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A multi-centre randomized controlled clinical trial on the treatment of intra-bony defects with enamel matrix derivatives/ synthetic bone graft or enamel matrix derivatives alone: results after 12 months

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Abstract

Objectives: Comparison of the clinical and radiographic outcomes of a combination of enamel matrix derivatives (EMD) and a synthetic bone graft (EMD/SBG) with EMD alone in wide ($\geqslant$2 mm) and deep ($\geqslant$4 mm) one- and two- wall intra-bony defects 12 months after treatment.

Materials and Methods: Seventy-three patients with chronic periodontitis and one wide ($\geqslant$2 mm) and deep ($\geqslant$4 mm) intra-bony defect were recruited in five centres in Germany. During surgery, defects were randomly assigned to EMD/SBG (test) or EMD (control). Assessments at baseline, after 6 and 12 months included bone sounding, attachment levels, probing pocket depths, bleeding on probing, and recessions. Changes in defect fill were recorded radiographically.

Results: Both treatment modalities led to significant clinical improvements. In the EMD/SBG group a mean defect fill of 2.7 $\pm$ 1.9 mm was calculated, in the EMD group the defect fill was 2.8 $\pm$ 1.6 mm. A mean gain in clinical attachment of 1.7 $\pm$ 2.1 mm in the test group and 1.9 $\pm$ 1.7 mm in the control group after 1 year was observed. Radiographic analysis confirmed for both groups that deeper defects were associated with greater defect fill.

Conclusion: The results show comparable clinical and radiographic outcomes following both treatment modalities 12 months after treatment.

Key words: bone replacement graft; enamel matrix derivative; intra-bony defects; periodontal regeneration; radiographic evaluation; randomized clinical trial

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Conflict of interest and source of funding statement

This study was supported by the Institut Straumann AG, Basel, Switzerland. Study design and development of protocol were performed in teamwork between the investigators of the five participating centres and an independent statistician. Authors had full control of the data and analyses and received no commercial input in the preparation of the paper. The authors declare that they have no conflict of interests.

During the past decades, different approaches and techniques have been used to regenerate lost periodontal structures (for a review, see Schallhorn & McClain 1993, Caton 1997, Becker & Becker 1999, Reddy & Jeffcoat 1999, Gestrelius et al. 2000, Needleman et al.

2001, Froum et al. 2002, Jepsen et al. 2002, Murphy & Gunsolley 2003, Esposito et al. 2005, Needleman et al. 2005, 2006, Bosshardt 2008, Sculean et al. 2008b).

In numerous studies, it has been demonstrated that enamel matrix derivatives (EMD) modulate the behaviour of cells in stimulating proliferation, inducing production of transforming growth factor-β as well as interleukin-6 and differentiation of immature cells in vitro (Schwartz et al. 2000, Tokiyasu et al. 2000, Van der Pauw et al. 2000, Hakki et al. 2001, Lyngstadaas et al. 2001, Giannobile & Somerman 2003, Okubo et al. 2003, Foster et al. 2006, 2008, Swanson et al. 2006, Sato et al. 2008).

EMD favour the formation of a new attachment apparatus *in vivo*, characterized by the presence of acellular and cellular cementum with inserting collagen fibres and new alveolar bone (Hammarstrom 1997, Hammarstrom et al. 1997, Heijl et al. 1997, Sculean et al. 2000, 2001, Jepsen et al. 2004, Meyle et al. 2004).

In several controlled clinical trials treatment of intra-bony defects with EMD resulted in significantly more attachment gain and bone fill than open flap debridement (Froum et al. 2001a, b, Tonetti et al. 2002, 2004a, b, Sanz et al. 2004, Esposito et al. 2005). EMD was also successfully used in class II furcation defects. Compared with guided tissue regeneration, EMD treatment resulted in reduced postoperative swelling and pain (Jepsen et al. 2004, Meyle et al. 2004, Hoffmann et al. 2006).

In wider defects, the viscous nature of EMD does not prevent the collapse of the soft tissue flap into the defect. Therefore, EMD have been combined with different space-maintaining products (e.g. membranes or bone substitutes) in order to enhance the space for periodontal regeneration (Pietruska 2001, Sculean et al. 2001, 2002, 2003, 2008b, Rosen & Reynolds 2002, Trombelli et al. 2002, Zucchelli et al. 2003, Dori et al. 2005, Donos et al. 2006, Trombelli & Farina 2008). Controlled clinical studies indicate that a combination of EMD and bovine-derived xenograft may enhance gain of clinical attachment (Lekovic et al. 2001, Zucchelli et al. 2002). It is still questionable, if graft materials are substituted by newly formed bone (Sculean et al. 2008c). Recently the combination of EMD with autogenous bone indicated that, this combined approach led to less recession as compared with EMD alone (Guida et al. 2007). Similar results were reported when EMD was compared with a bovine derived xenograft (Velasquez-Plata et al. 2002, Mellonig 2006). In a recent review, it was concluded that the additional use of a graft (autogenous bone, DFDBA, BPBM, bioactive glass) seems to enhance the clinical outcome of EMD over EMD alone (Trombelli & Farina 2008).

