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ORIGINAL ARTICLE

# Four-year results following treatment of intrabony periodontal defects with an enamel matrix derivative alone or combined with a biphasic calcium phosphate

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**Abstract** The aim of this study was to evaluate the 4-year clinical outcomes following regenerative surgery in intrabony defects with either EMD+BCP or EMD. Twenty-four patients with advanced chronic periodontitis, displaying one-, two-, or three-walled intrabony defect with a probing depth of at least 6 mm, were randomly treated with either EMD+BCP (test) or EMD alone (control). The following clinical parameters were evaluated at baseline, at 1 year and at 4 years after regenerative surgery: plaque index, gingival index, bleeding on probing, probing depth, gingival recession, and clinical attachment level (CAL). The primary

outcome variable was CAL. No differences in any of the investigated parameters were observed at baseline between the two groups. The test group demonstrated a mean CAL change from from 10.8±1.6 mm to 7.4±1.6 mm ( $p<0.001$ ) and to 7.6±1.7 mm ( $p<0.001$ ) at 1 and 4 years, respectively. In the control group, mean CAL changed from 10.4±1.3 at baseline to 6.9±1.0 mm ( $p<0.001$ ) at 1 year and 7.2±1.2 mm ( $p<0.001$ ) at 4 years. At 4 years, two defects in the test group and three defects in the control group have lost 1 mm of the CAL gained at 1 year. Compared to baseline, at 4 years, a CAL gain of  $\geq 3$  mm was measured in 67% of the defects (i.e., in 8 out of 12) in the test group and in 75% of the defects (i.e., in 9 out of 12) in the control group. There were no statistically significant differences in any of the investigated parameters at 1 and at 4 years between the two groups. Within their limits, the present results indicate that: (a) the clinical improvements obtained with both treatments can be maintained over a period of 4 years, and (b) in two- and three-walled intrabony defects, the addition of BCP did not additionally improve the outcomes obtained with EMD alone. In two- and three-walled intrabony defects, the combination of EMD+BCP did not show any advantage over the use of EMD alone.

**Keywords** Regenerative periodontal therapy · Intrabony defects · Enamel matrix derivative · Biphasic calcium phosphate · Combination therapy · Controlled clinical study

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## Introduction

The goal of regenerative periodontal surgery is to completely restore the tooth's supporting apparatus (i.e., root cementum, periodontal ligament and bone), which have been lost following periodontal disease or trauma [1].

Findings from histological studies in animals and humans have provided evidence for periodontal regeneration following treatment with an enamel matrix derivative (EMD) [2–11]. Results from controlled clinical studies have demonstrated that in intrabony defects, open flap debridement (OFD) followed by application of EMD may result in significantly higher improvements in terms of clinical attachment level gain and bone fill when compared to OFD alone [12–16].

Concerns have been expressed regarding the viscous nature of EMD, which may not be sufficient to prevent flap collapse in periodontal defects with a complicated anatomy. A collapse of the mucoperiosteal flap may limit the space available for the regeneration process, thus yielding less clinical improvements such as clinical attachment gain and defect fill [17–25]. In order to overcome this limitation and enhance the clinical outcomes obtained with EMD alone, different combinations of EMD and various types of grafting materials such as autogenous bone (AB) [17], demineralized freeze-dried bone allograft (DFDBA) [18, 19], anorganic bovine bone mineral (ABBM) [20–24], bioactive glass [25, 26], or beta tricalcium phosphate ( $\beta$ -TCP) [27] have been used with varying degree of success.

Recently, a biphasic calcium phosphate (BCP) with >99% crystallinity, consisting of 60% hydroxyapatite (HA) and 40%  $\beta$ -TCP in particulate form has been introduced as a grafting material in periodontal, peri-implant and bone defects [28–30]. Findings from animal studies have indicated that this ratio of HA and  $\beta$ -TCP may allow for a better control of the resorbability of the graft material, thus, resulting in accelerated new bone formation [28, 29].

