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Clinical outcomes after treatment of intra-bony defects with an EMD/synthetic bone graft or EMD alone: a multicentre randomized-controlled clinical trial

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Abstract

Objectives: Comparison of the outcomes of a combination of an enamel matrix derivative and a synthetic bone graft (EMD/SBC) with EMD alone in wide intra-bony defects.

Material and Methods: Seventy-three patients with chronic periodontitis were recruited in five centres in Germany. All patients had one wide intra-bony defect of $\geqslant$4 mm. Surgical procedures involved microsurgical technique and the modified papilla preservation flap. After debridement, defects were randomly assigned to EMD/SBC (test) or EMD (control). Assessments at baseline and after 6 months included bone sounding, attachment levels, probing pocket depths, bleeding on probing and recessions. Early wound-healing, adverse effects and patients' perceptions were also recorded.

Results: Both treatment modalities led to significant clinical improvements. Change in bone fill 6 months after surgery was 2.0 mm ($\pm$ 2.1) in the test group and 2.1 mm ($\pm$ 1.2) in the control group. A gain in clinical attachment of 1.3 mm ($\pm$ 1.8) in the test group and 1.8 mm ($\pm$ 1.6) in the control group was observed. One week after surgery, primary closure was maintained in 95% of the test sites and 100% of the control sites. No differences in patients' perceptions were found.

Conclusion: The results of the present study showed similar clinical outcomes following both treatment modalities.

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

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Histological findings in monkeys (Hammarström et al. 1997) and observations from human case reports (Heijl 1997, Mellonig 1999, Sculean et al. 2000) have demonstrated that treatment with an enamel matrix derivative (EMD) favours the formation of a new attachment apparatus, characterized by the presence of acellular and cellular cementum with inserting collagen fibres, and of new alveolar bone.

Controlled clinical studies have shown a significantly higher gain of clinical attachment and radiographic bone gain in intra-bony periodontal defects treated with open flap debridement combined with EMD when compared with open flap debridement alone (Heijl et al. 1997, Froum et al. 2001, Tonetti et al. 2002, Giannobile & Somerman 2003, Venezia et al. 2004, Trombelli 2005). EMD has also been successfully used in Class II furcation defects to reduce the horizontal furcation depth, post-operative swelling and pain (Jepsen et al. 2004).

As the formulation of EMD does not support the flap in wider defects, attempts have been made to combine EMD with different space-maintaining products (e.g. membranes or bone

Conflict of interest and source of funding statement

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substitutes). Controlled clinical studies have indicated that a combination of EMD and a bovine-derived xenograft may enhance clinical attachment level gain compared with EMD alone (Lekovic et al. 2000, Zucchelli et al. 2003). Considerable controversy still exists regarding the capability of these materials to be resorbed and substituted by newly formed bone. Very recently, the combination of EMD and an autogenous bone graft was compared with regenerative treatment with EMD alone (Guida et al. 2007). The results indicated that both treatment modalities resulted in comparable outcomes for attachment gain and bone fill; however, less recession was observed for the combined treatment.

Biphasic calcium phosphates 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, Daculsi et al. 1999). This study was designed to evaluate an EMD used in combination with a novel synthetic bone substitute composed of biphasic calcium phosphate in the treatment of wide intra-bony defects *versus* the use of an enamel matrix derivate alone.

