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# The combined use of enamel matrix proteins and a tetracycline-coated expanded polytetrafluoroethylene barrier membrane in the treatment of intra-osseous defects

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## Abstract

**Objectives:** The purpose of this split-mouth study was to evaluate the clinical response of enamel matrix proteins (EMPs, Emdogain Gel<sup>®</sup>) in intra-osseous defects with or without a combined application of a tetracycline-coated expanded polytetrafluoroethylene barrier membrane (e-PTFE, Gore-Tex<sup>®</sup>).

**Methods:** Twelve pairs of intra-osseous periodontal defects in 11 patients received the application of EMPs on the exposed root surface (EMP). One of the two defects received randomly, as an adjunct to EMP treatment, a tetracycline-coated e-PTFE membrane (MEMP). At baseline, 6- and 12-month probing pocket depth (PPD), clinical attachment level (CAL) and probing bone level (PBL) were measured.

**Results:** After 12 months, the EMP defects showed a significant mean PPD reduction of  $2.86 \pm 0.75$  mm, a mean gain in CAL of  $1.28 \pm 2.04$  mm, a mean PBL gain of  $1.63 \pm 1.21$  mm and a mean increase of recession (REC) of  $1.56 \pm 2.30$  mm. The MEMP defects showed a significant mean PPD reduction of  $3.02 \pm 1.55$  mm, a mean gain in CAL of  $1.65 \pm 1.29$  mm, a mean PBL gain of  $1.58 \pm 1.92$  mm and a mean increase of REC of  $1.38 \pm 1.63$  mm. Except for significantly more post-operative discomfort at the MEMP sites, no significant differences were found between EMP and MEMP defects.

**Conclusion:** Within the limits of this study, it is concluded that in the treatment of intra-osseous defects with EMP, the adjunctive use of a tetracycline-coated e-PTFE membrane failed to show more gain of CAL and PBL.

Key words: barrier membranes; enamel matrix proteins; GTR; intra-osseous defects; tetracycline

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A relatively new biological concept in the attempts to obtain periodontal regeneration is the use of enamel matrix proteins (EMP). In vitro studies have demonstrated that EMP stimulate attachment, growth and metabolism of periodontal ligament fibroblasts (Hoang et al. 2000, Van der Pauw et al. 2000,

Lyngstadaas et al. 2001), but not gingival fibroblasts (Gestrelus et al. 1997b). EMP absorb to hydroxyapatite, collagen and denuded dental roots (Gestrelus et al. 1997a). Furthermore, it has been suggested that EMP applied to teeth may have an inhibitory effect on the formation of the oral biofilm

(Arweiler et al. 2002, Spahr et al. 2002).

Comparable reduction of probing pocket depth (PPD), a gain in probing attachment level and, in some studies, a gain in alveolar bone have been accomplished after guided tissue regeneration (GTR) and application of EMP (Cortel-

lini et al. 1996, Bouchard et al. 1997, Caffesse et al. 1997, Hammarström et al. 1997, Heden et al. 1999, Heijl 1997, Heijl et al. 1997, Smith MacDonald et al. 1998, Mellonig 1999, Pontoriero et al. 1999, Sculean et al. 1999a,b,c, Heden 2000, Froum et al. 2001, Loos et al. 2002, Abbas et al. 2003). However, a variation of clinical outcome and predictability is reported for GTR as well as EMP (Tonetti & Cortellini 1997, Tonetti et al. 1998, 2002, Heden et al. 1999, Loos et al. 2002).

In contrast to EMP, barrier membranes are thought to control healing dynamics by covering the periodontal defect. The gingival tissues (epithelium and connective tissue) are prevented from contacting the root surface during early healing, and may thus provide space for cells originating from the periodontal ligament to re-populate the previously affected root surface. This may induce regeneration of the lost periodontal tissues (Gottlow et al. 1986). One important aspect jeopardizing a predictable and successful outcome concerns bacterial infection of the healing intra-osseous defect (Slots et al. 1999). It has been suggested that the immediate post-operative infection by periodontal pathogens of the treated defect and colonization of the membrane itself may be one reason for lack of results in some cases (Selvig et al. 1992, Mombelli et al. 1993). For example, periodontal pathogens may colonize membranes within 3 min. of intra-oral manipulation. The presence of bacteria on the membrane surface facing the gingiva at 6 weeks postoperatively has been shown to be a statistically significant negative predictor of gain in clinical attachment (Nowzari et al. 1996).