A recent controlled clinical study has evaluated the healing of intrabony defects treated with BCP alone, AB spongiosa, or OFD [30]. At 12 months following surgery, the results have shown similar clinical improvements in the groups treated with BCP and AB, which were superior to those obtained with OFD alone. On the other hand, results from another controlled, randomized multicenter clinical study comparing regenerative surgery with EMD alone to EMD+BCP have shown similar clinical outcomes following both treatments, without any advantage of the combination approach [31, 32]. Thus, at present, the potential advantage of combining EMD with BCP is still unclear. Moreover, to the best of our knowledge, no data are available evaluating over a longer period than 12 months the clinical outcomes obtained following this combination approach.

Therefore, the aim of this controlled clinical study was to evaluate the 4-year clinical outcomes following regenerative surgery in intrabony defects with either EMD+BCP or EMD.

## Materials and methods

### Study design and patient population

The study was designed as a prospective controlled clinical study with two treatment groups. Twenty-four non-smoking patients (14 females and 10 males) aged between 34 and 62 diagnosed from advanced chronic periodontitis [33] were included in this parallel design study (i.e., 12 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 and was reviewed and approved by the local ethical committee (Ethics Committee Nr.: R-I-003/350/2005). All patients were treated at the Medical Academy Bialystok, Poland by the same experienced surgeon (MP). The patients were consecutively enrolled in the study when following inclusion criteria were met: (1) no systemic diseases which could influence the outcome of the therapy, (2) a good level of oral hygiene ( $PI < 1$ ) [34], (3) compliance with the maintenance program, (4) presence of one intrabony defect with a probing depth of at least 6 mm and an intrabony component of at least 4 mm as detected on the radiographs. The following clinical parameters were assessed 1 week prior, at 1 year and at 4 years after the surgical procedure using the same periodontal probe (i.e., UNC 15, Hu-Friedy, Chicago IL, USA): plaque index (PI) [34], gingival index (GI) [34], bleeding on probing (BOP), pocket depth (PD), gingival recession (GR), and clinical attachment level (CAL). The measurements were made at six sites per tooth: mesiovestibular (mv), midvestibular (v), distovestibular (dv), mesiolingual (ml), midlingual (l), distolingual (dl) by a masked and calibrated investigator (JP). Examiner calibration was performed as follows: five patients, not enrolled in the study, and showing at least four teeth with probing depths  $\geq 6$  mm on at least one aspect of each tooth, were evaluated by the examiner on two separate occasions, 48 h apart. Calibration was accepted if measurements at baseline and at 48 h were similar to the millimeter at  $\geq 90\%$ .

The cemento-enamel junction (CEJ) was used as the reference point. In cases where the CEJ was not visible, a restoration margin was used for these measurements. The study reports only the measurements at exactly the same site (at baseline the deepest) of the selected defect. Pre- and postoperative radiographs were taken with the long-cone parallel technique. Randomization was computer-generated by using a block approach.

### Surgical procedure

Following local anesthesia and intracrevicular incisions mucoperiosteal flaps were raised vestibularly and orally. Vertical releasing incisions were performed if deemed

necessary for a better access to the surgical site or to achieve a better closure. All granulation tissue was removed from the defects and the roots were thoroughly scaled and planed by means of hand and ultrasonic instruments.

During surgery, the following measurements were made: distance from the CEJ to the bottom of the defect (CEJ–BD), distance from the CEJ to the most coronal extension of the alveolar bone crest (CEJ–BC). The intrabony component (INTRA) of the defects was defined as (CEJ–BD)–(CEJ–BC).

After defect debridement, in both groups, the root surfaces adjacent to the defects were conditioned for 2 min with ETDA gel (pH 6.7) (Straumann PrefGel™, Straumann, Basel, Switzerland) to remove the smear layer [35]. Subsequently, the defects and the adjacent mucoperiosteal flaps were thoroughly rinsed with sterile saline to remove all EDTA remnants.

Following root conditioning, in both the test and the control group, EMD (Straumann® Emdogain, Straumann, Basel, Switzerland) was applied onto the root surfaces and into the defects with a sterile syringe. In the test group, the defects were additionally filled up with the mixture of EMD+BCP (Straumann® BoneCeramic, Straumann, Basel, Switzerland).

If deemed necessary, periosteal releasing incisions were made to allow for a tension-free, coronally adaptation of the mucoperiosteal flaps. Finally, the mucoperiosteal flaps were closed with 5–0 monofilament vertical or horizontal mattress sutures and interrupted sutures.