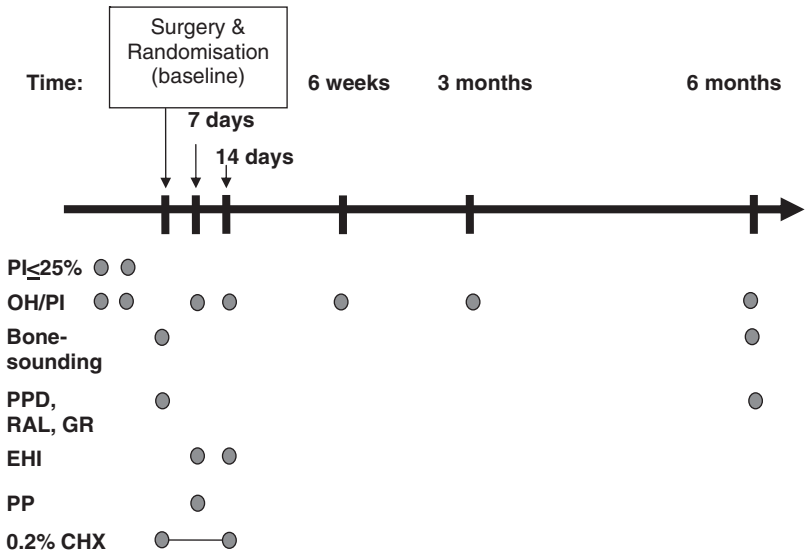

Fig. 1. Study design. Randomization into the two treatment groups during surgery (baseline). PI, plaque index (O'Leary et al. 1972); OH/PI, oral hygiene instructions, plaque index and gentle professional tooth cleaning; PPD, probing pocket depth; RAL, relative attachment level; GR, gingival recession; EHI, early wound-healing index (Wachtel et al. 2003); PP, patient perception; CHX, chlorhexidine gluconate 0.2%.

Material and Methods

Experimental design

A parallel group, randomized, prospective, multicentre and controlled clinical trial was designed to evaluate the amount of bone fill 6 months following two different treatment modalities of one- and two-wall intra-bony periodontal defects. In the test group (EMD/SBC), an access was prepared with a microsurgical technique and a papilla preservation flap (Cortellini et al. 1995, 1999). After debridement, application of an EMD (Straumann® Emdogain, Straumann, Basel, Switzerland) was followed by defect filling with a synthetic bone graft (Straumann® BoneCeramic, Straumann, Basel, Switzerland). SBC is a synthetic bone graft substitute of medical-grade purity in particulate form composed of biphasic calcium phosphate – a mixture of 60% hydroxylapatite, and of 40% of the beta form of tricalcium phosphate.

In the control group EMD, the same treatment was carried out, except for the use of SBC. Each patients who participated had a single defect. Clinical outcomes were evaluated at 6 months. This investigation was performed at five centres involving a total of five operators and five masked examiners. Each clinical centre was connected with and supervised by a central monitoring facility at the Institut Straumann AG, Basel, Switzerland. The study design is outlined in Fig. 1.

Investigators' meeting and calibration

An investigators' meeting was conducted to standardize examination and surgical procedures. A calibration exercise was performed to assure acceptable intra- and inter-examiner reproducibility for the measurement of probing pocket depth (PPD) and relative attachment level (RAL) from a customized acrylic stent. The sites measured were comparable to the sites to be measured in the study. Repeated measurements were performed at least 1 h later. The results differed within 1 mm in 89–100% of the repeated pocket depth measurements and in 91–100% of the repeated RAL assessments, and thus they were within the acceptance limits of 85% (PD) and 90% (CAL), respectively (Polson 1997).

Subject population

Patients were recruited at three German university dental clinics and two German private periodontal practices for the study. The age of the patients was limited to 18–70 years. Patients had to demonstrate good oral hygiene with a full-mouth plaque score $\leqslant 25\%$ before baseline (surgery). Only patients with a diagnosis of severe periodontitis treated previously by oral hygiene instructions and subgingival scaling and root planing were eligible for the study. The patients were informed in detail about the possible risks and benefits and were asked to give their consent to the trial. The study was performed in compliance with Good Clinical Practice and the Declaration of Helsinki, last revised Edinburgh 2000; the study protocol was reviewed and approved by the International Ethics Committee in Freiburg, Germany.

All patients had a radiographic intra-bony defect of a least 4-mm depth, and 2-mm width; defects extending into a furcation area were not included. The depth and width of the intra-bony component of the defect and absence of furcation involvement were evaluated during screening on radiographs, but had to be confirmed during surgery. Patients with uncontrolled or poorly controlled diabetes, unstable or life-threatening conditions, current pregnancy at the time of recruitment, or if they were smokers, were not recruited to the study. However, occasional smoking (1–30 cigarettes/month) was allowed (Table 1). A local inflammation or an

Table 1. Patient and defect characteristics at baseline

	Treatment	
variable	test (EMD/SBC)	control (EMD)
Demographic data		
n patients	38	35
Age (years, mean ± SD)	47.5 ± 12.0	46.2 ± 9.0
Men/women (*n*)	29/9	21/14
Smoking habits (*n*/%)		
No smoker	30 (78.9)	28 (80)
Former smoker	3 (7.9)	0 (0)
Occasional smoker	5 (13.2)	7 (20)
Measurements at defect sites		
PPD (mm)	6.9 ± 1.8	7.1 ± 1.5
Defect depth (mm)	6.7 ± 1.6	6.9 ± 2.2
Defect width (mm)	3.6 ± 0.7	3.6 ± 1.0

EMD, enamel matrix derivate; SBC, synthetic bone graft; PPD, probing pocket depths.

untreated endodontic or cariologic problem had to be eliminated before the patient could be included in the study.