Controlling the bacterial colonization in the early healing phase and reducing the spread of infections may increase the predictability of results (Zucchelli et al. 2000). Several antimicrobial agents have been shown to be inhibitory for bacteria associated with destructive periodontal disease (Walker et al. 1985). The use of systemic antibiotics have therefore been propagated by many researchers investigating regenerative procedures for the treatment of intra-osseous defects (Van Winkelhoff et al. 1996, Cortellini & Tonetti 2000, Kornman & Robertson 2000). Compared with systemically delivered antibiotics, locally controlled-release delivery devices applied professionally may exhibit several benefits: independence

of patient compliance, enhanced or improved pharmacokinetic response, greater access and the ability to position the drug adjacent to the disease and to deliver a lower total dosage of the drug to the patient but giving a more controlled concentration at the diseased site (Goodson 1989). Few studies are known using local antimicrobial devices in the treatment of intra-osseous defects. The use of topical 25% metronidazole gel in GTR was shown to be more effective in preventing membrane contamination, but did not improve clinical outcomes (Zucchelli et al. 1999). A study using a tetracycline-coated expanded polytetrafluoroethylene (e-PTFE) barrier membrane suggested that the antimicrobial properties of tetracycline during initial healing could result in additional gain of clinical periodontal attachment (Zarkesh et al. 1999).

Controlled clinical studies comparing the use of EMP and bioresorbable membranes and combinations of both showed no significant difference between any treatment modality in gain of clinical attachment and bone (Sculean et al. 1999a,b,c, 2000, 2001a,b, Silvestri et al. 2000). However, in these studies, the adjunctive use of tetracycline attached to an e-PTFE barrier membrane to control early membrane infection has not been investigated.

Therefore, the aim of this study was to evaluate, in a split-mouth randomized-controlled clinical trial, the clinical response of EMP in the treatment of intra-osseous defects with or without a GTR technique using a tetracycline-coated e-PTFE barrier membrane.

## Material and Methods

### Subjects and inclusion criteria

Initially, 18 consecutive untreated periodontitis patients, in the age range of 18–55 years, referred to the Department of Periodontology at ACTA, were recruited, in good general health, with at least two intra-osseous defects fulfilling the criteria of  $\geq 6$  mm pocket depth and  $\geq 4$  mm radiographic depth not adjacent to a molar furcation site. The defects could be located on any tooth, except the upper molars. The teeth at the defect sites had to have vital pulps, as assessed by sensitivity to a cold test using ethyl chloride to exclude endodontic complications. Smoking habits were recorded, and smoking was quantified as the number of packyears

(number of packs of cigarettes smoked per day  $\times$  number of years smoked) (Grossi et al. 1995). The patients were informed about the aim of the study verbally and in writing, and they signed an informed consent before participation.

### Pre-trial treatment

Each patient received a minimum of 6 h of pre-trial initial treatment consisting of meticulous oral hygiene instructions and supra- and subgingival debridement with manual and ultrasonic instruments using local anaesthetics. Patients who were culture positive for *Actinobacillus actinomycetemcomitans* 3 months after initial therapy received treatment of 1 h by means of re-scaling and root planing in conjunction with systemic antibiotics (375 mg amoxicillin TID and 250 mg metronidazole TID) for 1 week. Sites unrelated to the selected intra-osseous defects that showed at re-evaluation, i.e. 3 months after the completion of the initial therapy, residual PPD of  $\geq 6$  mm were treated by conventional periodontal surgery. The selected experimental intra-osseous sites and sites with a residual PPD of 4–5 mm received an extra session of subgingival debridement under local anaesthetics.

