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Four-year results of a prospective-controlled clinical study evaluating healing of intra-bony defects following treatment with an enamel matrix protein derivative alone or combined with a bioactive glass

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Abstract

Aim: To evaluate the 4-year results following regenerative periodontal surgery at intra-bony defects with either a combination of an enamel matrix protein derivative (EMD) and a bioactive glass (BG) or with EMD alone.

Methods: Twenty-five patients with one deep intra-bony defect each were randomly treated with either an EMD+BG (test) or with EMD alone (control). Measurements were recorded at baseline, at 1 and at 4 years following therapy. The primary outcome variable was the clinical attachment level (CAL).

Results: The test group demonstrated a mean CAL change from 10.3 ± 1.6 to 6.7 ± 1.2 mm ($p < 0.001$) and to 6.9 ± 1.0 mm ($p < 0.001$) at 1 and 4 years, respectively. No statistically significant differences were found between the 1- and 4-year results. The control group showed a mean CAL change from 10.4 ± 1.6 to 6.7 ± 1.1 mm ($p < 0.001$) at 1 year and 7.0 ± 0.9 mm ($p < 0.001$) at 4 years. The CAL change between 1 and 4 years did not present statistically significant differences. In each of the two groups, four defects have lost 1 mm of the CAL gained at 1 year. A CAL gain of 1 mm compared with the 1-year results was measured in only one defect of the test group. Compared with baseline, a CAL gain of $\geqslant 3$ mm was found at 4 years in 10 defects in both groups.

Between the treatment groups, no statistically significant differences in any of the investigated parameters were observed at 1 and at 4 years.

Conclusions: Within their limits, the present results indicate that the clinical improvements obtained with both regenerative modalities can be maintained over a period of 4 years.

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

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Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.
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Regenerative periodontal surgery with an enamel matrix protein derivative (EMD) has been proven to promote periodontal regeneration [i.e. new cementum, new periodontal ligament (PDL) and new alveolar bone] (Hammarström et al. 1997, Heijl 1997, Mellonig 1999, Sculean et al. 1999, 2000a, b, Yukna & Mellonig 2000, Majzoub et al. 2005). Findings from in vitro studies indicate that EMD may modulate the behaviour of a variety of dental and non-dental cell types by up-regulating cAMP levels, inducing synthesis and secretion of transforming growth factor-β (TGF-β) and interleukin-6 (IL-6) in periodontal ligament (PDL) cells and gingival fibroblasts and by stimulating proliferation of pre-osteoblasts and differentiation of immature osteoblasts (Schwartz et al. 2000, Van der Pauw et al. 2000, Lyngstadaas et al. 2001, Okubo et al. 2003). It has also been suggested that EMD may act as a cytostatic agent on cultured epithelial cells and may even inhibit dental plaque vitality (Kawase et al. 2000, Sculean et al. 2001, Arweiler et al. 2002). Thus, the available data seem to indicate that EMD may influence periodontal wound healing by an indirect stimulatory effect on the release of growth factors during periodontal wound healing and by inhibiting or at least retarding epithelial downgrowth (Kawase et al. 2000, Schwartz et al. 2000, Van der Pauw et al. 2000, Lyngstadaas et al. 2001, Okubo et al. 2003).

Results from controlled clinical studies have shown that treatment of intra-bony defects with open-flap debridement combined with EMD application may lead to higher clinical attachment gains than treatment with open-flap debridement alone (Tonetti et al. 2002, Esposito et al. 2005). Moreover, the histological and clinical results obtained following treatment with EMD are comparable with those obtained after guided tissue regeneration (GTR), still considered as one of the standard procedures in regenerative therapy (Esposito et al. 2005). Data from long-term follow-up studies have demonstrated that the clinical results obtained with EMD are stable throughout time (Heijl et al. 1997, Sculean et al. 2003, 2004, 2006, Francetti et al. 2004, Heden & Wennström 2006).