Biphasic calcium phosphates (BCP) have been used as bone substitutes in orthopaedic, cranio/maxillofacial, oral and periodontal surgery and have been shown to be biocompatible, safe, and effective scaffolds for the formation of new bone (Nery et al. 1992, Piattelli et al. 1996, Daculsi et al. 1999). Preclinical evidence suggests that a BCP with $>99\%$ crystallinity, consisting of 60% hydroxyapatite (HA) and 40% β-tri-calcium-phosphate (TCP) in particulate preparation may accelerate new bone formation (Nery et al. 1992).

In this study, we compared the clinical and radiographic outcomes of EMD in combination with synthetic bone substitute or EMD alone in the treatment of wide intra-bony defects after 12 months.

Materials and Methods

Experimental design

The amount of defect fill 6 and 12 months following two different regenerative treatments of one- and two- wall intra-bony periodontal defects was studied in a randomized, prospective, multi-centre controlled clinical trial. Details of the study protocol, statistical analysis, and clinical results after 6 months have been reported previously (Jepsen et al. 2008).

Briefly, an access flap was prepared with papilla preservation (Cortellini et al. 1995, 1999). After debridement, removal of granulation tissue and remaining subgingival calculus, EMD were applied (Straumann® Emdogain, Straumann, Basel, Switzerland). Subsequently, in the test group the defects were filled with a synthetic bone graft (SBG) (Straumann® BoneCeramic, Straumann), which had been mixed with EMD. In the controls, EMD was used alone. The flap was repositioned and closed with monofilament synthetic non-resorbable 5-0 and 6-0 suturing material (Ethicon Prolene, Ethicon Products, Norderstedt, Germany). All patients were controlled after 3, 6, 9, and 12 months. No subgingival instrumentation was performed at the surgical site.

Five centres participated involving a total of five operators and five masked examiners connected with and supervised by a central monitoring facility at the Institut Straumann AG, Basel, Switzerland.

Subject population

For a detailed description, see Jepsen et al. (2008). The study was performed in compliance with Good Clinical Practice and the Declaration of Helsinki lastly revised in Edinburgh 2000; the study protocol was approved by the International Ethics Committee in Freiburg, Germany.

Only patients with a diagnosis of severe periodontitis and a radiographic intra-bony defect of at least 4 mm depth, and 2 mm width without furcation involvement were included. Inclusion criteria were confirmed during surgery. Patients with uncontrolled or poorly controlled diabetes, unstable or life-threatening conditions, current pregnancy at the time of recruitment and smokers were not admitted. Only occasional smoking (1–30 cigarettes/month) was allowed.

All patients went through initial treatment including repeated oral hygiene instructions, professional tooth cleaning, and subgingival scaling and root planing. Patients had to demonstrate a full-mouth plaque index $\leqslant 25\%$ (O'Leary et al. 1972) at least one time out of two examinations before inclusion. At least two sessions of oral hygiene control were conducted.

Seventy-five patients gave informed consent and were enrolled. A randomization list was generated by an independent statistician based on one surgical site per patient for a total of 75 surgical sites. To conceal assignment, the investigator was instructed to assign a previously supplied sealed envelope containing the treatment assignment to the specific patient. The original randomization allocation could not be used for a replacement patient.

Clinical measurements

Clinical outcomes were evaluated after 6 and 12 months. The 6-month results have been reported previously (Jepsen et al. 2008). All measurements were

carried out using a customized acrylic stent with markings. Each of the centres had its own blinded and calibrated examiner. Full-mouth plaque scores (O'Leary et al. 1972) were recorded as the percentage of total surfaces (six aspects per tooth) that revealed plaque. The primary outcome variable was the change in bone fill after 6 months as measured by bone sounding. Secondary outcomes, i.e. probing pocket depths (PPD), relative attachment level (RAL) and gingival recessions (GR) were recorded with a computerized constant force probe (Florida Probe®, Gainesville, FL, USA) at six sites per tooth. Bleeding on probing was recorded concomitantly with PPD, RAL, and GR. All pocket depth and attachment measurements were adjusted to the nearest 0.2 mm. Following local anesthesia, vertical defect fill, as determined by bone sounding, was measured at the same six sites from the acrylic stent with a manual probe (PCP-UNC 15, Hu-Friedy, Leimen, Germany).