Sample size calculation

Two therapies, a combined application of EMD/SBC (test) or application of EMD (control) alone, were compared. A sample size of 58 patients was estimated to have 80% power to detect a difference in bone fill $>30\%$ between the two treatment groups, assuming a mean bone fill of 3.0 mm with an SD of 1.2 mm using a parametric test procedure with a level of $\alpha = 2.5\%$ (one-sided). Thus, 30% was the pre-defined non-inferiority margin. Twenty nine patients per group would have been necessary. For the compensation of possible dropouts, an overquotation of 10% was calculated, leading to a total sample size of $n = 32$ per group.

Pre-treatment procedures

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

Randomization and allocation concealment

After verification of the entry criteria, 75 patients gave informed consent and were enrolled into the study. All patients were assigned a patient number. Patients were enrolled sequentially. A randomization list was generated by an independent statistician based on one surgical site per patient for a total of 75 surgical sites (75 patients). Randomization envelopes were supplied to the centres and numbered sequentially containing the treatment allocation according to the randomization list. To conceal assignment from the investigator until the time during the surgical procedure that would require application of EMD/SBC or EMD, the investigator was instructed to assign a previously supplied sealed envelope containing the treatment assignment to the specific patient. Should a patient be found to be ineligible during the surgical intervention by the investigator, a new patient was recruited and a new randomization number was selected with a new envelope. Under no circumstances was the original randomization allocation used for a replacement patient. The clinical examiner was blinded throughout the study regarding treatment modality.

Clinical measurements

All measurements were carried out using a customized acrylic stent with markings. Each of the five 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 secondary outcomes PPDs, 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 anaesthesia, the primary outcome parameter vertical bone fill (bone sounding at baseline – bone sounding at 6 months) was measured at the same six sites at baseline and at 6 months from the acrylic stent with a manual probe (PCP-UNC 15, Hu-Friedy, Leimen, Germany). Recordings were collected again after 6 months. Seven days after surgery, patients were asked about pain, bleeding and swelling during the first week after treatment using a questionnaire. Post-operative healing was assessed by the ''Early wound-healing Index'' (EHI) at 7 and 14 days after surgery (Wachtel et al. 2003).

Surgical procedures

The surgical assess was identical for both groups. Following local anaesthesia, a full-thickness (muco-periosteal) access flap was elevated using the simplified or the modified papilla preservation technique (Cortellini et al. 1995, 1999). Granulation tissue and any remaining subgingival calculus were removed. Careful scaling and root planing was carried out with hand instruments and oscillating scalers. The morphology of the defect was then examined and recorded and the sealed envelope was opened.

In the test sites, the exposed root surface was conditioned with EDTA gel (sterile 24% EDTA gel, pH 6.7; PrefGel, Straumann, Basel, Switzerland) for 2 min in order to remove the smear layer (Blomlöf & Lindskog 1995, Blomlöf et al. 1996, Polson & Proye 1982, Polson et al. 1984). The root was then thoroughly rinsed with saline to remove all EDTA gel. Excess fluids were removed while making sure that no blood or saliva contaminated the root surface after the final rinse. EMD was then applied immediately, starting at the most apical end of the defect, covering the entire denuded root surface. The remaining EMD in the syringe was used to reconstitute the SBC. The combined EMD/SBC compound was gently packed into the defects and filled up to the most coronal levels of the defect walls.

The flap was subsequently replaced. Great care was taken to obtain complete closure of the inter-dental soft tissues

above the treated defect in order to achieve primary closure of the interdental area without any tension. Monofilament synthetic non-resorbable 5-0 and 6-0 suturing material was used (Ethicon Prolene, Ethicon Products, Norderstedt, Germany).

Intra-surgical clinical measurements

In both groups, the width and depth of the intra-bony defect were assessed with a manual probe (PCP-UNC 15, Hu-Friedy, Leimen, Germany). The following assessments were performed: (1) bone level (distance from the stent to the bottom of the defect), (2) defect depth (distance from the bone crest to the bottom of the 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).

