J Clin Periodontol 2005; 32: 225–230 doi: 10.1111/j.1600-051X.2005.00664.x

Journal of Clinical Periodontology

Enamel matrix proteins and systemic antibiotics as adjuncts to non-surgical periodontal treatment: clinical effects

A. Mombelli, P. Brochut, D. Plagnat, F. Casagni and C. Giannopoulou

School of Dental Medicine, University of Geneva, Geneva, Switzerland

Mombelli A, Brochut P, Plagnat D, Casagni F, Giannopoulou C: Enamel matrix proteins and systemic antibiotics as adjuncts to non-surgical periodontal treatment: clinical effects. J Clin Periodontol 2005; 32: 225–230. doi:10.1111/j.1600-051X.2005.00664.x. © Blackwell Munksgaard, 2005.

Abstract

Objectives: The purpose of the present study was to evaluate the clinical effects of non-surgical periodontal treatment, supplemented with enamel matrix derivative (EMD) and/or systemic antibiotics, in deep periodontal pockets of patients with chronic periodontitis.

Methods: This was a randomized, placebo-controlled longitudinal clinical trial of 12 months duration. Using a split-mouth design, 16 subjects were randomly assigned to scaling and root planing (SRP) with EMD or placebo in contra-lateral dentition areas. One half of the subjects received 250 mg metronidazole and 375 mg amoxicillin three times a day for 7 days and the other half received a placebo. One inter-proximal periodontal lesion was chosen as study site in each of the contra-lateral quadrants.

Results: Subjects treated with systemic antibiotics yielded significantly better clinical results than those treated with placebo. In these cases, probing pocket depth was reduced significantly more after 6 months (3.0 ± 2.1 mm *versus* 1.6 ± 1.4 mm, $p = 0.05$), and the mean clinical attachment gain was significantly greater after 6 months (2.3 ± 1.9 mm *versus* 0.7 ± 1.6 mm, $p = 0.02$) and 12 months (2.3 ± 3.5 mm *versus* 0.4 ± 3.8 mm, $p = 0.02$). Sites treated with the antibiotics plus EMD gained the largest amount of clinical attachment. There was no significant benefit of EMD adjunctive to SRP in subjects not treated with antibiotics.

Conclusions: The present study supports the notion that optimal repair and regeneration of the periodontium requires suppression of the microbiota causing periodontal disease.

Key words: amoxicillin; chronic periodontitis; enamel matrix proteins; metronidazole; non-surgical periodontal treatment

Accepted for publication 27 May 2004

Clinical studies in humans, and histological studies in animals, have demonstrated that enamel matrix derivative (EMD) has a positive effect on post-surgical wound healing, and may favour the formation of a new periodontal attachment apparatus (Hammarstrom et al. 1997, Heijl 1997, Heijl et al. 1997, Mellonig 1999, Sculean et al. 1999a, b, Yukna & Mellonig 2000, Tonetti et al. 2002). In the original studies demonstrating this effect, EMD was applied onto the root surface in an open procedure, i.e. following the elevation of a mucoperiosteal flap. Granulation tissue, plaque and calculus can be removed thoroughly under these circumstances and the root can be conditioned predictably (e.g. removal of smear layer). On the other hand, if essentially the same outcome could be achieved using a non-invasive approach, this would be preferable from the practitioner and patient's point of view. The effect of non-surgical periodontal therapy has been demonstrated clearly in numerous studies (van der Weijden & Timmerman 2002), but the limitations of this therapeutic approach are also known, particularly when used in deep periodontal pockets. In deep sites, persistence of calculus (Waerhaug 1978, Caffesse et al. 1986) and pathogenic microorganisms is not an unusual finding (Renvert et al. 1990, Mombelli et al. 1994, Mombelli et al. 2000). Since microorganisms cause periodontal diseases, one would think that an attempt to suppress the subgingival microbiota

as much as possible would favor repair and regeneration of the periodontium. Thus, adjunctive antimicrobial therapy may enhance the effect of EMD, particularly when applied in difficult situations, for example in deep pockets treated without elevating a mucoperiosteal flap.

The purpose of this study was to evaluate the effect of EMD, applied during non-surgical periodontal treatment, in deep periodontal pockets of patients with chronic periodontitis, with or without adjunctive systemic antibiotics.