### Final patient selection

The subjects had to demonstrate the ability to perform good oral hygiene, by having a full-mouth plaque score of  $< 20\%$  in two consecutive screening sessions prior to the final selection; if not, they were excluded from the study. Patients using antibiotics were admitted for the final selection at least 3 months after the last use of antibiotics. Only patients who showed at the final selection, after the pre-trial treatment, two non-adjacent, inter-proximal intra-osseous defects with a probing depth from the buccal and/or lingual aspect of  $\geq 6$  mm were included. The defects were required to show an angular bony defect of  $\geq 4$  mm on a new radiograph made at the definitive selection appointment.

### Clinical measurements

The level of oral hygiene and the degree of inflammation during the pre-trial period as well as during the actual trial was evaluated on the basis of full-mouth plaque and bleeding scores. After application of erythrosine dye (Merck KGaA, Darmstadt, Germany), the presence or

absence of plaque along the gingival margin was scored at the mesiobuccal, mid-buccal, distobuccal, mesiolingual, mid-lingual and distolingual sites of all teeth. Full-mouth plaque scores were calculated as the percentage of sites harbouring plaque. Full-mouth bleeding scores were calculated as the percentage of sites, measured at six sites for all teeth, that bled upon probing to the bottom of the pocket with a manual PQW probe (Hu-Friedy, Zweigniederlassung, Leimen, Germany). Clinical measurements of the experimental sites at baseline (i.e. directly prior to surgery), 6 and 12 months were performed by the same calibrated investigator (B. G. L.), who was blinded with respect to treatment modality. PPDs were measured with the use of a force-controlled probe (parallel tine, 0.25 N, 127 N/cm<sup>2</sup>) and automatic readout in one-tenth of a millimetre (Florida Probe<sup>®</sup>, Gainesville, FL, USA) (Gibbs et al. 1988). PPD and clinical attachment level (CAL) measurements were carried out at the selected intra-osseous defects from the buccal and lingual aspects. CAL measurements were performed from a custom-made stent margin. PPD and CAL measurements were performed in duplicate, according to the guidelines of the Florida Probe<sup>®</sup> software, and subsequently the mean of the two values was calculated. Probing bone level (PBL) measurements were performed with a PCP UNC-15 probe (Hu-Friedy) after application of local anaesthesia using as much force as needed to feel a bony resistance, and recorded to the nearest half millimetre with the stent margin as reference.

#### Membrane preparation

The tetracycline-coated e-PTFE membranes were prepared in the following way: type GTA 1 and GTA 2 e-PTFE (Gore-Tex<sup>®</sup>, W. L. Gore & Associates Inc., Flagstaff, AZ, USA) barrier membranes were submerged for 1 min. in 5% solution of tridodecylmethylammonium chloride (TDMAC) in absolute alcohol. After drying at room temperature, the TDMAC-coated membranes were immersed for 1 min. into a freshly prepared 3% tetracycline solution (250 mg dissolved in 7.5 ml distilled water, pH adjusted from 1.7 to 9.5 using sodium hydrochloride) and dried at room temperature. Tetracycline-coated e-PTFE membranes were stored in separate ster-

ile containers in the dark at 10°C (Zarkesh et al. 1999).

#### Surgical procedures

Each patient received a prescription of 0.2% chlorhexidine rinses (Corsodyl<sup>®</sup>, GSK, Zeist, The Netherlands) for two rinses a day to be started 1 day before the planned surgical procedure. All surgical procedures were performed by one experienced clinician (F. A.). Full-thickness flaps were raised around both defects. The modified or simplified papilla preservation technique was used depending on the width of the inter-dental space (Cortellini & Tonetti 2000). The flaps were extended to include at least one tooth mesial and distal to the experimental defect involved. These adjacent teeth would provide anchoring for the tetracycline-coated barrier membrane. In addition, the flaps were extended apically to ensure that the barrier at placement would rest on bone along its entire peri-defect extension. Granulation tissue was removed, and root surfaces were planed with hand instruments and ultrasonics with saline cooling (EMS<sup>®</sup>, Electro Medical Systems, Nyon, Switzerland). In order not to compromise the vascularization of the flaps, no horizontal periosteal-releasing incisions were performed.

Clinical measurements to describe the defect were recorded, including the number of walls, and the width of the defect. To evaluate to what extent measurements of the clinical PBL reflect the actual bone level, surgical PBLs (SPBL) were measured during surgery by measuring from the deepest point of the defect with a PCP UNC-15 probe to the nearest half millimetre with the stent margin as reference.