Post-surgical instructions and infection control

All patients were advised to rinse twice a day with 0.2% chlorhexidine gluconate solution for 2 weeks following surgery. After 14 days, sutures were removed and patients were instructed to switch from rinsing with chlorhexidine to a local application of 1% chlorhexidine gluconate gel for the subsequent 4 weeks. During the 4 weeks after suture removal in the area of surgery, they were allowed to clean occlusal areas gently with a supersoft toothbrush. After 7 days, 14 days and 6 weeks, all patients underwent gentle supragingival professional tooth cleaning and reinforcement of oral hygiene if necessary. Each patient was reinstructed in proper oral hygiene measures when toothbrushing was reinstated 6 weeks after surgery. A maintenance programme was set up for all patients at 3 and 6 months. However, no subgingival instrumentation was performed at the surgical site. Any adverse effect or post-surgical complications were recorded using a questionnaire.

Data management and statistical analysis

Data should be analysed according to the ''intent-to-treat-principle'', i.e. data representing all subjects were to be included in the analyses at each time point. However, two patients – one in each group – dropped out pre-maturely. One patient in the EMD/SBC group was lost to follow-up; the other patient randomly assigned to the EMD group had to have a root resection in an adjacent tooth. The two dropouts are included in the full analysis set and were not considered to be protocol violations. However, as no data for the efficacy variable were available after baseline (surgery), these two patients could not be considered for analysis according to the intent-to-treat-principle. Hence, the data analysis had to be limited to 73 subjects.

In order to ensure that the database reflected data accurately, a double data-entry procedure (data entered by different staff in two separate data files) was used. In addition to an electronic comparison of these two data files, a validation of data took place by checking the ranges, consistency and plausibility. Unclear data underwent a data-cleaning process. Any changes applied to data in the database during data cleaning were recorded in a special log file. After completion of data cleaning, the database was locked. For data processing and statistical evaluation, appropriate validated software was used (SPSS software package, version 13, SPSS, Chicago, IL, USA).

Descriptive summary statistics were computed for all the parameters documented. Quantative parameters were described by seven-point scales with mean, standard deviation, median, quartiles, minimum and maximum. For qualitative variables, absolute and relative frequencies were given. All descriptions were made separately for treatment groups and visits. The primary outcome variable in this clinical study was the change in bone fill as measured by bone sounding 6 months after surgery. The statistical plan comprised the testing of two hypotheses: (a) non-inferiority of EMD/SBC compared with EMD and (b) superiority of EMD/SBC compared to EMD. The second hypothesis should be tested only in the case of a suspected superiority, i.e. a greater change in bone fill under the combined treatment compared to EMD alone. For both hypotheses, a clinically relevant difference of 30% was pre-defined.

H_0 was the null hypothesis, claiming that the change in bone fill was more than 30% lower in the test group compared with the control group. Confirmative statistical testing of this hypothesis was performed by computing a 95% confidence interval (CI) for the mean change in bone fill after 6 months in the test group and comparing it with the mean change in bone fill after 6 months in the control group -30%. Adjustment of type I error due to multiple testing was not necessary due to a priori ordering of the hypotheses.

Secondary variables in the study included RAL, PPD and early wound healing. These variables were compared descriptively between the treatment groups. 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

A total of 75 patients (23 men, 52 women) were consecutively recruited from March 2005 and randomly assigned to one of two treatment procedures. The 6-month data were completed in November 2006. This study was conducted in five study centres comprising 75 patients. The largest centre treated and documented 20 patients; the smallest centre treated and documented nine patients. No centre effects could be demonstrated. Two patients – one in each group – dropped out before the 6-month visit after surgery was reached. Hence, the data analysis had to be limited to 73 subjects: 38 in the test group and 35 in the control group. Hence, 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 (Table 1).

According to inclusion criteria, patients' full-mouth plaque scores (O'Leary et al. 1972) were monitored twice before the baseline. The respective mean plaque scores were (first assessment) $18.7 \pm 9.4\%$ and (second assessment) $13.1 \pm 5.4\%$.

Defects were measured during surgery; the mean depth from the bone crest to the bottom of the debrided bone defect was 6.7 mm ($\pm$ 1.6) and 6.9 mm ($\pm$ 2.2) for the test and the control group, respectively. Defect width reached a mean of 3.6 mm ($\pm$ 0.7) in the test group and 3.6 mm ($\pm$ 1.0) in the control group. Distributions of defect types are displayed in Fig. 2.

