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Enamel matrix proteins in the treatment of intra-bony defects

A prospective 24-month clinical trial

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Abstract

Background: A growing flow of recent evidence indicates enamel matrix derivative (EMD, Emdogain®) as a useful tool for the regeneration of periodontal tissues. This prospective clinical study aimed to evaluate the efficacy of EMD combined with surgical treatment of periodontal intra-bony defects, as compared with surgery alone, up to 24 months of follow-up.

Methods: Twenty-four intra-bony defects were treated in 24 patients in a single clinical centre. Each defect had intra-bony depth (IBD) $\geqslant 4$ mm and probing pocket depth (PPD) $\geqslant 6$ mm. Patients were randomly assigned to either test or control group. Plaque index (PI), gingival index (GI), PPD and periodontal attachment level (PAL) were assessed at baseline pre-surgical examination at the site to be treated. Full mouth plaque score (FMPS) and full mouth bleeding score (FMBS) were also evaluated. Twelve patients were treated by simplified papilla preservation flap technique (control group), while 12 patients were treated with the same surgical technique plus EMD after ethylenediamine tetraacetic acid root conditioning (test group). Any probing at the involved sites was avoided in the first year post-surgery. Radiographs were taken at baseline, 12 and 24 months after surgery using customized bite blocks. Intra-bony defect depth (IBD) and angle (IBA) were measured from X-rays by a computer-aided technique. At 12 and 24 months post-surgery, FMPS, FMBS, PI, GI, PPD, PAL and radiographic IBD and IBA were assessed. The difference between each follow-up and baseline, and between groups at each follow-up was evaluated for the above parameters by standard statistical methods.

Results: In both groups, clinical and radiographic parameters were improved at either 12 or 24 months when compared with baseline. The test group displayed better outcomes when compared with the control group for IBD, PPD, and PAL gain at 12 months, and only for PAL and IBD gain at 24 months. No adverse event related to the use of EMD was reported.

Conclusions: The surgical procedure used in the present study, aiming for maximum preservation of the regenerative potential of periodontal tissues, showed per se excellent results. The use of EMD as an adjunct to periodontal surgery in the treatment of angular defects possibly enhances periodontal regeneration rate.

Key words: enamel matrix proteins; intra-bony defects; open flap debridement; periodontal attachment; periodontal disease; periodontal regeneration; periodontal surgery

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In the last few years, enamel matrix derivative (EMD, Emdogain®, Biora AB, Malmö, Sweden) has received great attention as a possible tool to enhance periodontal regeneration.

EMD is mainly composed of amelogenin and other related proteins, derived from embryonic porcine tooth germ. There is evidence that these proteins are important for the development of acellular cementum, functionally oriented periodontal ligament and alveolar bone (Hammarström 1997, Heden et al. 1999, Gestrelius et al. 2000).

The rationale of using EMD in the treatment of intra-bony defects is to mimic the biological process occurring during the development of the nascent root and periodontal tissues (Gestrelius et al. 2000). EMD consists of a protein-rich matrix that is laid on the exposed root surface. Such a matrix represents a substrate that would induce differentiation of mesenchimal stem cells of the

nearby periodontal tissues. The regeneration of new periodontal attachment should follow, similar to what occurs in odontogenesis (Araujo & Lindhe 1998, Gestrelius et al. 2000, Schwartz et al. 2000).

Clinical efficacy and safety of amelogenins have already been demonstrated in both animal (Hammarström 1997, Hammarström et al. 1997, Araujo & Lindhe 1998) and human (Heijl 1997, Heijl et al. 1997, Heden et al. 1999, Mellonig 1999) models. Most of the above studies also report histological evidence to the actual regenerative potential of EMD in periodontal tissues.

Many clinical trials reported efficacy of EMD when used as an adjunct to surgical treatment of intra-bony and furcation defects, caused by moderate to severe periodontitis (Hammarström 1997, Hammarström et al. 1997, Heijl 1997, Heijl et al. 1997, Heden et al. 1999, Mellonig 1999, Pontoriero et al. 1999, Sculean et al. 1999a, b, Heden 2000, Froum et al. 2001, Trombelli et al. 2002, Zucchelli et al. 2002).

The objective of this clinical study is to evaluate the periodontal tissue regeneration when using EMD combined with surgical treatment of intra-bony defects, as compared with periodontal surgery alone, up to 24 months after surgery.

Material and Methods

The study was performed at the Department of Odontology of the University of Milan. It was conducted according to an open-label, randomized parallel study protocol. The latter was approved by the Institutional Review Board of the University.

Patients of either sex, both smokers and non-smokers (or ex-smokers), were recruited by following specific inclusion and exclusion criteria. They were subsequently allocated to either test or control group in accordance with a 1 : 1 computer-generated randomization list. The allocation to treatment group was concealed from clinicians until the patients received the treatment. Patients were blinded as to treatment assignment throughout the study.