By an aselect numbers randomization procedure, it was determined as to which defect would receive treatment with EMP alone or EMP in conjunction with a membrane (MEMP). For the randomization procedure, a randomization list and randomization envelopes were prepared. EMP (Emdogain Gel<sup>®</sup>, Biora AB, Malmö, Sweden) was prepared 15 min. before surgical use. The sites were kept free from bleeding as much as possible. The root surface was conditioned for 2 min. with 24% ethylenediamine tetraacetic acid gel (pH 6.7) (PrefGel<sup>®</sup>, Biora AB) and after thoroughly rinsing with sterile saline, EMP was applied on the exposed root surface followed by sutur-

ing with e-PTFE sutures (Gore-Tex<sup>®</sup>, W. L. Gore & Associates Inc.). For the MEMP site, a tetracycline-coated e-PTFE barrier was trimmed for coverage of the selected defect and sutured around the neck of the tooth in a tent-like fashion and lifted prior to root conditioning and application of EMP. Flaps over both defects were sutured with the intention to achieve primary closure. The duration of surgery was recorded for the EMP and MEMP site separately. Finally, post-operative instructions for pain control with acetaminophen tablets (paracetamol 500 mg) were provided to the patient.

#### Post-operative procedures and follow-up

Each patient was instructed to continue chlorhexidine rinses for 8 weeks post-operatively. Control of chlorhexidine compliance, gentle polishing with minimum amounts of pumice to remove plaque and chlorhexidine staining were performed at week 1, 2 and 4 post-operatively. Sutures were removed after 2 weeks. Sutures that loosened prematurely were removed. The extent of membrane exposure was recorded in millimetres with a PQW probe at each visit. The barrier membranes were removed in a second-stage surgery after 6 weeks, using local anaesthesia. The sutures for the second stage were removed after 1 week. Patients were asked at each visit up to 2 months whether they felt any discomfort at one or both of the treated sites. The amount of acetaminophen used by the patient was documented. Oral hygiene compliance was checked, and gentle supragingival scaling and careful polishing of the tooth surfaces were performed monthly until month 6, followed by checkups at months 9 and 12.

#### Statistical analysis

From the baseline buccal and lingual mean PPD measurements of each intra-osseous lesion, the deepest was selected for further analysis throughout the study. From the PPD and CAL measurements, changes in the location of the gingival margin (gingival recession) were calculated (REC). The primary outcome variables were CAL and PBL. Furthermore, full-mouth percentual scores of plaque and bleeding were calculated as a marker of periodontal condition and patient compliance. EMP and MEMP sites were compared within patients. A 2 × 2 level repeated measure

Table 1. Mean values of the clinical parameters  $\pm$  standard deviation at baseline and 6 and 12 months ( $n = 24$  defects in 11 patients)

	Baseline	6 months	12 months	Difference baseline – 12 months	<i>p</i> -value*
PPD					
EMP	6.95 $\pm$ 1.06	4.69 $\pm$ 0.99	4.09 $\pm$ 1.17	2.86 $\pm$ 0.75	0.0005
MEMP	7.32 $\pm$ 1.24	4.37 $\pm$ 1.33	4.30 $\pm$ 1.08	3.02 $\pm$ 1.55	0.0005
CAL					
EMP	10.94 $\pm$ 1.59	10.00 $\pm$ 1.42	9.66 $\pm$ 1.75	1.28 $\pm$ 2.04	0.0499
MEMP	11.10 $\pm$ 1.60	9.65 $\pm$ 1.59	9.45 $\pm$ 1.74	1.65 $\pm$ 1.29	0.0005
PBL					
EMP	12.83 $\pm$ 2.31	11.42 $\pm$ 2.07	11.21 $\pm$ 1.88	1.63 $\pm$ 1.21	0.0029
MEMP	13.17 $\pm$ 1.96	11.92 $\pm$ 2.02	11.58 $\pm$ 1.88	1.58 $\pm$ 1.92	0.0156
REC					
EMP	3.99 $\pm$ 1.93	5.31 $\pm$ 1.08	5.55 $\pm$ 1.31	1.56 $\pm$ 2.30	0.0454
MEMP	3.77 $\pm$ 1.19	5.29 $\pm$ 1.48	5.15 $\pm$ 1.98	1.38 $\pm$ 1.63	0.0226