Table 2. Clinical outcomes at 6 months

	Treatment			
variable baseline – 6 months	test (EMD/SBC), $n = 38$		control (EMD), $n = 35$	
	baseline	6 months	baseline	6 months
Bone sounding (mm)	12.0 ± 2.1	9.9 ± 2.4	12.2 ± 2.0	10.2 ± 2.5
Mean difference ± SD	2.01 ± 2.1		2.07 ± 1.2	
RAL (mm)	9.3 ± 2.1	8.0 ± 2.2	10.1 ± 2.2	8.3 ± 2.5
Mean difference ± SD	1.31 ± 1.8		1.83 ± 1.6	
PPD (mm)	6.9 ± 1.8	5.0 ± 1.7	7.1 ± 1.5	4.5 ± 1.9
Mean difference ± SD	1.93 ± 1.8		2.55 ± 1.8	
GR (mm)	2.4 ± 1.3	3.0 ± 1.7	3.0 ± 1.6	3.8 ± 1.7
Mean difference ± SD	− 0.62 ± 1.1		− 0.72 ± 1.1	

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

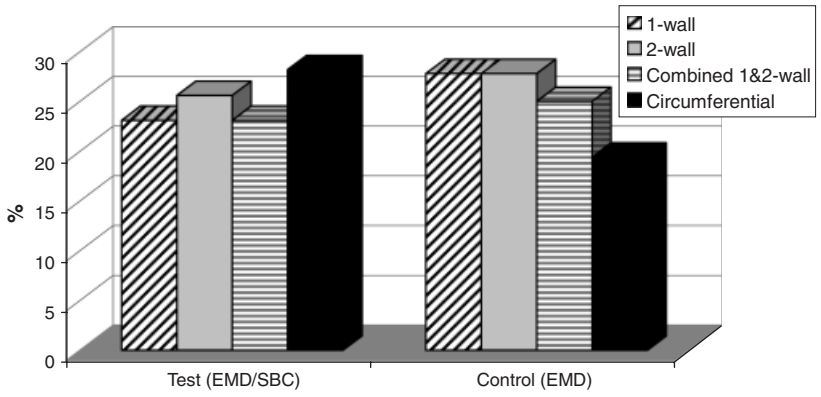

Fig. 2. Distribution (%) of defect types as assessed during surgical intervention.

Clinical outcomes

Both treatment modalities led to significant improvements for the primary outcome: bone fill measured by bone sounding at baseline and 6 months. In the EMD/SBC group, a mean defect fill of 2.01 mm [95%CI (1.32–2.71), $p < 0.001$, *t*-test] was calculated; the 95% CI included the mean of the control group (2.1 mm) minus 30% [1.45 mm; 95%CI (1.16–1.74),] thus demonstrating the clinical utility and proving the non-inferiority of the combination treatment. The statistical decision for the null hypothesis was herewith refuted; thus, non-inferiority can be claimed, because the mean difference of the EMD group minus 30% is within the CI of the mean difference of the EMD/SBC group. In the EMD group, a mean defect fill of 2.07 mm [95%CI (1.65–2.49), $p < 0.001$, *t*-test] was found (Table 2). Bone gain in the combined treatment group showed a higher variability as indicated by a higher standard deviation. Levene's test for equality of variances verfied this finding, showing a statistically significant difference between the variances ($F = 20{,}530$, $p < 0.001$).

A reduction of PPD was found after the combined treatment (mean difference of 1.93 mm) [95%CI (1.35–2.51), $p < 0.001$, *t*-test] as well as after EMD alone (2.55 mm) [95%CI (1.94–3.17), $p < 0$–001, *t*-test]. In the test group, a mean gain of attachment of 1.31 mm [95%CI (0.71–1.91), $p < 0.001$, *t*-test] was observed and in the control group it was 1.83 mm [95%CI (1.29–2.38), $p < 0.001$, *t*-test]. In the EMD/SBC-treated group, the mean GRs increased by 0.62 mm [95%CI (− 0.98 to − 0.26), $p < 0.001$, *t*-test] and in the control group EMD by 0.72 mm [95%CI (− 1.09 to − 0.34), $p < 0.001$, *t*-test], (Table 2). Both therapies resulted in significant reductions of PPD and gain of attachment. No significant differences were found for any of the variables between groups.

Full-mouth plaque scores ranged between 12.7% and 14.5% at all evaluation time points, with no significant differences between groups (Table 3). Local plaque scores at the experimental sites were 10 of 37 (19.4%) in the EMD/SBC group and 4 of 35 sites (11.4%) in the EMD group at baseline. At 6-month evaluation, the respective local plaque scores were 7 of 37 (18.9%) in the EMD/SBC group and 11 of 35 (31.4%) sites in the EMD group. These differences were not statistically significant (baseline: $p = 0.138$, 6 months $p = 0.278$, Fisher's exact test two-sided).

