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Healing of human intrabony defects following regenerative periodontal therapy with an enamel matrix protein derivative alone or combined with a bioactive glass

A controlled clinical study

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Abstract

Aim: The purpose of the present study was to compare clinically the treatment of deep intrabony defects with a combination of an enamel matrix protein derivative (EMD) and a bioactive glass (BG) to EMD alone.

Methods: Thirty patients (16 females and 14 males) suffering from advanced marginal periodontitis were included in this prospective, controlled parallel design multicenter study. In each of the patients, one intrabony defect was randomly treated with either EMD+BG (test) or with EMD alone (control). Clinical measurements were recorded at baseline and at 1 year following therapy.

Results: No differences in any of the investigated parameters were observed at baseline between the two groups. Healing was uneventful in all patients. At 1 year after therapy, the test group showed a reduction in mean probing depth (PD) from 8.5 ± 1.1 to 4.4 ± 1.2 mm ($p < 0.001$) and a change in mean clinical attachment level (CAL) from 10.4 ± 1.5 to 7.1 ± 1.5 mm ($p < 0.0001$). In the control group, the mean PD was reduced from 8.5 ± 1.5 to 4.0 ± 1.6 mm ($p < 0.001$) and the mean CAL changed from 10.2 ± 2.1 to 6.3 ± 2.2 mm ($p < 0.01$). In the test group, 12 sites (80%) gained at least 3 mm or more of CAL, whereas in the control group a CAL gain of 3 mm or more was measured at 13 sites (87%). No statistically significant differences in terms of PD reduction and CAL gain were found between the test and the control treatment.

Conclusions: Within the limits of the present study it can be concluded that: (i) at 1 year after surgery, both therapies resulted in significant PD reductions and CAL gains, and (ii) the combination of EMD+BG does not seem to additionally improve the clinical results.

Key words: bioactive glass; controlled clinical study; enamel matrix protein derivative; regenerative periodontal therapy

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The application of an enamel matrix protein derivative (EMD) onto a previously debrided and conditioned root surface has been shown to enhance the formation of a new connective tissue attachment (i.e. new cementum with inserting collagen fibers) and of new alveolar bone (Hammarström et al. 1997, Heijl 1997, Mellonig 1999, Yukna & Mellonig 2000, Sculean et al. 1999a, 2000a, b). Controlled clinical studies have indicated that in intrabony periodontal defects, open flap debridement (OFD) with the additional application of EMD may lead to significantly higher clinical attachment level (CAL) gains and defect fill than OFD alone (Heijl et al. 1997, Pontoriero et al. 1999, Okuda et al. 2000, Froum et al. 2001, Sculean et al. 2001, Silvestri et al. 2000, Tonetti et al. 2002, Zuchelli et al. 2002). Clinical reports have suggested that because of its fluid consistency, EMD possesses a limited space-making potential that in turn, may comport the risk of a flap collapse following its application (Mellonig 1999, Lekovic et al. 2000). In order to overcome this problem and to further improve the clinical outcome of the therapy, attempts have been made for combining EMD with either guided tissue regeneration (GTR) or with various types of bone substitutes (Lekovic et al. 2000, Sculean et al. 2000a, 2001, 2002a, b, 2003, Scheyer et al. 2002, Velasquez-Plata et al. 2002). Recent data from controlled histological and clinical studies have, however, failed to demonstrate an advantage of the combination EMD+GTR compared with EMD alone or to GTR alone (Sculean et al. 2000a, 2001). Furthermore, when treating intrabony defects, the combination of EMD+bone substitutes did not further enhance the healing process than treatment with bone substitutes alone (Scheyer et al. 2002, Sculean et al. 2002a, b, 2003). On the other hand, recent data have indicated that the combination of EMD and a cancellous bovine-derived xenograft (BDX) may lead to higher bone fill and less gingival recession (GR) compared with treatment with EMD alone (Lekovic et al. 2000, Velasquez-Plata et al. 2002). Another clinical study comparing EMD alone to BDX+GTR has indicated that both techniques may lead to significant improvements in clinical and radiographic parameters, although no significant differences between the treatments were found (Pietruska 2001).

