distribution of RAL gain (in %)

	EMD+AB ($N = 20$)	EMD ($N = 20$)
< 2 mm	0	0
2–3 mm	10	35
4–5 mm	85	55
6 mm	5	0

AB, autogenous bone; EMD, enamel matrix protein derivative; RAL, relative attachment level.

Table 5. Frequency distribution of PBL gain (in %)

	EMD+AB ($N = 20$)	EMD ($N = 20$)
< 2 mm	5	15
2–3 mm	10	60
4–5 mm	80	25
⩾6 mm	5	0

AB, autogenous bone; EMD, enamel matrix protein derivative; PBL, probing bone level.

Discussion

The present results have shown that the treatment of intrabony defects with both a combination of EMD+AB or with EMD alone may lead to statistically significant clinical improvements evidenced by reductions of PPD and gains of CAL and PBL. Moreover, the significant gains in PBL seem to indicate that both treatment modalities may facilitate hard tissue formation and defect fill in deep intrabony defects.

The lack of any adverse reactions such as allergies or abscesses in the patients is in agreement with previous data, thus supporting the excellent properties of EMD alone or combined with AB to enhance periodontal wound healing (Heijl et al. 1997, Pontoriero et al. 1999, Sculean et al. 1999, 2000b, 2006, 2007, 2008b, Tonetti et al. 2002, Trombelli et al. 2006, Guida et al. 2007, Cortellini et al. 2008). Important factors that have been shown to significantly influence the outcomes of regenerative periodontal surgery are plaque infection and smoking (Tonetti et al. 1995, 1996, Trombelli et al. 1997). Because no smokers were included in the present study population, it may be assumed that careful patient selection was also responsible for the positive outcomes obtained with both treatments.

The finding that the treatment of intrabony periodontal defects with EMD may result in statistically significantly improvements compared with baseline is in line with the conclusions of a systematic review, which has analysed the potential benefit of EMD when used in addition to OFD and shown that EMD-treated sites displayed statistically significant CAL improvements (i.e. mean difference 1.1 mm, 95% CI 0.61–1.55) when compared with OFD (Esposito et al. 2009).

The finding that treatment with EMD+AB resulted in statistically significant improvements in terms of PPD, reduction and gains in CAL and PBL when compared with treatment with EMD alone corroborates those of a recent controlled clinical study using a comparable study design (Guida et al. 2007). In that study, treatment with EMD+AB yielded in 50% of defects a CAL gain of ⩾6 mm while in 21% a CAL gain of 4–5 mm was obtained. In the group treated with EMD, CAL gains of ⩾6 mm and of 4–5 mm were obtained in 21% and 57% of the defects, respectively.

In the present study, treatment with EMD+AB yielded a RAL gain of 4–5 mm in 85% and of ⩾6 mm in 5% of the defects. In the group treated with EMD, a RAL gain of 4–5 mm was obtained in 55% of defects; however, in none of them was a RAL gain of

$\geqslant$6 mm measured. These slight differences between the two studies might be explained by differences in the initial depth of the defects (i.e. the deeper the defect, the greater the gain in CAL) and by the surgical technique used (Kahldahl et al. 1996, Cortellini et al. 1998, Cortellini & Tonetti 2005, 2007).

The present findings are also in line with those from controlled clinical studies evaluating the outcomes of regenerative surgery in intrabony defects using a combination of EMD and certain types of grafting materials such as AB, DFDBA, a natural bone mineral or a bioactive glass. In those studies, the combination treatment resulted in statistically significantly higher CAL gains and/or hard tissue fill compared with treatment with EMD alone, thus pointing to the possible advantage of a combination approach (Lekovic et al. 2000, Velasquez-Plata et al. 2002, Zucchelli et al. 2003, Gurinsky et al. 2004, Kuru et al. 2006, Guida et al. 2007). However, other controlled clinical studies have failed to show significant differences in the clinical outcomes following regenerative surgery using EMD alone or combined with certain types of alloplastic materials such a bioactive glass, β-tricalcium phosphate or calcium phosphate ceramic (Sculean et al. 2005, 2007, Bokan et al. 2006, Jepsen et al. 2008). The differences in the reported results among the studies evaluating various combinations of EMD and grafting materials are, at present, difficult to explain in the light of a biological rationale.