Table 3. Full-mouth plaque scores (mean ± SD)

	Treatment	
time	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

EMD, enamel matrix derivative; SBC, synthetic bone graft.

The results of the self-reported post-operative healing events within the first 7 days show that nearly the same percentage of patients of both groups reported no pain (EMD/SBC 43.6%, EMD 41.7%), no post-surgical bleeding (EMD/SBC 79.5%, EMD 80.6%) and no swelling (EMD/SBC 30.8%, EMD 33.3%) in the operated area (Fig. 3).

The percentage of patients with EHI score 1 (complete flap closure without fibrin line in the inter-proximal area) doubled in both groups from 7 to 14 days' evaluation (7 days: EMD/SBC 30.8%, EMD 36.1%; 14 days: EMD/SBC 64.1%, EMD 72.2%) (Fig. 4). The comparison of the results of EHI after 7 and 14 days showed no significant differences between groups (7 days: $p = 0.59$, Mann–Whitney; 14 days: $p = 0.29$, Mann–Whitney). A tendency for less bone fill was observed in those defects that exhibited an EHI of 3 as opposed to defects with EHI 1 or 2 (Table 4).

Discussion

The results of the present multicentre, randomized-controlled trial demonstrated favourable clinical outcomes following the application of EMD alone or in combination with SBC. After 6 months, both treatment modalities tested had led to comparable results for

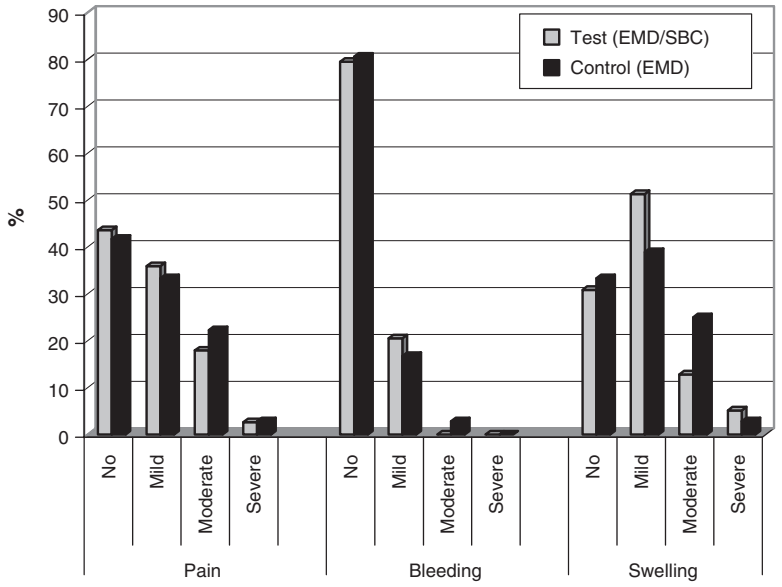

Fig. 3. Patients' perception of post-operative healing 7 days after surgery.

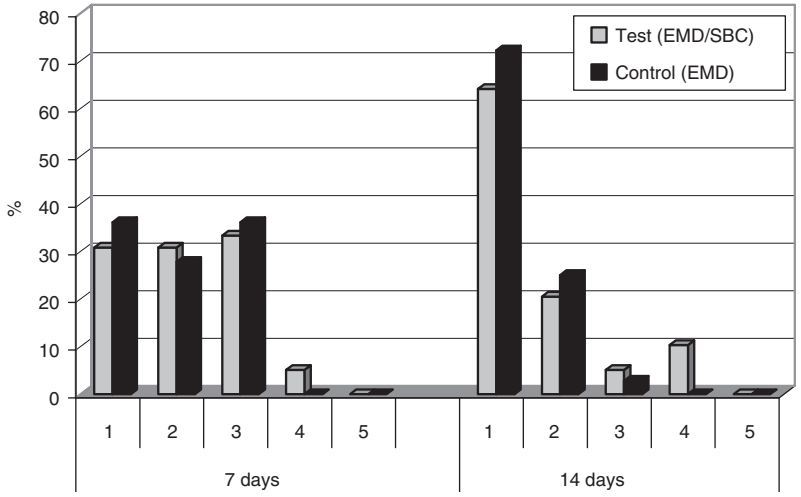

Fig. 4. Early wound-healing index as recorded 7 and 14 days after surgery (Wachtel et al. 2003).