The viscous nature of the EMD formulation may not provide the required soft tissue support in order to prevent gingival recession (GR) during healing (Mellonig 1999, Lekovic et al. 2000, Velasquez-Plata et al. 2002, Zucchelli et al. 2003, Gurinsky et al. 2004). This limited potential for soft tissue/flap support may compromise periodontal regeneration (Wikesjö & Selvig 1999). It was suggested that a combination of EMD and a bone replacement graft could represent a valuable approach to overcome this potential problem in non-contained periodontal defects requiring additional soft tissue support (Lekovic et al. 2000, Velasquez-Plata et al. 2002, Zucchelli et al. 2003, Gurinsky et al. 2004, Bokan et al. 2006). This may allow for a combination of the biologic properties of EMD with the tissue-supporting properties of a graft material, thus potentially improving the therapy outcome.

Bioactive glass (BG) is an alloplastic bone graft and a possible choice for a synthetic graft material to be mixed with EMD in order to overcome the possible limitations of the fluid nature of the EMD formulation. Results from clinical studies have indicated that treatment of intra-bony defects with BG alone may result in improved clinical outcomes as evidenced by probing depth (PD) reduction and clinical attachment-level (CAL) gain (Low et al. 1997, Zamet et al. 1997, Froum et al. 1998, Lovelance et al. 1998). This material has good clinical manageability, also possessing certain haemostatic properties (Low et al. 1997, Zamet et al. 1997, Froum et al. 1998, Lovelance et al. 1998, Sculean et al. 2002). A recent histologic study evaluating the healing of human intra-bony defects following treatment with EMD+BG or with BG alone has shown that the combined treatment approach resulted in the formation of new cementum with an associated PDL as well as enhanced mineralization around the BG particles while there was a low potential for BG alone to facilitate periodontal regeneration (Sculean et al. 2005a). The available data from short-term (8 months to 1 year) controlled clinical studies comparing the treatment of intra-bony defects with EMD+BG with EMD alone are somewhat controversial (Sculean et al. 2005b, Kuru et al. 2006). While some authors showed no added benefit of the combined approach (Sculean et al. 2005b), others suggested that the combined treatment seems to enhance the results in wide intra-bony defects (Kuru et al. 2006). However, to date, there are virtually no data on the long-term clinical outcome following treatment of intra-bony defects with EMD+BG.

The aim of this prospective, controlled clinical study is to present the 4-year clinical results following treatment of intra-bony defects with either EMD+BG or EMD alone.

Materials and Methods

The study design, patient population and the short-term (1-year data) results have been described in detail previously (Sculean et al. 2005b). Briefly, a total of 30 patients (16 females and 14 males) suffering from advanced chronic marginal periodontitis were included in this prospective, controlled parallel-design multicentre study. The study was in accordance with the Helsinki Declaration of 1975, as revised in 1983. However, only 25 patients (14 females and 11 males) with a mean age of (46 ± 7.5 years) (range 38–55 years) completed the 4-year evaluation. The other five patients were lost during follow-up for the following reasons: four patients refused to participate in the evaluation and preferred to visit their own dentists for regular care, and one patient moved away. Therefore, in the following only the data of the 25 available patients are presented. None of the patients was a smoker. The criteria needed for inclusion in the study were as follows: (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 programme and (4) the presence of one intra-bony defect with a PD of at least 6 mm and an intra-bony component of at least 3 mm as detected on the radiographs.

The following clinical parameters were assessed 1 week before, at 1 year and at 4 years after the surgical procedure using the same periodontal probe (UNC 15, Hu-Friedy, Chicago IL, USA): PI, gingival index (GI) (Löe 1967), bleeding on probing (BOP), PD,

GR and CAL. The measurements were made at six sites per tooth: mesiovestibular (mv), midvestibular (v), distovestibular (dv), mesiooral (ml), midoral (l) and distooral (dl) by two calibrated investigators (not listed as authors) 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 probing depths $\geqslant 6$ 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 millimetre 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, at baseline the deepest, point of the selected defect. Pre- and post-operative radiographs were taken with the long cone paralleling technique. Before surgery, the defects were randomly assigned by a toss of coin to the two treatment groups after controlling for the depth of the intra-bony component and CAL. The depth of the intra-bony component was estimated before surgery on radiographs.