During surgery, width and depth of the intra-bony defect was assessed with a manual probe (PCP-UNC 15, Hu-Friedy). The following assessments were performed: (1) bone level (distance from stent to bottom of the defect); (2) defect depth (distance bone crest to bottom of bone defect); (3) defect width (horizontally from the bone crest at the experimental site in a direction towards the centre of the tooth); and (4) determination of the defect type (one-wall, two-wall, combined one- and two-wall or circumferential). Any adverse effect or post-surgical complications were recorded using a questionnaire.

Radiographic examination

Seventy-five pairs of intra-oral periapical radiographs were obtained using XCP film holders (Kentzler & Kaschner, Ellwangen, Germany). The position of the film holder in relation to the teeth was fixed by an impression of elastic silicone. Film size (0 or 2) and exposure time were chosen according to tooth type. The radiographs were obtained immediately before and 12 months after surgery using F-speed films (Insight, Kodac, Rochester, CT, USA).

Radiographic evaluation

All radiographs were sorted in random order and numbered from 1 to 150 by the investigator of the radiographic analysis (P.E.), who also determined the coronal landmark [cement-enamel junction (CEJ) or restoration margin (RM)]. All radiographs where the anatomical landmarks or the defects could not be properly identified were excluded.

Radiographs were digitized using a computer program (SIDEXIS nextGeneration 1.51, Sirona, Bensheim, Germany) and a flatbed scanner (Microtek ScanMaker 4, Microtek, Hsinchu, Taiwan) with 600 dpi resolution and eight-bit grey values. The data were stored as TIFF files and analysed by the examiner using the computer program SIDEXIS and a 19′ flat screen (Totoku CCL 192 plus, Totoku Electric, Ueda, Japan) in a dark room.

Analysis started with number 1 in the order given by one examiner (C.M.) who was blinded to the clinical results and to the time point the particular radiographs had been taken (baseline, 12 months) (Eickholz et al. 2004a, b, Klein et al. 2001). Each radiograph was identified by its number.

For evaluation, the analysing tool of the program SIDEXIS was used. The images were magnified once using the ''zoom'' function. Then the distances CEJ/RM to alveolar crest (AC), CEJ/RM to BD, the depth of the intra-bony component (INTRA), and the angle between root surface and lateral bone wall were measured (Figs 1 and 2). If radiographs were too dark or had too low contrast to identify landmarks, the examiner was allowed to adjust brightness and contrast. If basic image enhancement functions (brightness, contrast) were insufficient to make landmarks visible the examiner was instructed to exclude these images from analysis.

Definition of radiographic landmarks

The radiographic landmarks were defined as follows: if the CEJ was destroyed by restorative treatment, it was replaced by RM (Fig. 2a). BD was defined as most coronal point where the periodontal ligament space showed a continuous width (Fig. 1). If no periodontal ligament space could be identified, the point where the projection of the AC crossed the root surface was used (Benn 1992). If both structures could be identified, the point defined by the periodontal ligament was used as BD and the crossing of the silhouette of the AC with the root surface was defined as AC. If several bony contours could be identified, the most apical one that crossed the root was defined as the BD and the most coronal one as AC (Eickholz et al. 1996). For all intra-bony defects, the distances CEJ/RM to AC and CEJ/RM to BD were measured using the measurement tool (Fig. 2a and b), also a first auxiliary line (AUX1) was drawn to represent the tooth axis (Fig. 2c). Then using the angle function a 90° angle was drawn

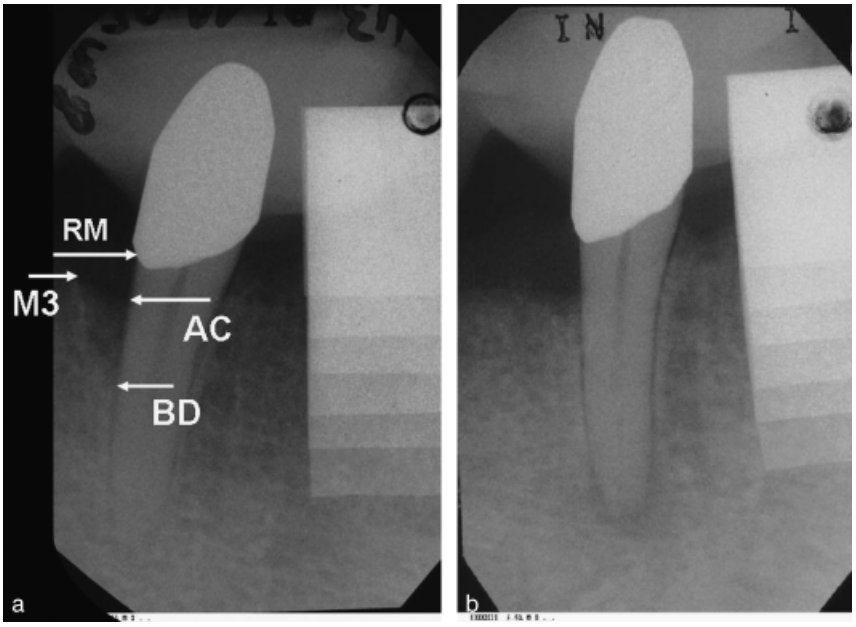

Fig. 1. (a) Maxillary left second pre-molar at baseline. (b) The same tooth 12 months after surgery: complete defect fill. CEJ, cemento-enamel junction; AC, alveolar crest; BD, most apical extension of bony defect; M3, most coronal extension of bone wall.