*Postoperative care*

All patients received antibiotics for 1 week (3×500 mg amoxicillin per day) starting 1 h preoperatively. The

postoperative care consisted of 0.2% chlorhexidine rinses twice a day for 4 weeks. Sutures were removed 14 days after the surgery. Recall appointments were scheduled weekly during the first 4 weeks after surgery and every 3 months following the rest of the observation period of 4 years. The recall appointments consisted mainly of reinforcement of oral hygiene measures and professional supragingival tooth cleaning.

*Statistical analysis*

The statistical analysis was performed with the package for social sciences 12.0 for Windows (SPSS®, Chicago, IL, USA). The primary outcome variable was the change in CAL. The secondary variables were PD reduction and GR change. Only one measurement per tooth, at the deepest site of the selected defect at baseline, was included into the calculations. For the statistical evaluation of the changes from baseline to 1 and 4 years, the paired *t* test was used. For the comparisons between the groups, the unpaired *t* test was used. The alpha error was set at 0.05.

Power calculation has demonstrated that in order to detect a statistically significant difference between the two groups, a much higher number of patients would have been needed (i.e., 54 patients for each group). The power of the study, given 1 mm as a significant difference between the groups, was calculated to be 0.70.

**Results**

The early wound healing phase was uneventful. Only adverse events were minor and were limited to usual

**Table 1** PI, GI, and BOP at baseline at 1 and at 4 years

	Parameter	Treatment	Baseline	1 Year	<i>P</i> value (baseline—1 year)	4 Years	<i>P</i> value (1–4 years)
No statistically significant differences between the EMD+BCP and EMD groups were found	PI						
		EMD+BCP ( <i>n</i> =12)					
	Mean (±SD)		0.6±0.2	0.5±0.3	n.s.	0.9±0.8	n.s.
		EMD ( <i>n</i> =12)					
	Mean (±SD)		0.7±0.1	0.6±0.2	n.s.	1.0±0.7	n.s.
	GI						
	EMD+BCP ( <i>n</i> =12)						
Mean (±SD)		1.0±0.2	0.5±0.2	<0.05	1.0±0.8	n.s.	
	EMD ( <i>n</i> =12)						
Mean (±SD)		1.1±0.2	0.6±0.3	<0.05	1.0±0.9	n.s.	
BOP							
	EMD+BCP( <i>n</i> =12)						
Mean (±SD)		36%	11%	<0.05	30%	n.s.	
	EMD ( <i>n</i> =12)						
Mean (±SD)		38%	12%	<0.05	35%	n.s.	

**Table 2** Distribution and configuration of treated defects

	EMD+BCP (N=12)	EMD (N=12)
Maxilla	6	5
Mandible	6	7
Anterior teeth	6	5
Premolars	4	5
Molars	2	2
1–2 wall	1	1
2 wall	9	8
3 wall	2	3

postoperative discomfort, swelling, and pain during the first days following surgery. No adverse reactions against any of the used regenerative materials were observed. There were no differences in the gender distribution between the groups (i.e., eight females and four males in each of the two groups).

The PI, GI, and BOP for both treatment groups at baseline, after 1 and 4 years are summarized in Table 1. Mean PI did not reveal a statistically significant difference between the two groups at baseline and after 1 and 4 years. At 4 years, mean PI increased slightly in both groups, but this difference was not statistically significant compared to baseline or to the 1 year results.

A statistically significant difference was observed within both treatment groups, when comparing the 1 and 4 year GI and BOP to the baseline values ( $P<0.001$ ). However, there were no statistically significant differences between the 1- and 4-year values (Table 1).

There were no differences in terms of defect distribution and configuration between the two groups (Table 2). The baseline defect characteristics are presented in Table 3. No statistically significant difference in the initial depth of the intrabony component was found between the two groups.

The PD, GR, and CAL at baseline, at 1 and 4 years are shown in Table 4. At 1 year, mean PD decreased statistically highly significantly in both groups ( $P<0.001$ ) (Table 4). Between the groups, no statistically significant difference was found. However, at 4 years, a slight, statistically not significant increase in PD was measured in both groups. At 4 years, the PD was still statistically highly improved compared to baseline ( $P<0.001$ ) (Table 4).