The biological rationale for selecting AB as a grafting material was based on the histological evidence reported for this material to promote periodontal regeneration (Dragoo & Sullivan 1973, Hiatt et al. 1978, Stahl et al. 1983). Moreover, histologic evidence for periodontal regeneration was demonstrated in monkeys following the combination of EMD and AB (Cochran et al. 2003). On the other hand, it needs to be pointed out that, until now, no data from human histological studies evaluating the healing following regenerative surgery with EMD+AB are available and thus, at present, no definitive conclusions regarding the type of healing following this combination can be made.

When interpreting the present findings, it should be realized that for the present study, the sample size was calculated for a clinically significant (relevant) difference of 1.0 mm. Therefore, the clinical relevance of the statistically significant additional RAL gain of 0.8 mm is unclear and should be interpreted with caution.

Another aspect that needs to be addressed when interpreting the results is related to the randomization method used. The present study used the toss of a coin as a randomization method, which is not the ideal method for randomly allocating patients to the two treatment groups. Consequently, this approach led to the last four patients being allocated to the control group without randomization. However, it is not possible to evaluate how much this ''lack of randomization'' of the last four patients might have influenced the results.

A thorough analysis of the available studies evaluating the benefit of using EMD in the treatment of intrabony defects has revealed a high heterogeneity in the treatment outcomes. It may thus be speculated that this heterogeneity may be partly due to differences in the defect configuration (Tonetti et al. 2002, Esposito et al. 2009). Because of its gel-like consistency, it cannot be excluded that the application of EMD, especially in so-called non-contained-type defects, cannot prevent a collapse of the mucoperiosteal flap, thus minimizing the space available for the regeneration process (Lekovic et al. 2000, Tonetti et al. 2002, Velasquez-Plata et al. 2002, Zucchelli et al. 2003, Gurinsky et al. 2004, Guida et al. 2007). Histological and clinical studies have provided evidence for comparable outcomes following regenerative surgery using EMD or GTR (Pontoriero et al. 1999, Sculean et al. 1999, 2000a, b, 2006, 2008b, Silvestri et al. 2003). This finding, coupled with the fact that the complications related to GTR (i.e. membrane exposure and subsequent bacterial colonization) are absent with EMD, appear to indicate that the use of EMD may, in certain clinical cases, replace the role of a barrier membrane, thus simplifying the surgical procedure (Trombelli et al. 1997, Sanz et al. 2004, Cortellini & Tonetti 2007, Cortellini et al. 2008).

The need for further clinical trials evaluating the potential benefit of a combination of EMD and grafting materials seems to be supported by recent findings from a systematic review of preclinical studies and from clinical studies indicating that in non-contained-type defects, a combination of grafting materials and barrier membranes may result in superior histological and clinical outcomes evidenced by less gingival recession and higher clinical attachment gains (Cortellini & Tonetti 2005, Sculean et al. 2008c).

In conclusion, within their limits, the present results indicate that (i) at 1 year after surgery, both therapies resulted in statistically significant clinical improvements compared with baseline and (ii) although the combination of EMD+AB yielded statistically significant higher soft and hard tissue improvements compared with the treatment with EMD, the clinical relevance of this finding is unclear.

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Clinical Relevance

Scientific rationale for the study: Histological findings from preclinical studies have provided evidence for periodontal regeneration in intrabony defects treated with a combination of an EMD and AB. This combination has also been shown to result in additional clinical improvements compared with treatment with EMD alone. However, the data evaluating the potential clinical benefits of this treatment approach are still limited.

Principal findings: Both therapies resulted in significant clinical improvements. The combination approach appeared to additionally improve the outcomes, although the clinical relevance of this finding is unclear.

Practical implications: Regenerative surgery with EMD+AB and EMD alone represent valuable treatment options for intrabony defects.