The inclusion criteria were: (1) presence of an intra-bony 1-, 2- or 3-wall defect, having a probing pocket depth (PPD) $\geqslant$6 mm and an intra-bony defect depth (IBD) $\geqslant$4 mm – these parameters were assessed prior to surgery, during the preliminary evaluation by clinical examination and periapical X-ray; (2) each patient had a single periodontal defect; (3) patients agreed to sign the informed consent.

The exclusion criteria were: (1) presence of severe systemic diseases or any chronic disease involving permanent drug consumption; (2) presence of mobility of third degree according to Miller's classification (Miller 1950), and/or tooth pulp necrosis, inappropriate restoration, primary or secondary occlusal trauma at the experimental tooth; (3) treatment for periodontitis in the last 36 months at the experimental tooth. Patients that clearly demonstrated poor interest and motivation in maintaining adequate oral hygiene regimen were also excluded from the study.

Preliminary clinical evaluation: Baseline examination occurred 7–14 days prior to surgery. Full mouth visible plaque score (FMPS) and full mouth gingival bleeding score (FMBS) were assessed. FMPS was defined as the % of total surfaces (4 surfaces/tooth) revealing the presence of plaque. FMBS was defined as the % of total sites (4 sites/tooth) showing bleeding on probing. Plaque index (PI) (Silness & Löe 1964) and gingival index (GI) (Löe & Silness 1963) were assessed at surgical sites. Probing pocket depth (PPD) was measured in millimetres as the distance from the gingival margin to the base of the pocket. Periodontal attachment level (PAL) was measured in millimeter as the distance from the cemento-enamel junction (CEJ) to the base of the pocket. PPD and PAL were recorded by a periodontal probe (PCP-UNC 15, Hu-Friedy) at six sites around each involved tooth. All measurements were rounded up to the nearest millimetre. The deepest value of both PPD and PAL at each defect was considered for the analysis. A single experienced examiner blind to treatment performed all the clinical measurements throughout the study.

A picture of the periodontal probe tip inserted in the defect was taken by a digital camera (Nikon Coolpix 950, Nikon, Japan). Fig. 1a shows an example of an infra-bony defect with the periodontal probe in situ. Periapical X-rays of each defect were taken to assess radiographic IBD at baseline.

At the end of this phase, all subjects received non-surgical periodontal therapy (a single session of whole mouth scaling and root planing). Detailed oral hygiene instructions were given and compliance was recommended in order to obtain at least an FMPS $\leqslant$20%, an FMBS $\leqslant$20%, a PI = 0 and a GI = 0 at the level of the surgical site by the day of surgery.

From the day before surgery, patients started antibiotic prophylaxis by consuming amoxycillin + clavulanic acid 1 g every 12 h.

Surgical procedure

Patients of both groups were treated with the simplified papilla preservation flap technique (SPP), aiming for the maximal preservation of the tissues (Cortellini et al. 1999).

A single operator with more than 10 years of clinical experience in periodontal surgery performed all the surgical procedures.

After flap elevation, debridement of the site was performed to remove calculus and bacterial plaque, and root surfaces were planed. Next, we evaluated the number of defect walls and, with a periodontal probe, measured (1) the distance from the CEJ to the bottom of the defect and (2) the distance from the CEJ to the most coronal portion of the bone crest. The infra-osseous component of the defect was calculated as the difference between the latter two measurements. Fig. 1b shows an example of intra-surgical probing at the defect site.

A CV-6 Gore-Tex® (Gore) suture joining the interproximal papillary tissues to the buccal flap was applied. The suture was not tightened at this instance. In the patients allocated to the test group, root surfaces of the experimental teeth were conditioned with 24% ethylenediamine-tetraacetic acid (EDTA) gel (PrefGel®, Biora AB, Malmö, Sweden) for 2 min to remove the smear layer. Root conditioning and untied sutures are visible in Fig. 1c. Next, a solution composed of 30 mg of sterile lyophilized EMD mixed with 1 ml of sterile gel of propylene glycol alginate (PGA, Biora AB, Malmö, Sweden) was placed on the exposed root-surfaces. The defect was completely filled with EMD. After that, the suture was accurately tightened, free of tension, obtaining a complete coverage of the intra-bony defects. Fig. 1d shows the sutured flap from the palatal side. The same kind of suture was used for the patients of the control group.