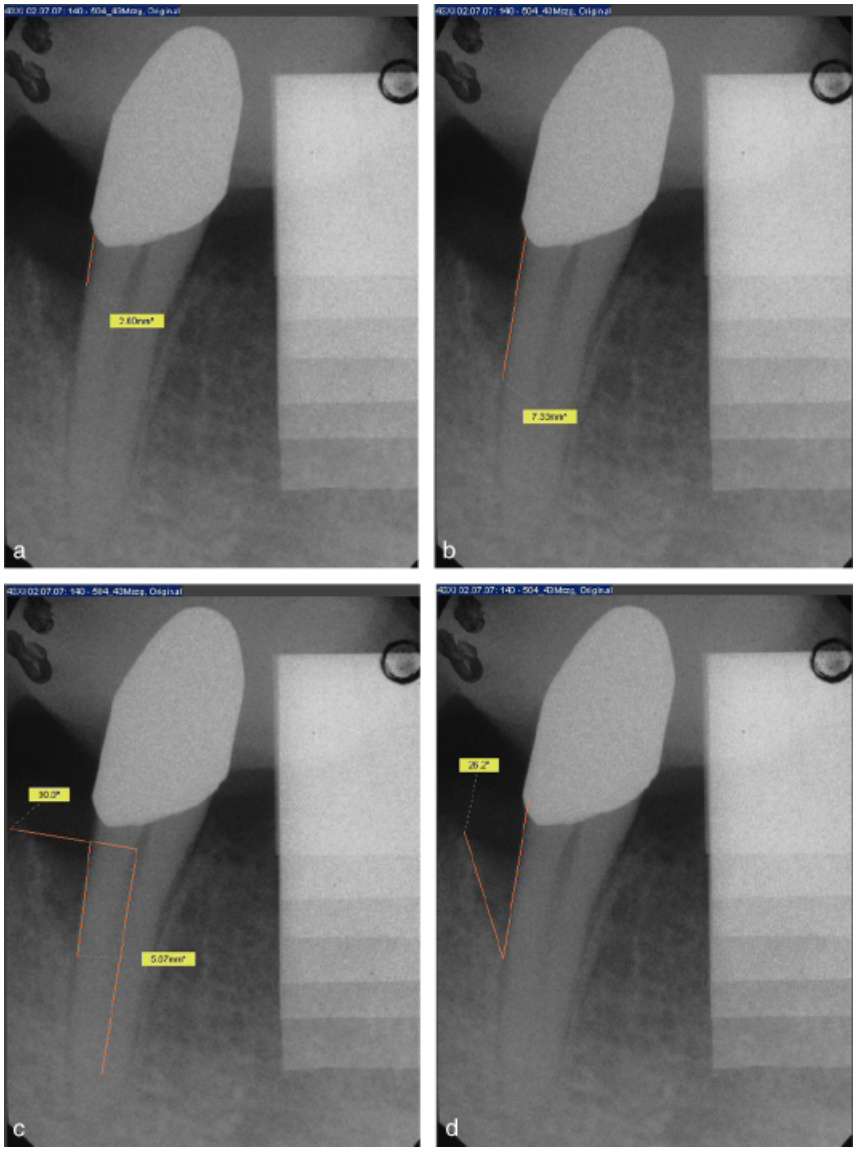

Fig. 2. Identification of landmarks for the evaluation of defect healing. (a) Distance RM to AC; (b) distance RM to BD; (c) definition of ''INTRA''; (d) definition and assessment of defect angle (for details, see text). RM, restoration margin; BD, most apical extent of bony defect; AC, alveolar crest; INTRA, depth of the intra-bony component of bony defects.

with AUX1 as one leg. This angle was moved along AUX1 until the other leg (AUX2) ran through the most coronal margin of the intra-bony defect (M3) (Figs 1a and 2c). The depth of the intra-bony defect (INTRA) was measured as distance between BD and the crossing of the silhouette of the root surface and AUX2 (Fig. 2c). Using the function ''angle'' the width of the intra-bony defect was assessed as an angle. One leg of this angle ran through BD and M3, the other through BD and CEJ/RM (Klein et al. 2001, Eickholz et al. 2004a, b, Pretzl et al. 2009) (Fig. 2d).

To assess intra-individual reproducibility, measurements were repeated in 20 radiographs (approximately each 10th radiograph) after all radiographs had been evaluated once.