Material and Methods

This was a randomized, placebo controlled longitudinal clinical trial of 12 months duration. Using a split-mouth design, each subject was randomly assigned to treatment with EMD or placebo in contra-lateral dentition areas. One half of the subjects received systemic antimicrobial therapy and the other half received a placebo.

Subjects

Sixteen systemically healthy patients with untreated moderate to advanced periodontitis were recruited into the study on the basis of the following criteria: Age 25–65 years, presence of inter-proximal periodontal lesions in each of two contra-lateral quadrants in the region including the canine, premolars, and the mesial aspect of the first molar, and presence of *Porphyromonas gingivalis* in a pooled subgingival plaque sample from this region. One inter-proximal periodontal lesion with radiographic evidence of an intrabony defect $\geqslant 2$ mm in depth, associated with a pocket probing depth (PPD) $\geqslant 5$ mm, clinical attachment level (CAL) $\geqslant 5$ mm, and bleeding on controlled force probing, was chosen as study site in each of the contra-lateral quadrants.

Exclusion criteria included the enrollment in another clinical trial (either medical or dental), systemic illnesses (i.e. diabetes mellitus, cancer, HIV, bone metabolic diseases or disorders that compromise wound healing, chronic high dose steroid therapy, radiation or immune-suppressive therapy), pregnancy or lactation, systemic antibiotics taken within the previous 2 months, confirmed or suspected intolerance to 5-nitroimidazole-derivatives or amoxicillin, and subgingival scaling in the last year. The smoking history was recorded (smoking was not an exclusion criterion).

Test products and handling

Sterile lyophilized EMD (Emdogain®, Biora AB, Malmø, Sweden), 30 mg per vial, to be reconstituted with 1.0 ml of sterile aqueous solution of Propylene Glycol Alginate (PGA; Biora AB), was used as test device. The vehicle, PGA, served as the placebo product for Emdogain®. Sterile ethylenediamine tetraaceticacid (EDTA)–gel 24%, pH 6,7 was used for root surface conditioning prior to experimental treatment (PrefGel™, Biora AB). 250 mg metronidazole (Flagyl, Rhône-Poulenc Rorer AG, Thalwil, Switzerland) and 375 mg amoxicillin (Clamoxyl, SmithKline Beecham, Thörishaus, Switzerland), or similarly looking placebos, were administered together three times a day for 7 days.

Clinical protocol

Subjects who met the criteria for inclusion, and had given written informed consent, were enrolled in the study and were given a patient number. After baseline examination and recording of all study parameters, the patients were instructed in proper oral hygiene. Excluding the selected study sites (one test, one control), all teeth with $\geqslant 4$ mm PPD were thoroughly scaled and root planed with ultrasonic and hand instruments. Treatment was continued until the operator felt that all tooth surfaces were clean, hard and smooth. This was accomplished in two to four treatment sessions, scheduled 1 week apart. The two study teeth were treated as follows in a single, separate session: Thorough scaling and root planing to the depth of the periodontal defect, followed by pocket irrigation with saline solution, manual compression of gingival tissues for 5 min, and the placement of a retraction chord containing 10% potassium sulphate (Gingibraid 3a, Van R Dental Products, Oxnard, CA, USA). Next, an envelope containing the code for the randomized supplementary treatment was opened, assigning Emdogain® to one, and placebo to the other side. The retraction chords were removed after 2 min in place, and then the sites were rinsed with saline. PrefGel was applied in the pockets during 2 min (Blomlöf & Lindskog 1995, Blomlöf et al. 1996), followed by another saline irrigation. With a blunt cannula inserted to the bottom of the pocket, either the test or the control substance was applied until spill over. At the end of the session, the subjects received a neutral package marked with their subject number, containing either metronidazole and amoxicillin or two placebos, and were advised to take one of each tablet three times a day for 7 consecutive days.

The patients were recalled after 10 days, as well as 2, 6, and 12 months after treatment. The oral hygiene was checked and reinforced, if necessary, and study parameters were recorded as detailed below. No instrumentation was performed below the gingival margin, but supragingival calculus was removed, if detected, and the teeth were polished.