PPD, probing pocket depth (mm); CAL, clinical attachment level (mm); PBL, probing bone level (mm); REC: recession (mm); dif, difference. \*Statistical testing of differences between baseline and 12 months. Wilcoxon signed rank test.

analysis was used to analyse the effect of several covariates on changes in CAL and PBL by entering baseline and 12-month values as dependent variables and entering baseline full-mouth plaque and bleeding scores, baseline PPD, defect dimensions, receiving antibiotics, smoking, age and gender as covariates. Since none of the covariates had a significant association with any of the effects in the analyses, all further analyses were performed using Wilcoxon tests, comparing various parameters at each assessment and comparing different assessments through time within defects.

Defects were paired in each patient. In one patient, two pairs were available; all other pairs were located in 10 different patients. The extra pair of defects in the 11th patient was entered into the analysis without further correction as the non-parametric analyses do not allow for it. Since the ratio is 1 extra against 10 in separate patients, the possible disturbing influence of within-patient dependence of the two pairs of defects could be considered as minor. *P*-values  $<0.05$  were accepted as statistically significant. The power of the study, given 2 mm as a significant difference in increments (from baseline to 12 months) between treatments, was calculated to be 0.8 for both CAL and PBL.

## Results

From the initial 18 selected subjects, 11 patients (four male, seven female) with, in total, 24 sites (one patient had two paired defects), entered and completed the study. Reasons for withdrawal were insufficient time ( $n = 2$ ), travelling dis-

tance ( $n = 1$ ) and insufficient compliance ( $n = 1$ ). Three patients showed reduction of PPD and radiographic defect depth after the pre-trial treatment, to such an extent that they did not fulfil the inclusion criteria anymore.

At baseline, the mean age of the patients was 34.8 years (range 18.0–51.0 years). Three patients were smokers with a mean of 7.5 packyears (range 2.0–20.0 packyears); one patient was a former smoker and stopped smoking 26 years ago (8.0 packyears). Seven out of the 11 patients entering the study who were culture positive for *A. actinomycetemcomitans* after initial therapy were treated by means of re-scaling and root planing in conjunction with systemic amoxicillin and metronidazole.

The patients maintained good levels of oral hygiene throughout the study. At baseline, the mean full-mouth plaque score was 5.4% (range 2.0–8.0%) and the full-mouth bleeding score was 6.7% (range 1.0–12.0%). At 6 months, the mean full-mouth plaque score was 11.8% (range 4.0–29.0%) and the mean full-mouth bleeding score was 3.9% (range 1.0–7.0%). At 12 months, the data showed a mean full-mouth plaque score of 12.5% (range 1.0–38.0%) and a mean bleeding score of 6.0% (range 1.0–17.0%).

The mean PPD at baseline was 7.0 mm (range 6.0–9.4 mm) for the EMP sites and 7.3 mm (range 6.1–10.6 mm) for the MEMP sites (Table 1). After 12 months, the EMP sites showed a mean PPD reduction of 2.9 mm (range 1.8–4.1 mm), a mean gain of CAL of 1.3 mm (range 3.5 mm loss–4.5 mm gain), a mean gain of PBL of 1.6 mm

(range 0.5 mm loss–3.0 mm gain) and a mean increase of REC of 1.6 mm (range 1.6 mm decrease–6.1 mm increase). The MEMP sites showed a mean PPD reduction of 3.0 mm (range 1.1–5.6 mm), a mean gain of CAL of 1.7 mm (range 0.1 mm–3.9 mm), a mean gain of PBL of 1.6 mm (range 0.5 loss–5.5 mm gain) and a mean increase of REC of 1.4 mm (range 1.8 mm decrease–3.8 mm increase). Again, no statistically significant differences in the amount of plaque and bleeding on a site level were found. No statistically significant difference was found between 6 and 12 months with regard to PPD, CAL, PBL and REC results. Twelve months after baseline, the clinical parameters PPD, CAL, PBL showed a significant improvement, together with a significantly increased amount of REC compared with baseline. However, no statistically significant differences were found between the two treatment modalities EMP and MEMP (Table 1).