1: complete flap closure – no fibrin line in the interproximal area
2: complete flap closure – fine fibrin line in the interproximal area
3: complete flap closure – fibrin clot in the interproximal area
4: incomplete flap closure – partial necrosis of the interproximal tissue
5: incomplete flap closure – complete necrosis of the interproximal tissue.

Table 4. Comparisons of bone fill (mm) after 6 months in relation to early wound-healing after 7 days

EHI	Test (EMD/SBC) mean ± SD (*n*/%)	Control (EMD) mean ± SD (*n*/%)
1	2.8 ± 2.3 (12/32)	2.3 ± 1.0 (13/37)
2	2.3 ± 2.2 (11/29)	2.7 ± 1.3 (9/26)
3	1.0 ± 1.7 (13/34)	1.4 ± 1.1 (13/37)
4	2.5 ± 0.7 (2/5)	–
5	–	–

bone gain, attachment gain and pocket reduction.

Clinical outcomes following regenerative therapy with EMD have been extensively studied and confirmed. Available data from systematic reviews (Venezia et al. 2004, Esposito et al. 2005, Trombelli 2005) showed that reconstructive treatment procedures with EMD produced a more favourable clinical improvement in hard and soft tissue healing compared with a conventional access flap (open flap debridement). The results following EMD application in the present study show significant improvements compared with the baseline and provide information on the applicability of EMD in the reconstructive treatment of one- and two-wall intra-bony defects. The magnitude of the observed effects has to be interpreted in light of the advanced defect morphology. Predominantly (approximately 75% of all defects) circumferential, one-wall or one- to two-wall defects, presenting with a mean width of 3.6 mm and a mean depth of 6.8 mm, had been included. Nevertheless, all defects responded favourably to the treatment with bone gains up to 5.5 mm in the control group and up to 8.5 mm in the test group.

In order to minimize any bias, measurements were performed by well-trained examiners. Furthermore, not only soft tissue but also hard tissue parameters were evaluated. As histological evidence for successful regeneration of intra-osseous periodontal defects is not a practical outcome variable for controlled clinical trials, any change in direct bone measurements (at surgery and by bone sounding 6 months later) serves as a primary outcome variable to evaluate clinical success (Machtei 1997). Hard tissue fill evident at follow-up is the only component of a regenerated periodontium that can be accurately assessed clinically. In addition, a change in the RAL and probing measurements served as secondary outcomes. In wide defects, a combination of EMD with space-maintaining products, like bone grafts or substitutes, could have the potential advantage of a better support of the flap, which EMD alone, due to its formulation, does not provide. Recent studies showed that the combination of EMD with bone substitutes, such as bovine bone mineral or demineralized freeze-dried bone allograft, had the potential to enhance the reconstructive outcome compared with EMD alone with regard to clinical attachment gain or bone fill (Lekovic et al. 2000, Zucchelli et al. 2003, Guinsky et al. 2004). A comparison of healing of deep intra-bony defects following treatment with EMD, combined either with a natural bovine bone mineral or ß-tricalcium phosphate, showed no significant differences between the groups with regard to PPD reduction or gain in attachment (Döri et al. 2005).

Taken together, these findings provided the principal rationale for the combined application of EMD and a graft in the test group of the present study. In the search for alternatives for bovine-derived bone substitutes or bone grafts from human donors, we investigated a new synthetic bone-replacement graft. So far, only one case series has tested this material in combination with autogenous bone and GTR (Zafiropoulos et al. 2007).

The observed lack of substantial additional benefits observed in the test group of the present study seems to confirm the biological potential of EMD per se to support the clinical reconstruction of the lost attachment apparatus. These results are in agreement with the findings of a recent study comparing the effect of EMD with EMD in combination with an autogenous bone graft in the treatment of intra-osseous defects, which showed equal clinical attachment gain, pocket reduction and radiographic bone gain for both therapies (Guida et al. 2007). The results of the present study support the findings in two other studies comparing EMD alone with EMD and a synthetical bone substitute. Both ß-TCP and bioactive glass, in combination with EMD, showed no significant differences in treatment outcomes compared with EMD alone (Bokan et al. 2006, Sculean et al. 2007). However, in both studies, significant improvements within treatment groups for PPDs and clinical attachment levels were found.

In the present study, bone gain in the combined treatment group showed a higher variability. However, these findings on variable defect fill are difficult to interpret due to lack of histological information on the healing and resorption pattern of the graft material in human periodontal defects. Future studies will have to identify the characteristics of defects that may benefit the most from a combined treatment strategy involving EMD and SBC.