Surgical procedure

The operative procedures were performed under local anaesthesia by two surgeons (A. S. and M. P.). From the present patient population, 13 were treated by A. S. in one centre (seven with EMD and six with EMD+BG) while 12 were treated by MP in the second centre (six with EMD and six with EMD+BG). Following intra-crevicular incisions 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. During surgery, the following measurements were made: distance from the CEJ to the bottom of the defect (CEJ-BD) and distance from the CEJ to the most coronal extension of the alveolar bone crest (CEJ-BC). The intra-bony component (INTRA) of the defects was defined as (CEJ-BD)–(CEJ-BC).

After defect debridement, the root surfaces adjacent to the defects were conditioned for 2 min. with ethylenediamine tetra acetic acid (ETDA) gel (pH 6.7) (PrefGel®, previously BIORA, Sweden now 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, in all defects the EMD gel was first applied on the root surfaces and then into the defects (Emdogain Gel®, BIORA Malmö, Sweden, previously BIORA, Sweden now Straumann, Basel, Switzerland). The test defects were additionally filled up with a mixture of EMD+BG (Emdogain Gel TS®, previously BIORA now Straumann). In both groups, the periostea at the base of the mucoperiosteal flaps were incised to allow tension-free coronal positioning. Finally, the flaps were closed with vertical or horizontal mattress sutures.

Post-operative care and maintenance

The post-operative care consisted of 0.2% chlorhexidine rinses twice a day for 4 weeks and administration of analgesics (2×600 mg Ibuprofen/day for 3 days). The sutures were removed 14 days after the surgery. Recall appointments were scheduled every second week during the first 6 months after surgery and then monthly during the first year after surgery. Neither probing nor subgingival instrumentation were performed during the first year after surgery. After the first year and during the rest of the observation period of 4 years, patients were recalled every 3 months. The recall appointments consisted mainly of reinforcement of oral hygiene measures and professional supragingival tooth cleaning.

Statistical analysis

The statistical analysis was performed using a commercially available software program (SPSS® for Windows, Chicago, IL, USA, 2003). In the statistical evaluation, only the baseline, 1- and the 4-year data of the 25 available patients have been considered. The primary outcome variable was the CAL. In the calculations, the same, at baseline the deepest, site per tooth was included. For the statistical evaluation of the changes from baseline to 1 year and baseline to 4 years, the paired *t*-test was used. For comparisons between the groups, the unpaired *t*-test was used. The α error was set at 0.05. The power of the study, considering 1 mm as a significant difference between the groups, was calculated to be 0.70.

Results

The post-operative healing was considered to be generally uneventful. Minor complications were related to usual post-operative swelling and occurred within the first days after surgery. The observations on the early post-operative healing have been described in detail elsewhere (Sculean et al. 2005b). No adverse reactions related to EMD or BG were observed.

At baseline, there were no statistically significant differences between the two groups in any of the investigated clinical parameters (Tables 1, 2 and 3).

The PI, GI and BOP for both treatment groups at baseline and after 1 and 4 years are summarized in Table 1. The mean PI did not reveal a statistically significant difference between the two groups at baseline and after 1 and 4 years. Although at 4 years the PI increased slightly in both treatment groups, this difference was not found to be statistically significant compared with the baseline or with the 1-year results. A statistically significant difference was observed within both treatment goups, when comparing the 1 and 4 years GI and BOP with the baseline values ($p < 0.001$). However, no statistically significant differences were observed between the 1- and the 4-year results (Table 1).

The baseline defect characteristics are presented in Table 2. No statistically significant difference in the initial depth of the intra-bony component was found between the two groups. The distribution of the defects according to their configuration is presented in Table 3.