Three clinicians were involved, working independently of each other, and at separate times, without discussing any clinical aspects of the trial: The study coordinator performed the screening visits, fabricated individual plastic stents to standardize radiographic film placement and RX projection geometry, took radiographs, scheduled the patients for all further procedures and supervised the study flow. The operator performed the treatments and dispensed the medicaments. The examiner recorded clinical parameters, took radiographs, and obtained samples of subgingival plaque and gingival fluid (results are reported in a companion paper, Giannopoulou et al. in preparation).

Study parameters were recorded at baseline, i.e. immediately before the treatment of the experimental units and at the four recall appointments mentioned above. The study sites were accessed from the buccal. Plaque Index (Silness & Löe 1964) and Gingival Index (Löe & Silness 1963) were recorded at every visit. Gingival fluid samples were taken using a Durapore strip (Millipore, Bedford, MA, USA) inserted 1 mm into the crevice and stored at -20°C. Thereafter, one sterile paper point was inserted to the bottom of the pocket to obtain a subgingival plaque sample.

PPD, bleeding upon probing (BOP) and the discrepancy between the gingival margin and the cemento-enamel junction (recession) were measured using a pressure sensitive probe set to 0.3 N probing force at baseline, and at months 2, 6 and 12. Standardized radiographs were obtained at baseline, and at months 6 and 12.

Radiographic evaluation

Serial dental radiographs were acquired using an individualized plastic positioning device linked to the dentition designed to minimize projection divergence (Graf et al. 1988, Dubrez et al. 1995). An individual plastic stent linked the device to the dentition. Each radiograph included an image of the same aluminum wedge. The radiographs were scanned on a flatbed scanner (Duoscan, Agfa-Gevaert N.V., Mortsel, Belgium) with a spatial resolution of 300 dpi, and pixel intensity coded in 16 bits gray scale. The intensity was calibrated using serial images of selected areas with defined thickness of the aluminum wedge. A robust B-splines multi-resolution registration algorithm was implemented to overcome the acquisition misalignment (Dornier et al. 2004). Radiographs taken at months 6 and 12 were subtracted from those taken at baseline to visually assess bone density evolution. Changes were expressed as gain or no gain dichotomously.

Microbiological procedures

Paper points were withdrawn from the periodontal pockets 10 s after insertion and were placed in 4 M guanidinium thiocyanate 2-mercaptoethanol. The samples were analysed using oligonucleotid probe technology (Dix et al. 1990). They were hybridized to a specific probe for the ssrRNA of *P. gingivalis* and to a universal bacterial probe (MicroProbe Corporation, Bothell, WA, USA, processed by IAI PadoTest, Zuchwil, Switzerland). Bacterial counts were calculated by comparison with homologous reference standards and expressed as count $\times 10^6$.

Statistical analysis

The Kruskal–Wallis one-way analysis of variance and the Mann–Whitney *U*-Test were used to determine differences between sites treated with or without EMD, and with or without systemic antibiotics at each time point. The longitudinal changes were analysed using the Wilcoxon-matched pairs signed ranks test. Stepwise multiple linear regression analysis was used to study the relationship between clinical outcome at months 6 and 12 and the other parameters assessed. *p*-values <0.05 were accepted for statistical significance. Two of the enrolled patients (one treated with systemic antibiotics, one without) were unavailable for the 12-month evaluation. Hence 32 sites were included in the analysis up to month 6, and 28 up to month 12.

Results

At baseline, the selected study sites had a mean PPD of 7.3 ± 1.6 mm (range 5–12 mm), with a mean CAL of 8.12 ± 2.5 mm. 94% of these sites bled upon probing, and 59% tested positive for *P. gingivalis*. Table 1 shows the baseline values separately for the four possible treatment assignments (subjects with or without adjunctive antimicrobial therapy: A, A0; sites treated with or without EMD: E, E0). The over-all pocket depth reduction from baseline to 6 and 12 months was highly significant ($p<0.001$). The mean PPD was 4.9 ± 1.6 mm after 6 months and 5.1 ± 1.6 mm after 1 year. A significant over-all gain in clinical attachment was noted over 6 months (1.5 ± 1.9, $p<0.001$) and 12 months (1.1 ± 2.3 mm, $p = 0.02$). Subtraction radiography confirmed an increase in bone density in 28% sites at month 6, and in 38% sites at month 12. None of the sites showed a decrease in bone density.