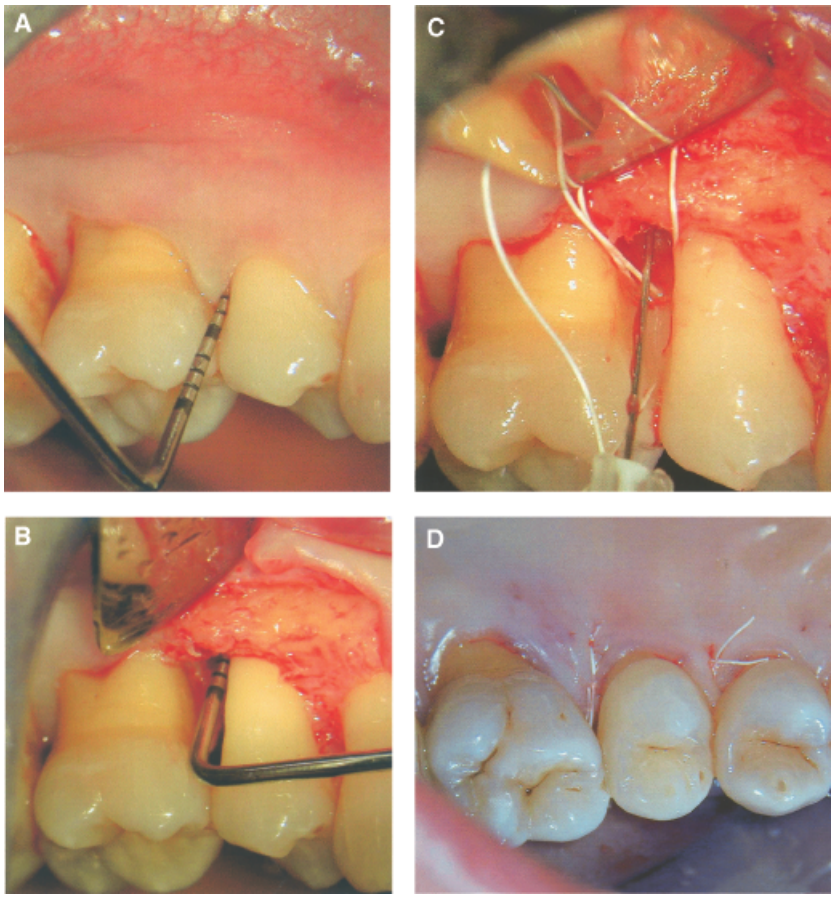

Fig. 1. (a) Photograph of an intra-bony defect; the periodontal probe tip is inserted into the pocket. The picture was taken before surgery was started. (b) Intra-surgical probing at the defect site after flap elevation. (c) Root conditioning; untied sutures are visible. (d) Palatal view of the surgical site after the suture was closed.

Post-surgical phase

At the end of the surgical phase, patients continued to take amoxycillin+clavulanic acid 1 g twice daily for 5 days. Nimesulide 100 mg twice daily for 2 days was also prescribed to the patients.

We recommended avoiding mechanical oral hygiene at the surgical area during the first 6 weeks post-operation. This was to ensure proper healing of the surgical site. Patients were instructed to rinse twice daily with a 0.12% solution of chlorhexidine digluconate (Ebur Os®, Dentsply). After 6 weeks, patients were allowed to mechanically clean the treated site gently, by using an ultra-soft toothbrush.

Sutures were removed 15 days after surgery. Patients were followed-up monthly during the first year, and then at 18 and 24 months after surgery. At each follow-up, PI and GI at the treated site were evaluated, as well as FMPS and FMBS. Professional oral hygiene was performed when needed. The occurrence of adverse events and/or complications was recorded.

During the first 12 months, we avoided probing at the treated site, as this might interfere with the healing process.

The first complete check-up was performed at the 12 months follow-up. FMPS and FMBS were assessed. PI and GI were evaluated at the treated site. The result of the regenerative therapy was assessed both by measuring PPD and PAL with a periodontal probe, and by evaluating radiographic parameters from periapical radiographs. Finally, we photographed each treated site, keeping the probe tip in the defect. The same evaluations were performed 24 months after surgery, at the second complete check-up.

Radiographic evaluation

Standardized periapical radiographs were taken at baseline evaluation, immediately before surgery and at 12 and 24 months follow-up. Individually customized bite blocks were used to obtain reproducible films at each radiographic control. X-rays served to evaluate the infra-bony radiographic parameters (defect depth and angle). Fig. 2 shows an example of the reproducibility of radiographs at three different controls (the day of surgery, 12 and 24 months after surgery).

All radiographs were evaluated by a single examiner blind to treatment. Infra-bony defect depth (IBD) and angle (IBA) were measured from periapical radiographs by a computer-aided technique, using an image analysis software (Scion Image, Scion Corporation, NIH, USA). The radiographs were previously scanned in digital format by a scanner (HP Scanjet 3c/t, Hewlett-Packard, Cernusco sul Naviglio, Milano, Italy) at a resolution of 600 dpi. A 10-mm caliper with ticks every 0.5 mm was also scanned at the same resolution and used for calibration. The precision obtained by the measuring system is accurate to within 0.01 mm for the linear measurement, and to within 0.01° for the angular one.