The PD, GR and CAL at baseline and at 1 and 4 years after treatment are presented in Table 4. At 1 year, the PD decreased statistically highly significantly in both groups ($p < 0.001$) (Table 4). Between the groups no statistically significant difference was found. At 4 years, a slight, statistically insignificant increase in PD was measured in both groups. At 4 years, the PD was still statistically highly improved compared

Table 1. Plaque Index (PI), Gingival Index (GI) and Bleeding on Probing (BOP) at baseline at 1 and at 4 years following treatment with test or control

Parameter	Treatment	Baseline	1 year	*p*-value (baseline–1 year)	4 years	*p*-value (1–4 years)
PI						
	Test ($n = 12$)					
	Mean (± SD)	0.8 ± 0.4	0.9 ± 0.4	NS	1.2 ± 0.8	NS
	Control ($n = 13$)					
	Mean (± SD)	0.7 ± 0.5	0.7 ± 0.5	NS	1.1 ± 0.7	NS
GI						
	Test ($n = 12$)					
	Mean (± SD)	1.7 ± 0.5	0.6 ± 0.4	<0.001	1.0 ± 1.0	NS
	Control ($n = 13$)					
	Mean (± SD)	1.8 ± 0.8	0.8 ± 0.6	<0.001	1.1 ± 0.9	NS
BOP						
	Test ($n = 12$)					
	Mean (± SD)	53%	34%	<0.001	38%	NS
	Control ($n = 13$)					
	Mean (± SD)	49%	36%	<0.001	40%	NS

No significant differences between the test and control groups were found.
BOP, bleeding on probing; GI, gingival index; PI, plaque index.

Table 2. Baseline defect characteristics expressed in mm (mean ± SD)

Treatment	PD (mm)	GR (mm)	CAL (mm)	CEJ-BD (mm)	CEJ-BC (mm)	INTRA (mm)
Test ($n = 12$)	8.6 ± 1.0	1.7 ± 1.0	10.3 ± 1.6	11.6 ± 1.4	7.2 ± 1.2	4.4 ± 1.5
Control ($n = 13$)	8.6 ± 0.9	1.8 ± 1.2	10.4 ± 1.6	11.8 ± 1.5	7.3 ± 1.3	4.5 ± 1.4

CAL, clinical attachment level; GR, gingival recession; PD, probing depth; CEJ, cemento-enamel junction.

Table 3. Distribution and configuration of treated defects

	Test ($N = 12$)	Control ($N = 13$)
1–2 wall	6	7
2 wall	5	5
3 wall	1	1

with baseline ($p < 0.001$) (Table 4). At 1 and 4 years, the GR values were statistically significantly increased compared with the baseline ($p < 0.001$) in both groups; however, the difference between the groups was not statistically significant (Table 4). At 4 years, the GR values improved in both groups compared with the 1-year results, but the difference did not reach statistical significance (Table 4). No statistically significant differences between the two groups were found at 4 years. At 1 and at 4 years the CAL improved statistically highly significantly within both groups compared with the baseline ($p < 0.001$) (Table 4). Within the groups, and between the two treatments, no statistically significant differences between the 1- and the 4-year results were found (Table 4).

At 4 years, in each of the two groups, four defects lost 1 mm of the CAL gained at 1 year (Table 5). A CAL gain of 1 mm compared with the 1-year results was measured in only one defect of the test group (Table 5). Compared with the baseline, a CAL gain of $\geqslant 3$ mm was found at 4 years in 10 defects in both groups (Table 6).

Discussion

The present results have shown that the treatment of intra-bony defects with both a combination of EMD+BG and EMD alone may lead to statistically significant PD reductions and CAL gains that can be maintained over a period of 4 years. No statistically significant differences between the two treatment modalities in any of the investigated clinical parameters were found at 1 and 4 years. In both groups a slight, statistically insignificant, loss of mean CAL was measured between the 1- and 4-year evaluation period. The observation that no adverse reactions such as allergies or abscesses occurred in any of the patients corroborates previous results and suggests that both EMD and the combination of EMD+BG is well tolerated (Heijl et al. 1997, Low et al. 1997, Zamet et al. 1997, Froum et al. 1998, Lovelance et al. 1998, Sculean et al. 1999, 2000a, 2002, 2005a, 2005b, Tonetti et al. 2002, 2004).