Subjects treated with metronidazole plus amoxicillin yielded significantly better clinical results than those treated with placebo (Fig. 1a,b). After 6 months PPD was reduced significantly more (3.0 ± 2.1 mm *versus* 1.6 ± 1.4 mm, $p = 0.05$), and the mean PPD was significantly smaller (Table 2, $p = 0.05$). Mean CAL gain was significantly greater after 6 months (2.3 ± 1.9 mm *versus* 0.7 ± 1.6 mm, $p = 0.02$) and 12 months (2.3 ± 3.5 mm *versus* 0.4 ± 3.8 mm, $p = 0.02$). On the other hand, although *P. gingivalis* was reduced considerably, this microorganism was not completely eradicated, even in the subjects treated with antibiotics.

A cross-sectional pairwise comparison of the clinical data from the sites treated with or without Emdogain®, independent of the antibiotic therapy, showed no significant difference (Table 3). However, when the four possible treatment assignments were compared for longitudinal changes, it became clear that the sites treated with the antibiotics plus EMD had gained the largest amount of clinical attachment (Fig. 2). Significant clinical attachment gain was obtained also in sites treated with antibiotics but without EMD ($p = 0.03$).

If the clinical goal of periodontal therapy is defined as absence of pockets deeper than 4 mm and absence of bleeding of probing, how many sites treated in this study reached this goal, and how could they be characterized? Twelve of the 32 investigated sites in fact reached a PPD of 4 mm or less at month 6, and five of them were also negative for BOP. Four of these five sites (baseline PPD: 6, 6, 7, 9 mm) had been subject to systemic antibiotic therapy, and three of them were also treated with EMD. On the other hand, eight of the 10 investigated sites still showing a PPD of 6 mm or more at month 6, had not been treated with systemic antibiotics, 50% had received EMD, and 50% not.

Four subjects treated without antibiotics, and two treated with antibiotics were smokers. With regards to pocket depth reduction and CAL gain there seemed to be a tendency of better clinical responses in non-smokers, but small numbers precluded a statistical evaluation.

Discussion

This randomized, placebo controlled longitudinal clinical trial of 12 months duration demonstrated the relative

Table 1. Baseline parameters for sites treated with SRP, with or without systemic antibiotics (A, A0), with or without Emdogain (E, E0)

	A0+E0	A+E0	A0+E	A+E
PPD (mm)	6.8 ± 1.4	7.1 ± 2.1	7.5 ± 1.3	7.7 ± 1.4
CAL (mm)	7.3 ± 1.9	8.0 ± 2.1	7.9 ± 2.7	9.3 ± 3.2
BOP (%)	100	88	100	88
Porphyromonas gingivalis				
Sites positive (%)	38	88	50	75
Count, if positive	$3.4 \pm 3.5 \times 10^6$	$1.3 \pm 1.5 \times 10^6$	$1.4 \pm 1.5 \times 10^6$	$5.3 \pm 3.7 \times 10^6$

No significant differences between groups for clinical parameters.
SRP, scaling and root planing; PPD, pocket probing depth; CAL, clinical attachment level; BOP, bleeding upon probing.