Table 2 shows data on PPD, CAL and PBL for individual sites. Of the MEMP sites, none of the defects experienced loss of clinical attachment, whereas of the EMP sites, three defects experienced loss of clinical attachment. In both groups, only one defect showed loss of PBL.

The anatomy of the intra-osseous component of the lesions was revealed to be three walled in all cases (Table 3). Comparable defect dimensions were found between EMP and MEMP sites. The average depth of the defect, measured from the bottom of the defect to the top of the crest (intra-osseous component), was 4.3 mm (range 3.0–6.5 mm) for the EMP sites and 4.3 mm (range 2.5–6.0 mm) for the MEMP sites (Table 2). Evaluation of the PBL and the SPBL measurements performed on the day of surgery showed no statistically significant difference.

In Table 4, the events related to the surgical treatment are presented. The mean time needed for the surgical procedures was 64.8 min. (range 35.0–115.0 min.) for the EMP site and 73.3 min. (range 28.0–125.0 min.) for the MEMP site. Evaluation of the extent of membrane exposure 6 weeks after surgery showed minor membrane exposure (1–2 mm) in eight MEMP sites and more advanced exposure ( $\geq 3$  mm) in two MEMP sites. Assessment of post-operative discomfort showed that one patient had a loose suture at the EMP site within 1 week, one patient complained about minor post-operative haemorrhage at

Table 2. Individual data on baseline PPD and increments for CAL and PBL for each treatment defect

Site no.	MEMP						EMP					
	patient	tooth	location	baseline PPD	12 months dif CAL	12 months dif PBL	tooth	location	baseline PPD	12 months dif CAL	12 months dif PBL	
1	1	36	Mesial	10.6	3.9	5.5	46	Distal	6.3	1.8	3.0	
2	2	37	Mesial	6.3	0.1	0.0	46	Mesial	6.0	-0.1	-0.5	
3	3	44	Mesial	7.0	2.9	1.0	36	Mesial	8.1	1.3	3.0	
4	4	33	Mesial	7.4	0.5	-0.5	43	Mesial	7.6	4.5	1.5	
5	5	36	Mesial	7.2	1.8	0.0	46	Mesial	7.2	-0.9	0.5	
6	6	24	Mesial	7.6	2.6	2.5	15	Distal	6.2	1.1	1.5	
7	7	36	Mesial	7.4	1.6	1.5	46	Mesial	7.7	3.0	1.0	
8	8	25	Mesial	6.7	3.3	0.0	36	Distal	6.2	1.5	3.0	
9	9	46	Distal	6.5	1.7	1.5	36	Mesial	6.3	2.3	0.0	
10	10	46	Mesial	8.6	0.5	3.5	36	Mesial	6.0	2.4	2.5	
11*	11	37	Distal	6.1	0.3	0.0	46	Distal	9.4	-3.5	2.5	
12*	11	44	Distal	6.4	0.6	4.0	34	Distal	6.4	2.0	1.5	

\*Two pairs of defects in one patient. PPD, probing pocket depth; CAL, clinical attachment level; EMP, enamel matrix protein; MEMP, EMP in conjunction with a membrane.

Table 3. Clinical characteristics of inter-proximal defects at baseline ( $n = 24$  defects in 11 patients)

	EMP	MEMP
Defect dimension (mean $\pm$ SD)		
Width defect bucco-lingual in mm (BL)	9.4 $\pm$ 0.9	9.2 $\pm$ 1.1
Width defect mesio-distal in mm (MD)	3.5 $\pm$ 0.9	3.6 $\pm$ 1.2
Depth defect buccal in mm	4.8 $\pm$ 1.7	4.4 $\pm$ 1.0
Depth defect lingual in mm	3.8 $\pm$ 1.7	4.1 $\pm$ 1.8
Average depth buccal and lingual in mm (D)	4.3 $\pm$ 1.3	4.3 $\pm$ 1.0
Volume defect in mm <sup>3</sup> (BL $\times$ MD $\times$ D)	144.2 $\pm$ 76.2	147.0 $\pm$ 72.1
Probing bone level in mm (mean $\pm$ SD)	12.7 $\pm$ 2.3	12.9 $\pm$ 2.1
Surgical probing bone level in mm (mean $\pm$ SD)	12.1 $\pm$ 2.3	12.6 $\pm$ 2.7

No statistical significant difference between MEMP and EMP defects.