The finding that treatment of intra-bony defects with EMD may result in statistically significantly improvements in PD and CAL on a short-term basis (up to 1 year) compared with baseline is in agreement with previous results (Tonetti et al. 2002, Esposito et al. 2005). The present 1-year results obtained with EMD are in line with the conclusions of a recent systematic review that has analysed the potential benefit of EMD when used in addition to OFD (Esposito et al. 2005). The results have shown that EMD treated sites displayed statistically significant CAL improvements [i.e. mean difference 1.2 mm, 95% confidence interval (CI) 0.7–1.7] when compared with OFD (Esposito et al. 2005). The 4-year results obtained with EMD group are also in agreement with other long-term data (Heijl et al. 1997, Sculean et al. 2003, 2004, 2006, Heden & Wennström 2006). In a controlled clinical trial comparing treatment of intra-bony defects with EMD with that with flap surgery, Heijl et al. (1997) reported, at 8 months, a mean CAL gain of 2.1 mm after treatment with EMD and of 1.5 mm after flap surgery alone (control). In a 4-year follow-up study, a total of 46 intra-bony defects in 33 patients were consecutively treated with EMD (Sculean et al. 2003); the results have indicated stable results at 1 year post-operatively. Moreover, the re-entry surgery performed after 4 years in one case has demonstrated an almost complete fill of the intra-bony component. Thus, the present 4-year results indicate that the clinical improvements obtained following EMD treatment may be maintained on a long-term basis, provided that an adequate plaque control programme is maintained (Heijl et al. 1997, Sculean et al. 2003, 2004, 2006, Heden & Wennström 2006).

The 1-year results obtained in the EMD+BG group are in agreement with the findings from a previous controlled clinical study where a mean CAL gain of 3.2 mm was obtained and no post-operative complications occurred (Sculean et al. 2002). When interpreting the clinical results obtained with EMD+BG, the findings of a previous human histological study should be

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

Parameter	Treatment	Baseline	1 year	*p*-value (baseline–1 year)	4-years	*p*-value (1–4 years)
PD						
	Test ($n = 12$)					
	Mean (± SD)	8.6 ± 1.0	4.1 ± 1.0	<0.001	4.5 ± 1.0	NS
	Control ($n = 13$)					
	Mean (± SD)	8.6 ± 0.9	3.9 ± 0.6	<0.001	4.4 ± 0.6	NS
GR						
	Test ($n = 12$)					
	Mean (± SD)	1.7 ± 1.0	2.6 ± 0.9	<0.01	2.4 ± 0.6	NS
	Control ($n = 13$)					
	Mean (± SD)	1.8 ± 1.2	2.8 ± 1.0	<0.01	2.7 ± 0.8	NS
CAL	Test ($n = 12$)					
	Mean (± SD)	10.3 ± 1.6	6.7 ± 1.2	<0.001	6.9 ± 1.0	NS
	Control ($n = 13$)					
	Mean (± SD)	10.4 ± 1.6	6.7 ± 1.1	<0.001	7.0 ± 0.9	NS

No significant differences between the test and control groups were found.
CAL, clinical attachment level; GR, gingival recession; PD, probing depth.

Table 5. Frequency distribution of CAL change from 1 year to 4 years in the test and control groups

CAL change (mm)	Test ($n = 12$)		Control ($n = 13$)	
	No	%	No	%
− 1	4	33	4	31
0	7	59	9	69
1	1	8		

CAL, clinical attachment level.