Very recently, a combination of EMD and a bioactive glass (BG) was suggested for preventing the collapse of the mucoperiosteal flap, thus minimizing soft-tissue recession (Sculean et al. 2002a). Results from a controlled clinical study have failed to demonstrate that the combination of EMD+BG may result in greater improvements than those obtained following treatment with BG alone (Sculean et al. 2002a). However, at the time being, to the best of our knowledge there are no published studies comparing the treatment of intrabony defects with a combination of EMD+BG to EMD alone.

Therefore, the aim of this controlled clinical study was to compare the treatment of intrabony defects with EMD+BG to EMD alone.

Material and Methods

Thirty patients (16 females and 14 males) suffering from advanced marginal periodontitis were included in this prospective, controlled parallel design multicenter study (i.e. 15 patients in each group) after having signed an informed consent form. None of the patients included was a smoker. The study was performed according to the declaration of Helsinki as revised in 1983. The criteria needed for inclusion in the study were: (1) no systemic diseases which could influence the outcome of the therapy, (2) a good level of oral hygiene plaque index (PI < 1) (Löe 1967), (3) compliance with the maintenance program, (4) presence of one intrabony defect with a probing depth (PD) 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 1 week prior and 1 year after the surgical procedure using the same periodontal probe (CP-15UNC Probe, Hu–Friedy, Chicago, IL, USA): PI, gingival index (GI) (Löe 1967), bleeding on probing (BOP), PD, GR, and CAL. The mea-

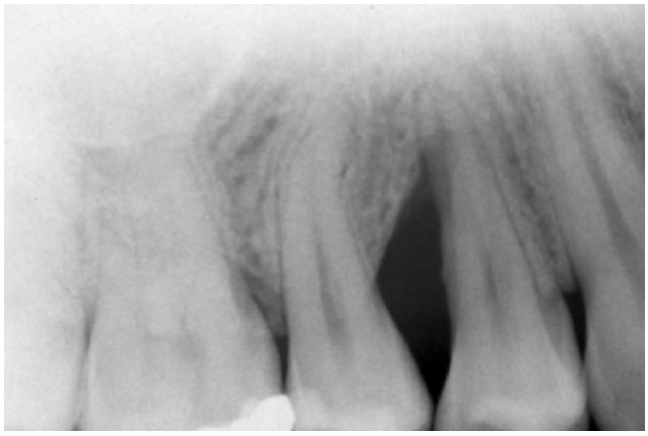

Fig. 1. The preoperative X-ray revealed the presence of an intrabony defect.

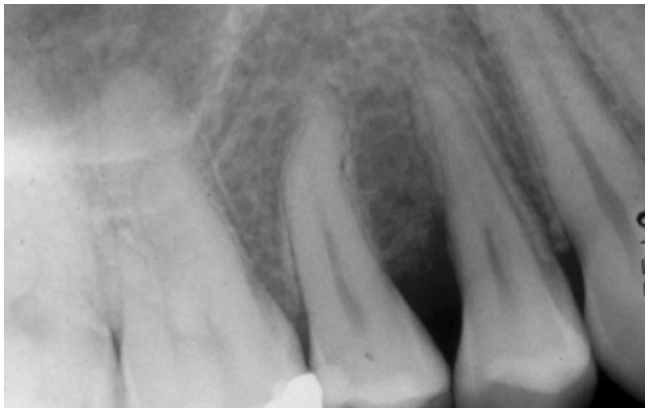

Fig. 2. At 1 year after treatment, the X-ray demonstrates a hard tissue fill of the intrabony component.

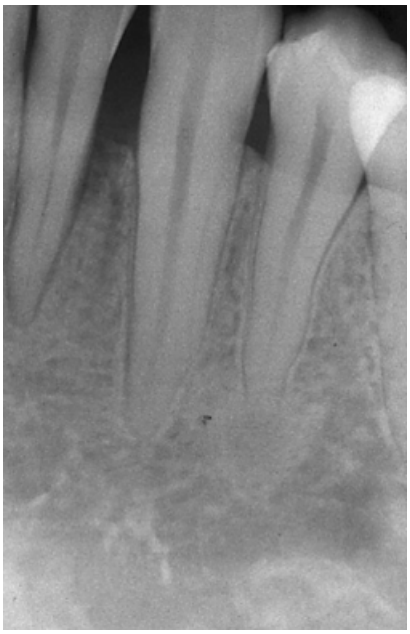

Fig. 3. The preoperative X-ray demonstrated the presence of an intrabony defect.