Both (investigator and examiner of the radiographic analysis) were blinded for the clinical parameters and treatment assignment as well as the time point the radiographs had been taken (baseline, 12 months). Using 20 radiographs of intra-bony defects unrelated to this study, the examiner was calibrated before evaluation by the investigator of the radiographic analysis in finding the anatomical landmarks and measurement of respective distances. Both evaluated the 20 radiographs (measurement of CEJ/RM-BD, CEJ/RM-AC, INTRA, angle) and repeated all measurements approximately 2 weeks later.

Data management and statistical analysis of clinical data

Statistical management of data for the 6 months results has been reported previously (Jepsen et al. 2008). Statistical analysis after 12 months was mostly of descriptive nature. Based on the study protocol, testing the hypothesis of non-inferiority of EMD/SBG compared with EMD had been performed after 6 months. Two patients – one in each group – dropped out prematurely. As no data for the efficacy variable was available after baseline (surgery), these two could not be considered for analysis according to the intention-to-treat-principle. Hence, the data analysis had to be limited to 73 subjects. For data processing and statistical evaluation, appropriate validated software was used (SPSS software package, version 13, SPSS, Chicago, IL, USA).

The primary outcome variable was the change in defect fill recorded by bone sounding 6 months after surgery. Bone sounding values at baseline and after 12 months were compared by *t*-test in both treatment groups. Mean changes and 95% confidence intervals were computed.

Secondary variables included RAL, PPD 12 months after surgery, which were compared descriptively between the treatment groups. Secondary variables were also the differences between the distances from the CEJ to the most apical extension of the bony defect (BD) on radiographs obtained before and 12 months after surgery. All radiographic measurements were entered in a database (MS Excel 2000, Microsoft Co., Redmond, WA, USA) and transferred to an independent statistician. Intra-individual reproducibility was calculated for both examiners as standard deviation of single measurements (Cohen & Ralls 1988). For the distances CEJ/RM-BD, CEJ/RM-AC, and INTRA the inter-individual reproducibility was assessed as amount of differences >1.0 mm.

The patient was looked upon as statistical unit. The outcome variable of the radiographic evaluation was the difference between the distance CEJ/RM to BD at baseline and 12 months after surgery (absolute defect fill). Baseline and 12 months results were compared using the Wilcoxon test. Between-group differences (EMD *versus* EMD/SBG)

were tested using the Mann–Whitney *U*-test. Factors influencing defect fill (change of distance CEJ/RM-BD from baseline to 12 months after therapy) were identified using multiple linear regression analysis including the following independent variables: therapy (EMD *versus* EMD/SBG), baseline INTRA, baseline defect angle. The full analysis was described in detail in a specific statistical analysis plan before unblinding data. The significance level was set at $p < 0.05$.

Results

Patient and defect characteristics

This study was conducted in five study centres comprising 73 patients. No centre effects could be demonstrated. The per protocol population consisted of 23 men and 50 women, with a mean age of 46.9 years (median 48.2; range 21.1–66.7 years), 12 of the patients were occasional smokers. The 6-month data have been reported previously (Jepsen et al. 2008).

Clinical outcomes

Both treatment modalities led to significant improvements measured by bone sounding. The mean defect fill in the EMD/SBG group was 2.7 mm [95%CI (2.03–3.26), $p < 0.001$, *t*-test], and 2.8 mm [95%CI (2.26–3.36), $p < 0.001$, *t*-test] in the EMD group (Table 1). Bone gain in the combined treatment group showed a higher variability as indicated by a higher standard deviation. A reduction of PPD was found after the combined treatment (2.8 ± 2.1 mm; $p < 0.001$, *t*-test) as well as after EMD alone (2.9 ± 1.8 mm; $p < 0.001$, *t*-test). In the test group, a mean gain of attachment of 1.7 ± 2.1 mm ($p < 0.001$, *t*-test) was observed and in the control group of 1.9 ± 1.7 mm ($p < 0.001$, *t*-test). In the EMD/SBG treated group, mean GR increased by 1.1 ± 1.3 mm and in the control group (EMD) by 1.0 ± 1.1 mm (Table 1). Both therapies resulted in significant reductions of PPD and gain of attachment. Between groups no differences were found for any of the variables as well as for the changes of each variable. As compared with the 6-month data, a slight (insignificant) increase in attachment gain (EMD: 1.8–1.9 mm and EMD/SBG: 1.3–1.7 mm) and pocket reduction (EMD: 2.6–2.9 mm and EMD/SBG: 1.9–2.8 mm) was observed.

Full-mouth plaque scores ranged between 12.2% and 14.5% at all time points with no significant differences between groups (Table 2). At baseline local plaque scores at the experimental sites were 10 of 37 (27.0%) in the EMD/SBG group and four of 35 sites (11.4%) in the EMD group. Twelve months after surgery, the respective values were seven of 37 (18.9%; EMD/SBG) and five of 35 (14.3%; EMD). These differences were not statistically significant [Fisher's exact test (two-sided)].