At 1 and 4 years in both groups, mean GR was statistically significantly increased compared to baseline

( $P<0.01$ ). No statistically significant differences between the two groups were found at 1 and 4 years (Table 4).

Mean CAL demonstrated statistically significant improvements in both groups at 1 and at 4 years compared to the baseline ( $P<0.001$ ) (Table 4). No statistically significant differences were observed at 1 and at 4 years within and between the two treatment groups (Table 4). The frequency distribution of CAL gain at 1 and 4 years are depicted in Tables 5 and 6. At 1 year, a CAL gain of  $\geq 3$  mm was measured in 75% of the defects (i.e., in 9 out of 12) in the EMD+BCP group and in 83% of the defects (i.e., in 10 out of 12) in the EMD group. At 4 years, a CAL gain of  $\geq 3$  mm was measured in 67% of the defects (i.e., in 8 out of 12) in the test group and in 75% of the defects (i.e., in 9 out of 12) in the control group. At 4 years, two defects in the EMD+BCP group and three defects in the EMD group have lost 1 mm of the CAL gained at 1 year.

## Discussion

The results of this study have shown that treatment of intrabony defects with both, a combination of EMD+BCP and EMD alone may lead to statistically significant PD reductions and CAL gains which can be maintained over a period of 4 years. However, the statistical analysis has failed to reveal significant differences between the two treatment modalities in any of the investigated clinical parameters at 1 and at 4 years. In both groups, a slight, statistically insignificant, loss of mean CAL was measured between the 1- and 4-year evaluation period. This loss of mean CAL was due to a CAL loss of 1 mm in two defects in the test group and in three defects in the control group. Compared to baseline, at 4 years, a CAL gain of  $\geq 3$  mm was measured in 67% of the defects (i.e., in 8 out of 12) in the test group and in 75% of the defects (i.e., in 9 out of 12) in the control group.

It should, however, be emphasized that the study does not have the statistical power to rule out the possibility of a difference between the two groups. Further studies, with a higher number of patients and defects would be needed to detect an eventual difference between the treatments [36]. On the other hand, according to the best of our knowledge, at present, there are no other studies evaluating the clinical performance of a combination of EMD+BCP over a period of 4 years, and therefore, the findings may bear clinical relevance despite

**Table 3** Baseline defect characteristics expressed in millimeters (mean $\pm$ SD)

No statistically significant differences between the EMD+BCP and EMD groups were found

Treatment	PD (mm)	CAL (mm)	CEJ-BD (mm)	CEJ-BC (mm)	INTRA (mm)
EMD+BCP (N=12)	8.8 $\pm$ 1.5	10.8 $\pm$ 1.6	11.8 $\pm$ 1.5	6.2 $\pm$ 1.4	5.6 $\pm$ 1.4
EMD (n=12)	8.8 $\pm$ 1.0	10.4 $\pm$ 1.3	11.7 $\pm$ 1.2	6.0 $\pm$ 1.2	5.7 $\pm$ 1.3
<i>P</i> value	n.s.	n.s.	n.s.	n.s.	n.s.

**Table 4** Clinical parameters at baseline at 1 and at 4 years

Parameter	Treatment	Baseline	1 Year	<i>P</i> value (baseline—1 year)	4 Years	<i>P</i> value (1–4 years)
PD						
EMD+BCP ( <i>n</i> =12)						
Mean (±SD)		8.8±1.5	4.3±0.9	<0.001	4.7±0.8	n.s.
EMD ( <i>n</i> =12)						
Mean (±SD)		8.8±1.0	4.1±0.5	<0.001	4.4±0.8	n.s.
GR						
EMD+BCP ( <i>n</i> =12)						
Mean (±SD)		2.1±1.0	3.2±1.1	<0.01	3.0±1.1	n.s.
EMD ( <i>n</i> =12)						
Mean (±SD)		1.6±1.0	2.9±0.9	<0.01	2.8±0.8	n.s.
CAL						
EMD+BCP ( <i>n</i> =12)						
Mean (±SD)		10.8±1.6	7.4±1.6	<0.001	7.6±1.7	n.s.
EMD ( <i>n</i> =12)						
Mean (±SD)		10.4±1.3	6.9±1.0	<0.001	7.2±1.2	n.s.