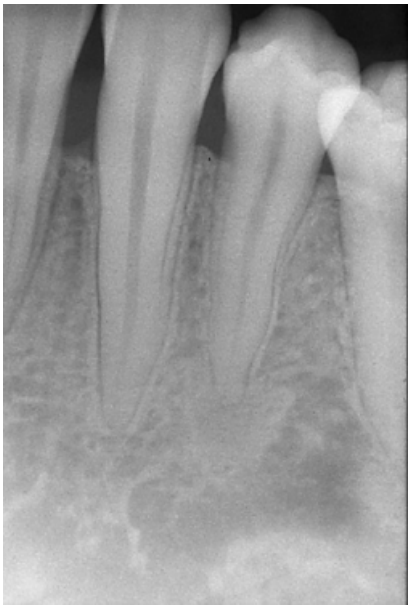

Fig. 4. At 1 year after treatment with enamel matrix protein derivative the X-ray demonstrates a hard-tissue fill of the intrabony component.

surements were made at six sites per tooth: mesiovestibular, midvestibular, distovestibular, mesio-oral, midoral, disto-oral by two calibrated investigators who were not the same as the surgeons. Examiner calibration was performed as follows: five patients, not enrolled in the study, and showing at least four teeth with PDs $\geqslant$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 $\geqslant$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 measurements at the same deepest point of the selected defect. Pre- and postoperative radiographs were taken with the long cone paralleling technique (Figs 1–4). Before surgery, the defects were randomly assigned by a toss of coin to the two treatment groups after controlling for the depth of the intrabony component and CAL. The depth of

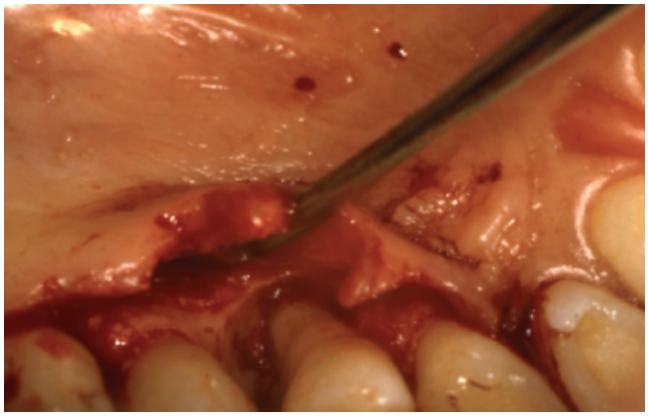

Fig. 5. Intraoperative view of the defect.

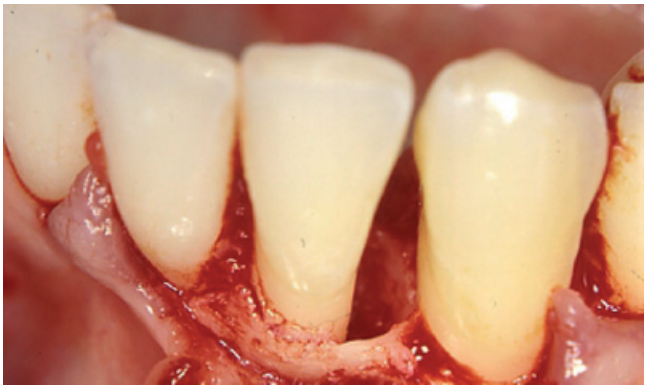

Fig. 6. Intraoperative view prior to application of enamel matrix protein derivative.