EMP, enamel matrix protein; MEMP, EMP in conjunction with a membrane.

the MEMP site for 2 days, one patient had headaches for 1 week and one patient had a limited mouth opening for 1 week. A significantly shorter period of post-operative pain (mean 1.4 days, range 0–4.0 days) was found in the EMP sites as compared with the MEMP sites (mean of 3.5 days, range 1.0–7.0 days). Relatively few days of discomfort were recorded at the MEMP sites after membrane removal (mean 0.2 days, range 0–1.0 days). During the first 7 post-operative weeks, a mean of 8.2 tablets (range 0–34 tablets) of acetaminophen was used. Because of the fact that both treatment modalities were carried out within each patient, the use of tablets could not be evaluated for EMP and MEMP sites separately.

The repeated measures analysis did not show a significant influence of baseline full-mouth plaque and bleeding scores, baseline PPD, defect dimensions, receiving of antibiotics, smoking, age and gender on changes of CAL and PBL in the analysis.

## Discussion

The results of the present investigation showed that treatment of deep inter-proximal intra-osseous defects with EMP with or without an adjunctive tetracycline-coated barrier membrane resulted in a statistically significant mean PPD reduction, gain in attachment level, gain in bone level and some gingival recession. There were, however, no significant differences between treatment results when comparing the two treatment modalities. The results of the present MEMP procedure are comparable with other GTR studies (Kersten et al. 1992, Weltman et al. 1997, Mayfield et al. 1998, Zucchelli et al. 1999, Loos et al. 2002, Minabe et al. 2002). In contrast to the present findings, much larger attachment level gains have been reported by a number of other clinical trials using GTR (Cortellini et al. 1995), EMP (Heden et al. 1999, Sculean et al. 1999a, b, c, Heden 2000) or both (Pon-toriero et al. 1999, Sculean et al. 2001a).

A number of factors that may have influenced the present results can be suggested. First, a major difference between the present study and other reports relates to the pre-trial treatment. The current pre-trial treatment included not only the use of systemic antibiotics in a number of subjects but also the re-treatment of the selected experimental sites by scaling and root planing. It has been demonstrated that the presence of *A. actinomycetemcomitans* may have a negative influence on the outcome of periodontal therapy (Machtei et al. 1994). Therefore, it was decided to treat all patients who were culture positive for *A. actinomycetemcomitans* after initial therapy by means of re-scaling and root planing in conjunction with systemic amoxicillin and metronidazole. This treatment has been shown to be highly effective in eradicating *A. actinomycetemcomitans* (Pavicic et al. 1994, Berglundh et al. 1998).

It has been suggested that the pre-trial treatment negatively influences the potential of defects to regenerate. Inflamed lesions may respond more favourably to surgery than lesions that have been extensively instrumented prior to surgery; this may be because of more open marrow spaces, presence of immature collagen and tissue factors (Prichard 1957, Becker et al. 1986, Becker 1994). Results of the present study might indeed corroborate the suggestion that extensive scaling and root planing of an intra-osseous site prior to regenerative procedures could result in limited gains in clinical attachment and new bone. Thus, one has to keep in mind that extensive pre-trial treatment actually changes the baseline values of all

Table 4. Events related to surgical treatment (mean  $\pm$  SD)