As regards patient-centred outcomes and evaluation of adverse effects of regenerative treatment, favourable results have been reported previously (Jepsen et al. 2008).

Radiographic outcomes

During radiographic analysis, three pairs of radiographs were excluded because of excentric projection and overlapping of crowns. One pair of radiographs was not evaluated, because the 12 months radiograph exhibited a bending mark within the defect and another pair could not be evaluated because the 12 months radiograph was lost. Finally a total of 136 radiographs (68 pairs) were analysed.

Intra-individual reproducibility of calibration measurements assessed as standard deviations of single measurements was 0.27 mm (CEJ/RM-BD), 0.49 mm (CEJ/RM-AC), 0.25 mm (INTRA), and 1.22° (angle), respectively. Intra-individual reproducibility for the investigational radiographs was 0.46 mm (CEJ/RM-BD), 0.34 mm (CEJ/RM-AC), 0.44 mm (INTRA), and 4.21° (angle), respectively.

Some minor differences in defect fill were observed depending upon the topography (Fig. 3a and b). In circumferential defects, the variation was higher than in others without reaching statistical significance. Both treatment modalities resulted in significant defect fill. This led to a significant ($p < 0.001$) reduction of CEJ/RM-BD (EMD/SBG: 1.77 ± 1.92 mm; EMD: 1.40 ± 1.93 mm) and INTRA (EMD/SBG: 2.19 ± 2.21 mm; EMD: 1.49 ± 1.89 mm), which was also reflected in a significant ($p < 0.01$) increase of the defect angle (EMD/SBG: $9.0 \pm 14.4°$; EMD: $6.7 \pm 13.0°$). However, statistical analysis failed to reveal differences between both treatment modalities (Table 3). Multiple linear regression analysis identified only baseline INTRA to influence bone fill, i.e. the deeper the defect the more defect fill may be expected (Table 4).

Discussion

Clinical results

The results of the present, randomized-controlled trial demonstrate favourable outcomes after 12 months.

Both treatment modalities resulted in statistically significant defect fill with significant reductions of the distance

Table 1. Clinical outcomes at 12 months; mean differences are calculated as baseline–6 months and baseline-12 months respectively

Variable	Treatment					
	test (EMD/SBC), $n = 38$			control (EMD), $n = 35$		
	baseline	6 months	12 months	baseline	6 months	12 months
Bone sounding (mm)	12.0 ± 2.1	9.9 ± 2.4	9.3 ± 2.2	12.2 ± 2.0	10.2 ± 2.5	9.4 ± 2.3
Mean difference ± SD		$2.01 \pm 2.1/2.65 \pm 1.9$			$2.07 \pm 1.2/2.81 \pm 1.6$	
RAL (mm)	9.3 ± 2.1	8.0 ± 2.2	7.6 ± 2.3	10.1+2.2	8.3 ± 2.5	8.2 ± 2.5
Mean difference ± SD		$1.31 \pm 1.8/1.69 \pm 2.1$			$1.83 \pm 1.6/1.93 \pm 1.7$	
PPD (mm)	6.9 ± 1.8	5.0 ± 1.7	4.1 ± 1.7	7.1 ± 1.5	4.5 ± 1.9	4.2 ± 1.9
Mean difference ± SD		$1.93 \pm 1.8/2.80 \pm 2.1$			$2.55 \pm 1.8/2.90 \pm 1.8$	
GR (mm)	2.4 ± 1.3	3.0 ± 1.7	3.5 ± 1.7	3.0 ± 1.6	3.8 ± 1.7	4.0 ± 1.8
Mean difference ± SD		$-0.62 \pm 1.1/-1.11 \pm 1.3$			$-0.72 \pm 1.1/-0.97 \pm 1.1$	

PPD, probing pocket depths; RAL, relative attachment level; GR, gingival recessions; EMD, enamel matrix derivative; SBC, synthetic bone graft.

from apical to coronal radiological landmarks as well as the intra-osseous depth of defect, which was also reflected in an increase of the defect angle. No differences were found between treatment modalities.

The results of the present investigation are confirmed by several other studies and systematic reviews (Pontoriero et al. 1999, Froum et al. 2001b, Tonetti et al. 2002, Wachtel et al. 2003, Esposito et al. 2005, Francetti et al. 2005, Sculean et al. 2008a). In 2008, Sculean et al. demonstrated that the treatment of intra-bony defects with enamel matrix proteins may result in a reduction of pocket probing depth and gain of clinical attachment, which could be maintained over a period of 10 years. The present results confirm that after 12 months significant improvements in clincial parameters can be obtained in one- and two-wall intra-bony defects after treatment with enamel matrix proteins and a bone replacement graft. Since hard tissue fill is the only component of regenerated peridontium which can be assessed clinically, bone sounding was performed and served as primary outcome variable (Machtei 1997).