Secondary outcomes in the present study included an evaluation of early wound healing and patients' perception of post-operative healing. The results could demonstrate very good flap closure during the first and second post-operative week in the vast majority of defects treated, as well as very favourable post-operative healing with regard to pain, bleeding and swelling as perceived by the patients during the first post-operative week.

Lack of pain, improved healing of soft tissues and limited inflammation of operated areas have been common clinical observations following surgical periodontal therapy with EMD. In fact, at a recent consensus conference, it was stated that more emphasis should be given to patient-centred outcomes and evaluation of adverse effects of regenerative therapy (Lindhe & Palmer 2002). The favourable wound-healing parameters observed in both groups of this study correspond well with common clinical observations following surgical periodontal therapy with EMD, such as lack of pain, improved healing of soft tissues and limited inflammation of operated area. Data from a study investigating wound healing in the dentogingival region following soft tissue curettage and EMD application (Wennström & Lindhe 2002) support the observations in the present study, where after 1 week, 41.7% and 43.6% of the patients reported no pain after treatment in the EMD and in the EMD/SBC group, respectively. These findings correspond well with another study using EMD in intra-bony defects in which 1 week after surgery, no pain was recorded in 50% of the patients (Tonetti et al. 2004). In a previous investigation on buccal furcations, we observed an absence of pain in even 62% of patients 1 week after surgery with EMD (Jepsen et al. 2004). However, in that study, a flap was elevated on the buccal aspect only, in contrast to the present study, in which wide and partly circumferential intra-bony defects were accessed following buccal and oral flap elevation.

Only a few previous studies have investigated the early wound-healing events following re-constructive periodontal surgery with EMD (Tonetti et al. 2004, Wachtel et al. 2003). An early wound-healing index was used to evaluate healing after a microsurgical access flap with or without the use of EMD (Wachtel et al. 2003). Both treatment approaches resulted in a high percentage of primary flap closure after 1 and 2 weeks. In the current study, there was also a high percentage of flap closure after 1–2 weeks. No differences were found between both treatment groups, accentuating that flap closure as well as the pain and swelling parameters were not negatively influenced by the combination with a bone-graft substitute. To the best of our knowledge, no other studies have investigated the influence of graft material combined with EMD on early healing events. Clinical and pre-clinical studies seem to strongly support a role for EMD in soft tissue wound healing (Mirastschijski et al. 2004, Okuda et al. 2001, Wennström & Lindhe 2002). The biological mechanisms behind these effects of EMD on the early phase of healing, however, are presently not understood. EMDs were thought to be present at the instrumented site for a period of 1–2 weeks (Gestrelius et al. 1997), but in a later report it could be demonstrated by means of immunohistochemistry that EMDs are present on treated root surfaces for up to 4 weeks following periodontal surgery (Sculean et al. 2002). Even though no conclusions can be drawn from these studies regarding the persistence of EMD in the soft and hard tissues surrounding the periodontal defects, it has to be assumed that EMD has an effect on the critical steps of periodontal wound healing that occur during the early healing phase. Further studies are needed to elucidate the mechanisms behind the influence of EMD on soft tissue wound healing.

The results of the present study, which carefully examined the early wound-healing events with regard to flap closure and patients' perception, could clearly demonstrate that the beneficial effects of EMD on wound healing following periodontal surgery were not impaired by the additional placement of a synthetic bone substitute. However, it must be kept in mind that the population of the present study consisted predominantly of non-smoking patients with an optimal oral hygiene, factors that favour outcome after periodontal treatment.

In summary, the data provided by this large multicentre randomized trial support the effectiveness and safety of regenerative procedures based on EMD application, either alone or in combination with a SBC, in the treatment of deep and wide intra-osseous defects with a predominant one- to two-wall component.

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Clinical Relevance

Scientific rationale for the study: To enhance regenerative outcomes in deep one- or two-wall intra-bony defects treated with EMD, the additional placement of bone grafts has been advocated. A novel synthetic bone substitute has not yet been evaluated.

Principal findings: The results could demonstrate favourable clinical results for both treatments and could not confirm any differences in the clinical outcomes between the two treatment modalities in a population of predominantly non-smoking patients with good oral hygiene.

Practical implications: A synthetic bone substitute resulted in no additional benefit when used with EMD for the regenerative treatment of advanced intra-bony defects.