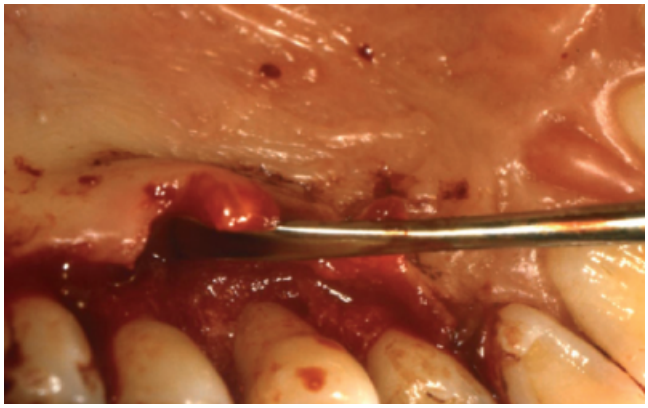

Fig. 7. Following removal of granulation tissue, root planing and conditioning with ethylenediaminetetraacetic acid, the defect was filled with the combination of enamel matrix protein derivative+bioactive glass.

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

	EMD	EMD+BG
Plaque index scores		
baseline	0.4 ± 0.2	0.5 ± 0.3
12 months	0.4 ± 0.3	0.4 ± 0.4
Gingival index scores		
baseline	1.1 ± 0.3	1.2 ± 0.4
12 months	0.4 ± 0.4	0.5 ± 0.4
Bleeding scores		
baseline	50%	52%
12 months	22%	28%

EMD, enamel matrix protein derivative; BG, bioactive glass.

Table 2. Distribution and configuration of treated defects ($n = 15$ for each group)

	EMD+BG	EMD
1–2 wall	6	7
2 wall	7	6
3 wall	2	2

EMD, enamel matrix protein derivative; BG, bioactive glass.

the intrabony component was estimated before surgery on radiographs.

Surgical procedure

The operative procedures were performed under local anesthesia by two surgeons (A.S. and M.P.). Following intracrevicular incisions, full thickness mucoperiosteal flaps were raised vestibularly and orally. Vertical releasing incisions were performed only if necessary for a better access or, to achieve a better closure of the surgical site. All granulation tissue was removed from the defects and the roots were thoroughly scaled and planed using hand and ultrasonic instruments. After defect debridement, the root surfaces adjacent to the defects were conditioned for 2 min with EDTA gel (pH 6.7) (PrefGel®, BIORA, Malmo, Sweden) 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 ethylenediaminetetraacetic acid residues.

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) (Figs 5 and 6).

Table 3. Baseline defect characteristics expressed in mm (mean ± SD)

Treatment	PD (mm)	GR (mm)	CAL (mm)	CEJ-BD (mm)	CEJ-BC (mm)	INTRA (mm)
EMD+BG ($n = 15$)	8.5 ± 1.1	1.9 ± 1.1	10.4 ± 1.5	11.3 ± 1.3	7.0 ± 1.2	4.3 ± 1.0
EMD ($n = 15$)	8.5 ± 1.5	1.5 ± 1.4	10.2 ± 2.1	11.2 ± 1.4	7.1 ± 1.3	4.1 ± 1.1

EMD, enamel matrix protein derivative; BG, bioactive glass; PD, probing depth; GR, gingival recession; CAL, clinical attachment level; CEJ-BD, cemento-enamel junction to the bottom of the defect; CEJ-BC, cemento-enamel junction to the bone crest; INTRA, intrabony component.

Table 4. Clinical parameters at baseline and 1 year for the test and control groups ($n = 15$ for each group)

	Baseline	1 year	Difference	Significance (p)
Probing depth				
EMD+BG	8.5 ± 1.1	4.4 ± 1.2	4.2 ± 1.4	<0.001
EMD	8.5 ± 1.5	4.0 ± 1.6	4.5 ± 2.0	<0.001
			NS	
Gingival recession				
EMD+BG	1.9 ± 1.1	2.8 ± 0.9	1.1 ± 0.8	<0.01
EMD	1.5 ± 1.4	2.4 ± 1.6	0.9 ± ± 0.7	<0.01
			NS	
Clinical attachment level				
EMD+BG	10.4 ± 1.5	7.1 ± 1.5	3.2 ± 1.7	<0.001
EMD	10.2 ± 2.1	6.3 ± 2.2	3.9 ± 1.8	<0.001
			NS	

EMD, enamel matrix protein derivative; BG, bioactive glass; NS, not significant.