Osseous regeneration after treatment with EMD in combination with a BCP in humans requires more than 9 months as outlined in a human histological analysis by Sculean et al. (2008c).

Lekovic et al. (2000) reported about significant improvements if EMD were combined with bovine porous bone mineral. The improvements were observed on the buccal and lingual sites despite the fact that inter-proximal defects were treated (Lekovic et al. 2000). Zucchelli et al. (2003) reported about significantly greater attachment gain and bone gain with a combination of EMD and bone mineral. It is obvious that the defect characteristics were different from our study. The authors reported about a mean intra-bony defect depth of 6.8 mm whereas in our study this was 5.9 and 5.6 mm, respectively. In other trials, only slight differences between the two treatment groups (EMD *versus* EMD+SBG) were observed (Bokan et al. 2006) A systematic review has shown that clinical parameters are improved when intra-bony defects are treated with bone fillers (Reynolds et al. 2003). Similar results were described by Yilmaz et al. (2010), who compared EMD combined with/without autogenous bone in two- to three-wall intra-bony defects (Yilmaz et al. 2010). They reported about a small but significantly higher gain of RAL. In general, it appears that the combination of EMD with bone grafts or autogenous bone seems to be more favourable than a combination of EMD with barrier membranes if intra-bony lesions are treated (Tu et al. 2010).

According to our data in wide one- and two-wall defects the effect of EMD cannot be improved by adding a SBG. After 12 months, there was no substantial improvement as compared with 6-month data (Jepsen et al. 2008).

Table 2. Full-mouth plaque scores (mean±SD)

Time	Treatment	
	test (EMD/SBC)	control (EMD)
2 weeks	12.7 ± 9.2%	12.7 ± 9.5%
6 weeks	14.5 ± 8.6%	13.9 ± 10.0%
3 months	13.2 ± 7.7%	14.5 ± 10.6%
6 months	13.6 ± 7.0%	13.7 ± 9.3%
9 months	12.2 ± 7.0%	12.8 ± 7.7%
12 months	13.9 ± 11.0%	13.2 ± 11.3%

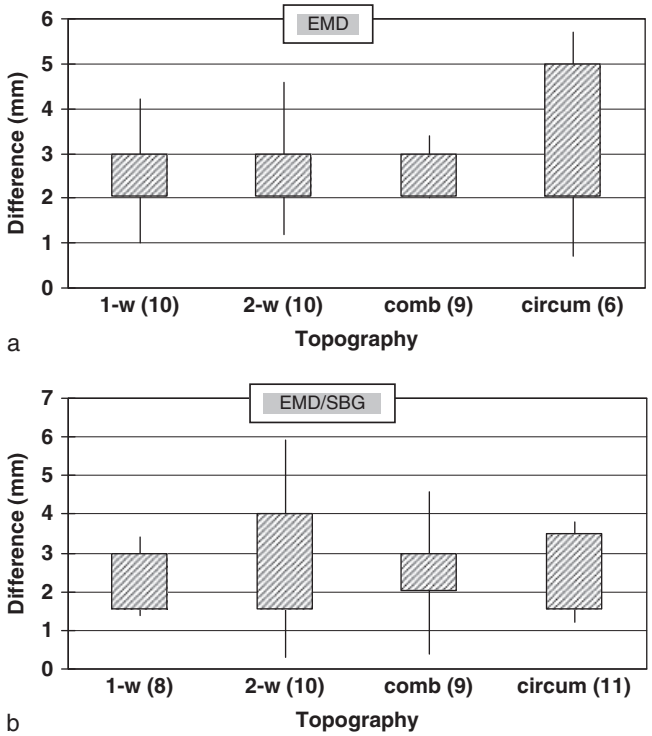

Fig. 3. Defect fill (measured as differences in bone sounding) in millimeters at the test sites after 12 months Tukey's plots (25% and 75% percentiles and standard deviations). 1-w, predominantly one-wall defect (>2/3); 2-w, predominantly two-wall defect (>2/3); comb, combined one-wall and two-wall defect; circum, circumferential defect; number of defects in parentheses. (a) Regenerative treatment with Emdogain (EMD). (b) Regenerative treatment with Emdogain and synthetic bone ceramics (EMD/SBG).

Radiographic results

For calibration, the radiographic examiner achieved better reproducibility than during evaluation of the investigational radiographs. The radiographs chosen for training and calibration were of optimal and in some cases better quality than the investigational radiographs regarding projection, brightness, and contrast. This may explain the differences.