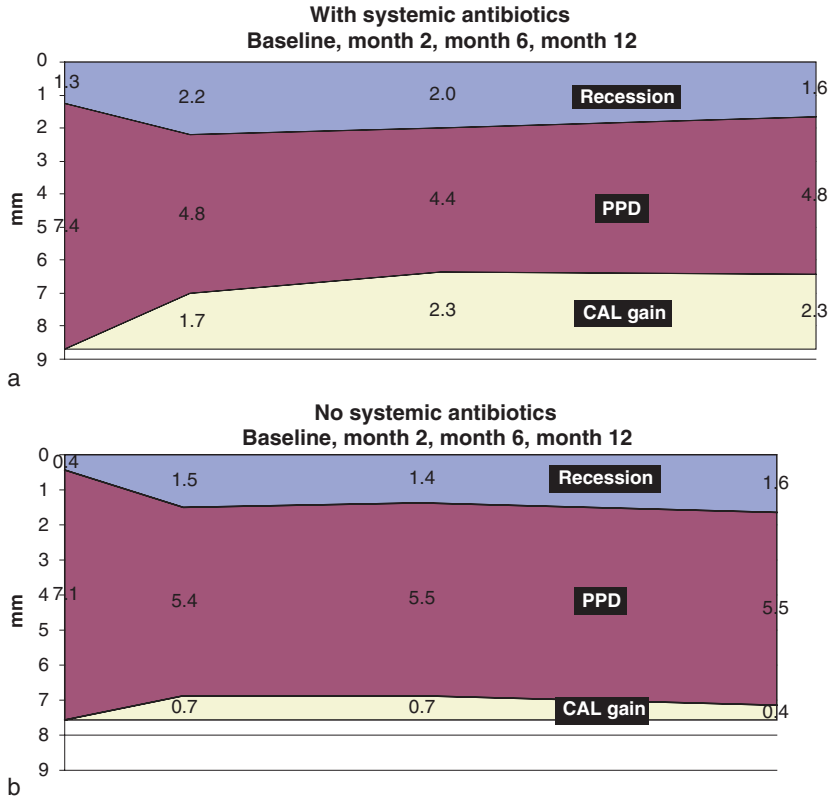

Fig. 1. a, b: Mean changes in recession, pocket probing depth and clinical attachment level in subjects treated with (a) and without (b) adjunctive systemic antibiotics (0 mm corresponds to the cemento-enamel junction).

Table 2. Parameters for patients treated with SRP alone, or with adjunctive systemic antimicrobial therapy (SRP+AB)

		Baseline	Month 2	Month 6	Month 12
PPD (mm)	SRP	7.1 ± 1.4	5.4 ± 1.3	5.5 ± 1.7*	5.5 ± 1.7
	SRP+AB	7.4 ± 1.8	4.8 ± 1.3	4.4 ± 1.3*	4.8 ± 1.5
CAL (mm)	SRP	7.6 ± 2.3	6.9 ± 1.6	6.9 ± 2.2	7.1 ± 1.6
	SRP+AB	8.7 ± 2.7	7.0 ± 2.1	6.4 ± 2.0	6.4 ± 2.4
BOP (%)	SRP	1.0	0.8	0.7	0.8
	SRP+AB	0.9	0.5	0.5	0.6
Porphyromonas gingivalis					
Positive (%)	SRP	44	0	25	50
Count, if positive		$2.2 \pm 3.1 \times 10^6$	–	$0.5 \pm 0.9 \times 10^6$	$0.5 \pm 0.7 \times 10^6$
Positive (%)	SRP+AB	81	13	25	13
Count, if positive		$2.6 \pm 3.3 \times 10^6$	$<10^5$	$0.3 \pm 0.4 \times 10^6$	$<10^5$

*Significant difference between groups, $p = 0.05$.
SRP, scaling and root planing; PPD, pocket probing depth; CAL, clinical attachment level; BOP, bleeding upon probing.

benefits of adjunctive EMD and systemic antibiotics to non-surgical therapy of deep lesions in patients with chronic periodontitis. While the effect of systemic adjunctive antibiotics was clearly visible, the effect of EMD emerged only in cases also receiving antibiotics, indicating the possibility for a combined effect of antibiotic and EMD (Fig. 3). In fact, when all subjects were pooled together, a pairwise comparison showed no significant difference between the clinical data from the sites treated with or without Emdogain®. This is in accordance with recently published data from a longitudinal trial of three months duration showing no significant difference between sites treated with or without EMD as an adjunct to non-surgical periodontal therapy (Gutierrez et al. 2003). It should also be noted that in our study the sites treated with antibiotics and EMD appeared to have the deepest mean PPD and CAL (Table 1) at baseline. Although this imbalance between treatment groups was not statistically significant, the higher clinical attachment gain observed in those sites (Fig. 2) needs to be interpreted with caution. Since the study was completely blinded there was no way to interfere with the distribution of cases to better balance the groups. In fact, these non-significant differences were only revealed after completion of the entire clinical part of the study and data locking.