Table 6. Frequency distribution of CAL change after 4 years in the test and control groups

CAL change (mm)	Test ($n = 12$)		Control ($n = 13$)	
	No	%	No	%
− 1	1	8	0	0
0	0	0	0	0
1	0	0	1	8
2	1	8	2	15
3	3	25	3	23
4	5	42	6	46
5	2	17	0	0
6	0	0	1	8

CAL, clinical attachment level.

pointed out that have demonstrated periodontal regeneration following treatment of advanced intra-bony defects with this combination approach. It may thus be suggested that the regenerative potential reported for EMD does not seem to be blocked if EMD is combined with BG (Sculean et al. 2005a). Taken together, the data from human histologic material suggest that the clinical improvements obtained with EMD alone or a combination of EMD+BG may reflect a regenerative type of healing characterized by the formation of cementum, PDL and bone (Heijl 1997, Mellonig 1999, Sculean et al. 1999, 2000a, 2005a, Yukna & Mellonig 2000, Majzoub et al. 2005). On the other hand, observations from human histologic studies have failed to demonstrate periodontal regeneration following treatment of intra-bony defects with BG alone (Nevins et al. 2000, Sculean et al. 2005a). In the present study, the additional placement of BG did not further improve the clinical results obtained with EMD. When interpreting this finding, it should be kept in mind that in the present study the number of available defects was rather limited and thus, the study may not have the statistical power to rule out the possibility of a difference between the two groups (Gunsolley et al. 1998). Furthermore, the limited number of defects and their heterogenity in configuration (i.e. one, two or three walls) have made further analysis of the possible influence of defect parameters upon the clinical outcomes not possible. Further prospective, randomized, controlled clinical studies with a sufficient number of patients and defects with well-defined inclusion criteria in terms of defect depth, width and number of bony walls are needed to allow for adequate analysis of a possible influence of defect parameters upon the clinical outcome (Gunsolley et al. 1998, Tsitoura et al. 2004). The need for further trials evaluating the potential benefit of a combination of EMD+BG seems to be supported by recent findings from a controlled clinical study indicating that in deep and large intra-bony defects a combination of EMD+BG may indeed result in less GR and higher CAL gain compared with treatment with EMD alone (Kuru et al. 2006).

The findings that the plaque and bleeding scores were not statistically significantly increased at 4 years compared with the 1-year data indicate that throughout the entire observation period, an optimal level of plaque control was maintained. Another important factor that strongly influences the outcome of regenerative periodontal treatment is smoking (Tonetti et al. 1995). As there were no smokers in the present patient population, it may be assumed that the long-term stability of the obtained results can mainly be attributed to the careful patient selection and maintenance of an optimal level of plaque control. When interpreting the present findings, it needs to be emphasized that long-term stability has also been demonstrated following conventional periodontal surgery, which indicates that from a clinical point of view, the main role of regenerative periodontal therapy is to achieve more support for the tooth and not to increase the stability against further progression of periodontal disease (Tonetti et al. 1996).

In conclusion, within their limits, the present results indicate that the clinical improvements obtained with both regenerative modalities can be maintained over a period of 4 years.

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Clinical Relevance

Scientific rationale for the study: Regenerative treatment with an EMD has been shown to promote periodontal regeneration in intrabony defects. Owing to the viscous nature of EMD formulation it was suggested that a combination of the EMD and a bone replacement graft may additionally improve the clinical outcome. Until now, there are limited data on the long-term clinical outcome following treatment of intra-bony defects with EMD and various types of bone replacement grafts. The purpose of the present study was to evaluate the 4-year results following treatment of intrabony defects with either a combination of EMD+BG or with EMD alone.

Principal findings: Both treatments showed at 1 and at 4 years statistically significant improvements in terms of CAL gain compared with baseline ($p<0.001$). There were no statistically significant differences in any of the two groups between the 1- and 4-year results. Between the treatment groups, no statistically significant differences in any of the investigated parameters were observed at 1 and at 4 years.

Practical implications: The present results suggest that the clinical improvements obtained with both regenerative modalities can be maintained over a period of 4 years.