Following root conditioning, in all defects the EMD gel was first applied on the root surfaces and then into the defects (Emdogain Gel®, BIORA). The test defects were additionally filled up with the mixture of EMD+BG (Emdogain Gel TS®, BIORA) (Fig. 7). In both groups, the mucoperiosteal flaps were repositioned coronally and fixed with vertical or horizontal mattress sutures.

Postoperative care

The postoperative care consisted of 0.2% chlorhexidine rinses twice a day for 4 weeks. The sutures were removed 14 days after the surgery. Recall appointments were scheduled every second week during the first 6 months after surgery and monthly for the rest of the observation period. Neither probing nor subgingival instrumentation were performed during the first year after surgery.

Statistical analysis

The statistical analysis was performed using a commercially available software program (SPSS® for Windows, Chicago 1997). The primary outcome variable was the CAL. In the calculations, the deepest site per tooth was included. 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. 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 0.80.

Results

The postoperative healing was considered as generally uneventful. Minor complications were related to usual postoperative swelling and occurred within the first days after surgery. The mean PI, GI and BOP at the treated sites, for each of the two groups, at baseline and after 1 year are summarized in Table 1.

The mean PI did not reveal a statistically significant difference in any of the two groups when compared with baseline or between the groups. In both groups, the GI and BOP improved significantly compared with baseline ($p < 0.001$). However, at 1 year the difference between the groups was not statistically significant.

Table 5. Frequency distribution of CAL gain in the test and control groups ($n = 15$ for each group)

CAL gain (mm)	Test (EMD+BG)		Control (EMD)	
	N	%	*N*	%
−1	1	7	0	0
1	1	7	2	13
2	1	7	0	0
3	5	33	5	33
4	5	33	3	20
5	1	7	2	13
6	1	7	2	13
7	0	0	0	0
8	0	0	1	7

EMD, enamel matrix protein derivative; BG, bioactive glass; CAL, clinical attachment loss.

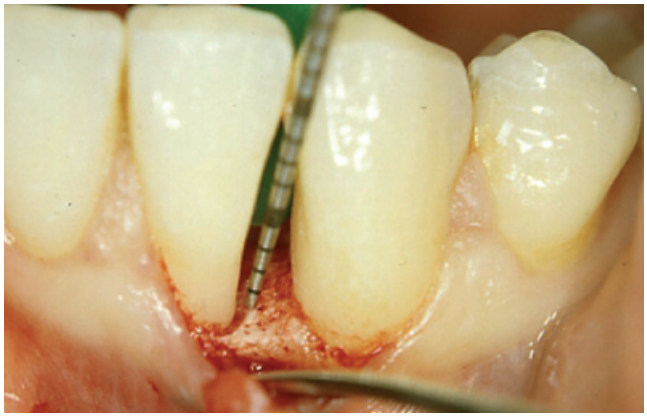

Fig. 8. At re-entry, an almost complete fill of the intrabony defect component was evident.

The distribution of the defects according to their configuration is presented in Table 2. No differences in the distribution of the defects were found between the two groups.

Baseline defect characteristics are presented in Table 3. At baseline, no differences in the depth of the intrabony component were found between the two groups.

The clinical results at 1 year after treatment are presented in Table 4.

At 1 year after therapy, the EMD + BG group showed a reduction in mean PD from 8.5 ± 1.1 to 4.4 ± 1.2 mm ($p < 0.001$) and a change in mean CAL from 10.4 ± 1.5 to 7.1 ± 1.5 mm ($p < 0.001$). In EMD control group, the mean PD was reduced from 8.5 ± 1.5 to 4.0 ± 1.6 mm ($p < 0.001$) and the mean CAL changed from 10.2 ± 2.1 to 6.3 ± 2.2 mm ($p < 0.001$). The frequency distribution of CAL gain for both treatment groups is shown in Table 5. In the test group, 12 sites (80%) gained at least 3 mm or more of CAL, whereas in the control group a CAL gain of 3 mm or more was measured at 13 sites (87%). No statistically significant differences in terms of PD reduction and CAL gain were observed between the two groups.