The improvements from baseline clinical parameters, obtained overall in the present trial, compare favourably with previous reports on the effect of non-surgical periodontal therapy. A systematic review on the clinical efficacy of subgingival debridement in the treatment of chronic periodontitis, including 26 papers (van der Weijden & Timmerman 2002), calculated a weighted mean of CAL in pockets originally deeper than 4 mm of 0.64 mm. In the present study, 1.5 ± 1.9 mm were gained over 6, and 1.1 ± 2.3 mm over 12 months in total. When looking at those sites treated with neither adjunctive antibiotics nor EMD, however, only 0.25 mm CAL gain was noted over 6 months. The same systematic review presented a PPD reduction of 1.18 mm. In the present study, the mean PPD reduction over 6 months amounted to 2.3 ± 1.9 mm and to 1.9 ± 1.8 mm over 12 months in total; in those sites treated with neither antibiotics nor EMD the pockets were reduced by 1.3 ± 0.9 mm over 6, and by 1.6 ± 1.3 mm over 12 months.

Adjunctive benefits of systemic amoxicillin and metronidazole to mechanical therapy of periodontal diseases have been recorded in several trials (Herrera et al. 2002). The significant differences among subjects treated with or without these antimicrobials in our study, in line with other trials (Rooney et al. 2002), corroborate the benefits of adjunctive systemic amoxicillin and metronidazole to SRP in the treatment of advanced chronic periodontitis.

Experiments testing bacterial vitality in dental plaque after exposure to several substances have indicated that EMD and PGA have a certain antimicrobial effect (Sculean et al. 2001, Arweiler et al. 2002). One study demonstrated a specific inhibitory effect of EMD on the growth of *A. actinomycetemcomitans*,

Table 3. Parameters for sites treated with SRP, or SRP plus EMD (SRP+EMD)

		Baseline	Month 2	Month 6	Month 12
PPD (mm)	SRP	6.9 ± 1.7	5.1 ± 1.3	5.1 ± 1.5	4.9 ± 1.4
	SRP+EMD	7.6 ± 1.3	5.1 ± 1.4	4.8 ± 1.7	5.4 ± 1.9
CAL (mm)	SRP	7.6 ± 2.0	6.9 ± 1.5	6.7 ± 2.0	5.8 ± 2.5
	SRP+EMD	8.6 ± 2.9	6.9 ± 2.2	6.6 ± 2.3	6.1 ± 3.4
BOP (%)	SRP	0.9	0.7	0.6	0.6
	SRP+EMD	0.9	0.6	0.6	0.8

SRP, scaling and root planing; PPD, pocket probing depth; CAL, clinical attachment level; BOP, bleeding upon probing; EMD, enamel matrix derivative.

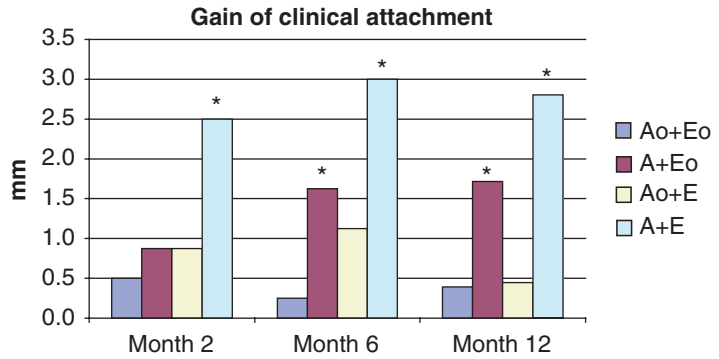

Fig. 2. Clinical attachment gain 2, 6 and 12 months after scaling and root planing in sites treated with or without enamel matrix derivative, in subjects with or without adjunctive antimicrobial therapy (A, systemic antibiotics; E, Emdogain®; A0, E0, placebos; *: $p < 0.03$).