However, the intra-individual reproducibility of the measurement of the distance CEJ/RM-AC with a standard deviation of single measurements of 0.34 mm was comparable or better than measurement errors reported by other authors (Hausmann et al. 1989, Benn 1992, Wolf et al. 2001: 0.35–0.56 mm). For the assessment of the distance CEJ/RM-BD only within intra-bony defects, the measurement error was also comparable or better (Wolf et al. 2001: 0.70–0.82 mm). The computer-assisted method has been used before for the evaluation of regenerative therapy (Pretzl et al. 2009) and demonstrated good validity as compared with the gold standard of intra-surgical assessments (Tihanyi et al. 2011).

Table 3. Change of intra-bony defects by therapy as evaluated on intra-oral radiographs

Variable	Treatment			
	test (EMD/SBG), $n = 35$		control (EMD), $n = 33$	
	baseline	12 months	baseline	12 months
CEJ/RM – BD (mm)	9.8 ± 3.2	8.0 ± 2.8	10.1 ± 2.5	8.7 ± 2.6
Mean difference ± SD	1.77 ± 1.9		1.40 ± 1.9	
CEJ/RM – AC (mm)	6.1 ± 2.7	5.3 ± 2.3	6.4 ± 2.5	6.0 ± 2.6
Mean difference ± SD	0.73 ± 2.0		0.37 ± 1.7	
INTRA (mm)	5.9 ± 2.6	3.7 ± 2.3	5.6 ± 2.1	4.2 ± 2.2
Mean difference ± SD	2.19 ± 2.2		1.49 ± 1.9	
Defect angle (°)	25.8 ± 9.9	34.8 ± 14.4	28.0 ± 10.2	34.8 ± 16.0
Mean difference ± SD	− 9.0 ± 14.4		− 6.7 ± 13.0	

CEJ, cemento-enamel junction; RM, restoration margin; BD, most apical extent of bony defect; AC, alveolar crest; INTRA, depth of the intra-bony component of bony defects; EMD, enamel matrix derivative; SBG, synthetic bone graft.

Table 4. Multiple linear regression analysis

Dependent variable: change of distance CEJ/RM − BM; $n = 68$; $R^2 = 0.125$; $R^2_{\text{adjusted}} = 0.112$; standard error of estimate = 1.809

	coefficient	s.e.(b)	t	P
Constant	− 0.094	0.591	− 0.159	0.874
INTRA at baseline	0.202	0.095	3.068	0.003

Analysis of variance

Model	sum of squares	df	MSQ	F-ratio	P
Regression	30.801	1	30.801	9.410	0.003
Residual	216.033	66	3.273		

CEJ, cemento-enamel junction; RM, restoration margin; INTRA, depth of the intra-bony component of bony defects; df, degrees of freedom; MSQ, mean of squares.

Radiographic defect fill as evidenced by reduction of the distances CEJ/RM-BD (EMD/SBG: 1.77 mm, EMD: 1.40 mm) and INTRA (EMD/SBG: 2.19 ± 2.21 mm; EMD: 1.49 ± 1.89 mm) 12 months after therapy corresponds well to results reported 12 months after GTR therapy of intra-bony defects with non-resorbable barriers [CEJ/RM-BD: ePTFE: 1.9 mm (Eickholz et al. 1996)] as well as with resorbable membranes [CEJ/RM-BD: Polyglactin 910: 1.4 mm (Eickholz et al. 1996)].

Eight and 16 months after use of EMD in one- and two-walled intra-bony defects radiographic bone gain of 0.9 and 2.2 mm was reported (Heijl et al. 1997). Better radiographic defect fill was observed 12 months after therapy of three-wall intra-bony defects with non-resorbable barriers or EMD [ePTFE: 2.9 mm, EMD: 2.4 mm (Crea et al. 2008)]. Comparison of these results is difficult. Reduction of the distance CEJ/RM-BD represents exclusively defect fill, whereas reduction of INTRA represents levelling of the defect due to a combination of apical defect fill and marginal resorption. From a technical point of view, radiographic changes are more trustworthy if they were obtained with individual stent film holders as in this study (Eickholz et al. 1996, Pretzl et al. 2009, Crea et al. 2008).

Not only after 6 months but also after 12 months the data support the effectiveness and safety of regenerative procedures based on EMD application. The differences in attachment gain between our study and previous investigations can easily be explained by defect topography: in our study wide ($\geqslant 2$ mm) uncontained (one- and two-walled) intra-bony defects were treated.

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Clinical Relevance

Scientific rationale for the study: Clinical and in particular radiographic comparison of a combination of an EMD/SBG with EMD alone in wide and deep uncontained intrabony defects 12 months after treatment.

Principal findings: The follow-up data reported 12 months after the use of EMD alone and a EMD/SBG in wide and deep intra-bony defects demonstrate significant clinical and radiographic improvements, compared with baseline as well as minor insignificant improvements (stability) compared with the 6-month results.

Practical implications: Local defect characteristics have an impact on treatment outcome irrespective of the mode of regenerative treatment, i.e. if EMD is combined with a SBG or not.