A re-entry surgery was performed in four cases (two treated with EMD+BG and two with EMD) indicating a fill of the osseous defects (Fig. 8).

Discussion

The results of this study have demonstrated that treatment of deep intrabony defects with both, the combination of EMD+BG and EMD alone may lead to statistically significant PD reductions and CAL gains. No statistically and clinically significant differences in any of the investigated parameters were observed between the two treatment modalities. It should, however, be pointed out 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 much higher number of patients and defects would be needed to detect an eventual difference between the treatments (Gunsolley et al. 1998). Based on the histological evidence from human material it may be assumed that the clinical improvements following both treatments may represent, at least to some extent, a real periodontal regeneration characterized by formation of cementum, periodontal ligament and bone (Heijl 1997, Mellonig 1999, Sculean et al. 1999a, 2000b, in press, Yukna & Mellonig 2000). The observations made in the cases where a re-entry surgery was performed, seem also to indicate that both treatments may facilitate hard-tissue formation and defect fill in deep intrabony defects.

The results obtained in the EMD+BG group corroborate the findings from a recent controlled clinical study where, at 1 year after treatment a mean CAL gain of 3.2 mm was obtained and no postoperative complication occurred (Sculean et al. 2002a).

Similarly, the clinical results obtained following application of EMD are in agreement with previously published data (Heijl et al. 1997, Heden et al. 1999, Pontoriero et al. 1999, Sculean et al. 1999b, c, 2001, Okuda et al. 2000, Silvestri et al. 2000, Froum et al. 2001, Tonetti et al. 2002, Trombelli et al. 2002, Zuchelli et al. 2002).

On the other hand, it is important to point out that in the present study the additional placement of BG did not seem to further improve the results. There might be several explanations for these findings. First of all, the number of treated cases was rather limited and thus, it cannot be excluded that a higher number of cases might have allowed for assessing eventual differences between the two groups (Tonetti et al. 2002). Secondly, because of the configuration of the defects (i.e. predominantly 2 or 1–2 walled) a collapse of the mucoperiosteal flaps during the healing phase might have been prevented by the bony walls, thus allowing for sufficient stability of the blood clot (Wikesjö & Selvig 1999). However, to the best of our knowledge, until now there are no other data from controlled clinical studies comparing treatment with EMD+BG to EMD alone, and therefore a comparison with other studies with a similar design is not possible. In this context, it should be kept in mind that the available clinical and histological data following treatment of intrabony defects with various types of combination appro-

aches such as EMD+bone substitutes or EMD+GTR do not seem to clearly indicate more favorable clinical and histological outcomes when compared with treatment with EMD alone, GTR alone or bone substitutes alone (Lekovic et al. 2000, Pietruska 2001, Scheyer et al. 2002, Velasquez-Plata et al. 2002, Sculean et al. 2000a, 2001, 2002a, b, 2003). Results from controlled histological and clinical studies have failed to show any additional improvements when comparing the combination of EMD+GTR to EMD alone or to GTR alone (Sculean et al. 2000a, 2001). When comparing the combination of EMD+bone substitutes to bone substitutes alone, no differences between the treatments were found (Scheyer et al. 2002, Sculean et al. 2002a, b, 2003). On the other hand, when comparing treatment of intrabony defects with EMD alone to the combination of EMD+a cancellous BDX, statistically significant differences for GR and bone fill, yielding a more favorable outcome towards the combined approach were reported (Lekovic et al. 2000, Velasquez-Plata et al. 2002). However, results from a controlled clinical study comparing treatment with EMD to that with BDX covered by a resorbable collagen membrane have indicated that both techniques may lead to significant and similar improvements in clinical and radiographic parameters (Pietruska 2001).

In conclusion, within their limits, the present results indicate that: (i) at 1 year after surgery both therapies resulted in significant PD reductions and CAL gains, and (ii) the combination of EMD+BG does not seem to additionally improve the clinical results.

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