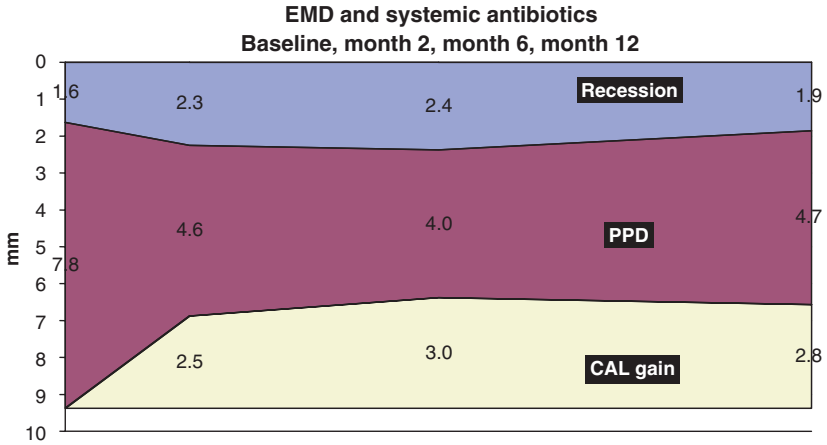

Fig. 3. Mean changes in Recession, pocket probing depth and clinical attachment level following scaling and root planing plus enamel matrix derivative and adjunctive systemic antibiotics (0 mm corresponds to the cemento-enamel junction).

P. gingivalis and *P. intermedia* in vitro (Spahr et al. 2002). Because PGA was applied to test and control sites, the relative effect of PGA alone could not be evaluated in the present study. Nonetheless, the markedly better clinical outcomes of sites treated with adjunctive systemic antibiotics, together with the absence of a significant benefit of EMD adjunctive to SRP in subjects not treated with antibiotics, questions the clinical relevance of Emdogain's® antibacterial properties for long-term success. The bacteriostatic effect of EMD seems to be as a result of contact inhibition and probably affects the microbiota in vivo only during the first days after application. The present study supports the notion that optimal repair and regeneration of the periodontium requires suppression of the microbiota causing periodontal disease to a maximum.

Acknowledgements

This study was supported by a grant from Biora AB, Malmø, Sweden.

References

Arweiler, N. B., Auschill, T. M., Donos, N. & Sculean, A. (2002) Antibacterial effect of an enamel matrix protein derivative on in vivo dental biofilm vitality. *Clinical Oral Investigations* **6**, 205–209.

Blomlöf, J. P. S., Blomlöf, L. B. & Lindskog, S. F. (1996) Smear removal and collagen exposure after non-surgical root-planning followed by etching with EDTA gel preparation. *Journal of Clinical Periodontology* **67**, 841–845.

Blomlöf, J. & Lindskog, S. (1995) Root surface texture and cell colonization after different etching modalities. *European Journal of Oral Sciences* **103**, 17–24.

Caffesse, R. G., Sweeney, P. L. & Smith, B. A. (1986) Scaling and root planing with and without periodontal flap surgery. *Journal of Clinical Periodontology* **13**, 205–210.

Dix, K., Watanabe, S. M., McArdle, S., Lee, D. I., Randolph, C., Moncla, B. & Schwartz, D. E. (1990) Species-specific oligodeoxynucleotide probes for the identification of periodontal bacteria. *Journal of Clinical Microbiology* **28**, 319–323.

Dornier, C., Dorsaz-Brossa, L., Thévenaz, P., Casagni, F., Brochut, P., Mombelli, A. & Vallée, J. P. (2004) Geometric alignment and chromatic calibration of serial radiographic images. *Dentomaxillofacial Radiology* **33**, 220–225.

Dubrez, B., Jacot-Descombes, S. & Cimasoni, G. (1995) Reliability of a paralleling instrument for dental radiographs. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **80**, 358–364.

Graf, J. M., Mounir, A., Payot, P. & Cimasoni, G. (1988) A simple paralleling instrument for superimposing radiographs of the molar regions. *Oral Surgery, Oral Medicine, and Oral Pathology* **66**, 502–506.

Gutierrez, M. A., Mellonig, J. T. & Cochran, D. L. (2003) Evaluation of enamel matrix derivative as an adjunct to non-surgical periodontal therapy. *Journal of Clinical Periodontology* **30**, 739–745.

Hammarstrom, L., Heijl, L. & Gestrelius, S. (1997) Periodontal regeneration in a buccal dehiscence model in monkeys after application of enamel matrix proteins. *Journal of Clinical Periodontology* **24**, 669–677.

Heijl, L. (1997) Periodontal regeneration with enamel matrix derivative in one human experimental defect. A case report. *Journal of Clinical Periodontology* **24**, 693–696.

Heijl, L., Heden, G., Svardstrom, G. & Ostgren, A. (1997) Enamel matrix derivative (EMDOGAIN) in the treatment of intrabony periodontal defects. *Journal of Clinical Periodontology* **24**, 705–714.