Duration surgery (min)							
EMP		64.8 $\pm$ 26.6					
MEMP		73.3 $\pm$ 29.0					
		Membrane removal					
		week 1	week 2	week 4	week 6	total	
Exposure membrane (no. of sites)							
MEMP 1–2 mm		3	6	7	8	8	
MEMP $\geq$ 3 mm		1	1	2	2	2	
		Week 1	Week 2	Week 4	Week 6	Week 7	Total
Post-operative pain (no. of days)							
EMP		1.1 $\pm$ 0.8	0.2 $\pm$ 0.6	0	0	0.2 $\pm$ 0.6	1.4 $\pm$ 1.4
MEMP		2.6 $\pm$ 1.7	0.7 $\pm$ 1.7	0	0	0.2 $\pm$ 0.4	3.5 $\pm$ 2.2*
Acetaminophen (no. of tablets)							
EMP+MEMP		7.3 $\pm$ 8.4	0.3 $\pm$ 0.6	0	0	0.5 $\pm$ 1.0	8.2 $\pm$ 9.6

\*Statistically significant more pain MEMP sites; Wilcoxon signed rank test  $p$ -value 0.0078.

EMP, enamel matrix protein; MEMP, EMP in conjunction with a membrane.

clinical parameters. Measures of PPD and CAL will already be more favourable than before pre-trial treatment. When comparing data from studies that provide no or limited pre-treatment of experimental sites to studies that perform extensive pre-treatment, the baseline values before all treatment should be taken into account. In that case, conclusions should be based on the total change of parameters because of the whole series of treatments provided. In the present study, data do not allow for such a comparison.

Of the 12 membrane-treated sites, 10 showed exposure of the membrane to some degree. This occurrence could have a negative effect on the treatment outcomes since it has been shown that exposure of the membrane is related to less gain in attachment level (Becker et al. 1988, Cortellini et al. 1990, 1993, De Sanctis et al. 1996). However, the tetracycline coat may have limited these potential negative effects.

Defect morphology has been shown to influence the potential for regeneration (Renvert et al. 1985, Tonetti et al. 1993, Pontoriero et al. 1999). In the present study, all lesions were three-wall intra-osseous defects providing enough support for the membrane in order not to collapse, and providing stabilization of the intra-osseous cloth formation (Trombelli et al. 1997, Mayfield et al. 1998).

No effects of smoking on the CAL or PBL were found in the present data. This is in contrast to several previous studies, which showed that smoker

patients demonstrated less gain in CAL and PBL than non-smoker patients (e.g. Tonetti et al. 1998, 2002, Loos et al. 2002). Most likely, the small number of smokers ( $n = 3$ ) in the current study is responsible for this lack of effect.

In the present study, defects were treated either by EMP or by EMP plus a membrane coated with tetracycline. EMP on itself has been reported to have positive and stimulatory effects on cells originating from the periodontal ligament (Van der Pauw et al. 2000, Lyngstadaas et al. 2001). Moreover, EMP has inhibitory effects on bacteria, in particular, it may help to prevent bacterial colonization of the root surface that is targeted for regeneration, i.e. deposition of new cementum with insertion of new periodontal ligament fibres (Arweiler et al. 2002, Spahr et al. 2002). It is generally thought that periodontal ligament cells cannot re-populate the root surface where bacteria form colonies. While EMP acts supposedly at the level of the root surface, it has been our thought that the local application of tetracycline from the membrane as a vehicle prevents infection of the blood clot and infection of flap margins. Tetracycline is bacteriostatic and effective against Gram-positive bacteria, and many periodontal pathogens (Walker et al. 1981, Baker et al. 1985a, b). Tetracycline has the characteristic to adhere to dentin surfaces, known as substantivity (Stabholz et al. 1993). It has been shown to inhibit tissue collagenase activity, reduce alveolar bone resorption (Golub et al. 1984) and pro-

mote fibroblast adhesion and growth (Terranova et al. 1986). The tetracycline coat to the membrane has been shown to have an in vitro antimicrobial activity for 2–6 weeks, demonstrating its excellent potential to prevent bacterial colonization of the membrane (Zarkesh et al. 1999). The extended antibacterial activity is obtained by the strong coupling to the membrane and slow release. Thus, a combined periodontal ligament stimulatory and bacteriostatic approach was hypothesized to enhance GTR results. However, this hypothesis is not substantiated by the results; sites treated with the combination do not respond substantially better than sites treated with EMP alone.

In conclusion, within the limits of this study, it is concluded that in the treatment of intra-osseous defects with EMP, the adjunctive use of a tetracycline-coated e-PTFE membrane failed to show more gain of clinical attachment and bone.

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