No statistically significant differences between the EMD+BCP and EMD groups were found

the relatively small number of patients and defects. Furthermore, the present results are in line with findings from previous controlled clinical studies which have failed to show significant differences in the clinical outcomes following regenerative surgery using EMD alone or combined with different alloplastic materials such as bioactive glass, beta tricalcium phosphate, or bone ceramic graft [26, 27, 31, 32]. In a recent multicenter, randomized, controlled clinical study comparing treatment with EMD+BCP (test) to EMD alone (control), the obtained mean CAL gain measured at 1 year after therapy 1.7±2.1 mm in the test and 1.9±1.7 mm in the control group, respectively [32]. These values are somewhat lower than those obtained in the present study where treatment with EMD+BCP yielded a mean CAL gain of 3.4±1.0 mm, whereas the corresponding value in the EMD group was 3.5±0.9 mm. These slight differences may, on one hand, be related to differences in the initial depth of the defects and, on the other, to differences in defect configuration. It is well documented that in deeper defects, a greater CAL gain may be achieved [37, 38]. In the present study, baseline PD was 8.8±1.5 mm in the test group and 8.8±1.0 mm in the control group, while in the study referred to,

the corresponding values measured 6.9±1.8 mm and 7.1±1.5 mm, respectively.

Moreover, when comparing the two studies, it needs to be mentioned that findings from preclinical and clinical studies have provided evidence for superior healing in so-called contained-type defects (i.e., three- and two-walled) compared to “non-contained” (i.e., one-walled or one- and two-walled) ones [39, 40]. In the study referred to the great majority of the defects were classified as either one-walled, combined one- and two-walled or circumferential, while in the present study, most of the defects displayed a two-walled configuration [31, 32].

Other key factors which have been shown to profoundly affect wound healing following conventional and regenerative periodontal surgery are infection control and smoking [38, 41]. The pivotal role of careful patient selection and strict maintenance program is further supported by the present findings where the patient population did not include any smokers, while in both groups, the plaque and bleeding values remained unchanged throughout the entire observation period of 4 years.

**Table 5** Frequency distribution of CAL gains at 1 year in the EMD+BCP and EMD groups

CAL gain (mm)	EMD+BCP ( <i>n</i> =12)		EMD ( <i>n</i> =12)	
	No.	Percent (%)	No.	Percent (%)
2	3	25	2	17
3	3	25	3	25
4	4	33	6	50
5	2	17	1	8

**Table 6** Frequency distribution of CAL gains at 4 years in the EMD+BCP and EMD groups

CAL gain (mm)	EMD+BCP ( <i>n</i> =12)		EMD ( <i>n</i> =12)	
	No.	Percent (%)	No.	Percent (%)
1	1	8		
2	3	25	3	25
3	3	25	2	17
4	4	34	7	58
5	1	8		



A recent human histologic study evaluating the healing of intrabony defects treated with EMD+BCP revealed formation of new cementum with an associated periodontal ligament in six out of the nine biopsies, while formation of new bone or of a mineralized bone-like tissue around the graft particles was only occasionally observed. Furthermore, in most specimens, the graft particles were still present at 9 months following surgery without signs of resorption or bone formation [42]. These observations appear to suggest that adding BCP to EMD may not additionally improve the healing modulated by EMD alone, thus corroborating the present and previously reported findings [31, 32].

When interpreting the present results, it should be pointed to the results from controlled clinical studies comparing various combination protocols of EMD with AB, ABBM, or DFDBA to EMD alone and have shown higher clinical improvements following the combination approach [17, 18, 22–24]. On the other hand, in most other studies where EMD was combined with different alloplastic grafts, no additional benefits were detected [25–27, 31, 32]. Thus, all these findings appear to suggest that the grafting material itself may also influence the healing process and, subsequently, the clinical outcomes. However, further research is necessary to elucidate the exact biological mechanisms behind these combinations.

Taken together, our results indicate that: (a) the clinical improvements obtained with both treatments can be maintained over a period of 4 years, and (b) in two- and three-wall intrabony defects, the addition of BCP did not additionally improve the outcomes obtained with EMD alone.

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

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