Herrera, D., Sanz, M., Jepsen, S., Needleman, I. & Roldán, S. (2002) A systematic review on

the effect of systemic antimicrobials as an adjunct to scaling and root planing in periodontitis patients. *Journal of Clinical Periodontology* **29**, Suppl. 3, 136–159.

Löe, H. & Silness, J. (1963) Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontologica Scandinavica* **21**, 533–551.

Mellonig, J. T. (1999) Enamel matrix derivative for periodontal reconstructive surgery: technique and clinical and histologic case report. *International Journal of Periodontics and Restorative Dentistry* **19**, 8–19.

Mombelli, A., Gmür, R., Gobbi, C. & Lang, N. P. (1994) *Actinobacillus actinomycetemcomitans* in adult periodontitis. I. Topographic distribution before and after treatment. *Journal of Periodontology* **65**, 820–826.

Mombelli, A., Schmid, B., Rutar, A. & Lang, N. P. (2000) Persistence patterns of *Porphyromonas gingivalis*, *Prevotella intermedia/nigrescens*, and *Actinobacillus actino-mycetem-comitans* after mechanical therapy of periodontal disease. *Journal of Periodontology* **71**, 14–21.

Renvert, S., Wikstrom, M., Dahlen, G., Slots, J. & Egelberg, J. (1990) Effect of root debridement on the elimination of Actinobacillus actinomycetemcomitans and Bacteroides gingivalis from periodontal pockets. *Journal of Clinical Periodontology* **17**, 345–350.

Rooney, J., Wade, W. G., Sprague, S. V., Newcombe, R. G. & Addy, M. (2002) Adjunctive effects to non-surgical therapy of systemic metronidazole and amoxycillin alone and combined. A placebo controlled study. *Journal of Clinical Periodontology* **29**, 342–350.

Sculean, A., Auschill, T. M., Donos, N., Brecx, M. & Arweiler, N. B. (2001) Effect of an enamel matrix protein derivative (Emdogain) on ex vivo dental plaque vitality. *Journal of Clinical Periodontology* **28**, 1074–1078.

Sculean, A., Donos, N., Windisch, P., Brecx, M., Gera, I., Reich, E. & Karring, T. (1999a) Healing of human intrabony defects following treatment with enamel matrix proteins or guided tissue regeneration. *Journal of Periodontal Research* **34**, 310–322.

Sculean, A., Reich, E., Chiantella, G. C. & Brecx, M. (1999b) Treatment of intrabony periodontal defects with an enamel matrix protein derivative (Emdogain): a report of 32 cases. *International Journal of Periodontics and Restorative Dentistry* **19**, 157–163.

Silness, J. & Löe, H. (1964) Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica* **22**, 121–135.

Spahr, A., Lyngstadaas, S. P., Boeckh, C., Andersson, C., Podbielski, A. & Haller, B. (2002) Effect of the enamel matrix derivative Emdogain on the growth of periodontal pathogens in vitro. *Journal of Clinical Periodontology* **29**, 62–72.

Tonetti, M. S., Lang, N. P., Cortellini, P., Suvan, J. E., Adriaens, P., Dubravec, D., Fonzar, A., Fourmousis, I., Mayfield, L., Rossi, R., Silvestri, M., Tiedemann, C., Topoll, H., Vangsted, T. & Wallkamm, B. (2002) Enamel matrix proteins in the regenerative therapy of deep intrabony defects. *Journal of Clinical Periodontology* **29**, 317–325.

van der Weijden, G. A. & Timmerman, F. A. (2002) A systematic review on the clinical efficacy of subgingival debridement in the treatment of chronic periodontitis. *Journal of Clinical Periodontology* **29**, 55–71.

Waerhaug, J. (1978) Healing of the dento-epithelial junction following subgingival plaque control. As observed on extracted teeth. *Journal of Periodontology* **49**, 119–134.

Yukna, R. A. & Mellonig, J. T. (2000) Histologic evaluation of periodontal healing in humans following regenerative therapy with enamel matrix derivative. A 10-case series. *Journal of Periodontology* **71**, 752–759.

Address:
Andrea Mombelli.
University of Geneva
School of Dental Medicine
19 rue Barthélemy-Menn
CH-1211 Geneva 4
Switzerland
E-mail: Andrea.mombelli@medecine.unige.ch