The reliability of the technique was previously assessed by examining 20 periapical radiographs from other patients rehabilitated by implant therapy. Forty-five endosseous oral implants of different size and manufacturer were examined. The examiner had to measure the length and width of each implant by using a caliper for calibration, as described above. He was blind to the implant dimensions. Measures were rounded to the nearest 0.1 mm. Correlation analysis showed 97.8% and 98.3% of concordance between digital measurements and, respectively, actual length and width of the implants.

Fig. 3 is a simple drawing illustrating the parameters measured in the radiographic analysis. The infra-bony defect depth was assessed as the vertical distance (in millimetres) between the crestal bone level and the most apical contact between bone and root surface.

The infra-bony defect angle was measured as the angle formed between the major axis of the root and the oblique surface of the defect, as seen on the radiograph. Deeper defects are often associated with more acute angles. In the authors' experience, an improved

bone condition is usually accompanied by an increase of the angle (a less vertical bone defect profile), as schematically shown in Fig. 3 (right panel).

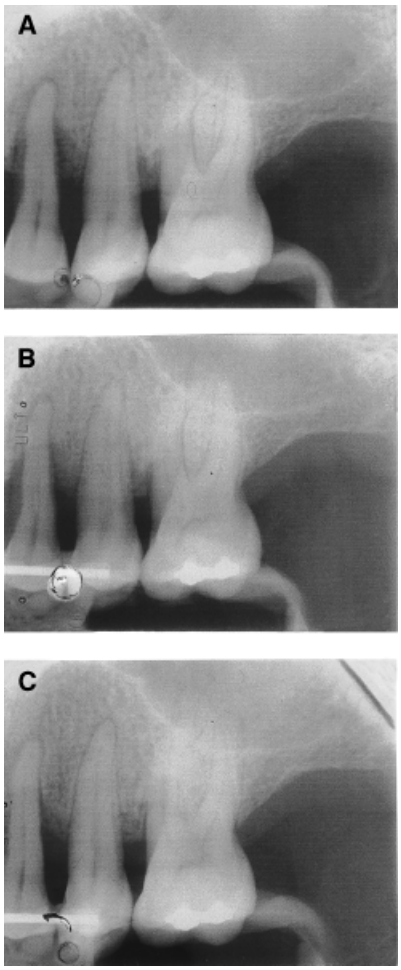

Fig. 2. Example of the reproducibility of radiographs obtained using the individual bite block. (a) the day of surgery; (b) 12 months post-surgery; (c) 24 months post-surgery.

Data analysis

Data were expressed as the mean value ± 1 SD. The overall data were analysed for their statistical significance by standard statistical methods. We considered a value of $p = 0.05$ as the level of significance. The unpaired *t*-test was used to assess homogeneity between test and control group at baseline for all parameters. One-way ANOVA was used to evaluate differences in the clinical parameters between baseline, 12 and 24 months (within-group), considering raw data. The paired *t*-test was then used to compare 12-month and 24-month versus baseline evaluations for both groups. For a given parameter the ''gain'' was defined as the difference between a given follow-up and baseline, while the ''gain%'' was defined as the ratio between gain and the baseline value. Gain% at 12 and 24 months was tested for significance with respect to zero (within groups) by the paired *t*-test. Both the unpaired *t*-test and the Mann–Whitney test were used to compare test and control group for gain and gain% at 12 and 24 months. In fact, though parametric tests should be preferred for the statistical evaluation of difference when comparing quantitative variables, given the low sample size and the high variability of distributions, we also used non-parametric tests as a further check.

The following comparisons were made: (1) 12 and 24 months versus baseline, and (2) test versus control group at 12 and 24 months after surgery, for PAL, PPD, IBD and IBA. The first comparison assesses the improvement of the periodontal condition along time, that is the clinical success of the surgical treatment. The second one tests the null hypothesis of equivalence between the two treatments (EMD combined with SPP as compared with SPP alone).

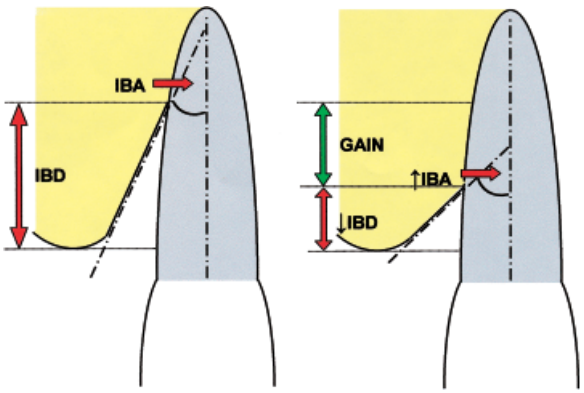

Fig. 3. Schematic drawing showing the parameters measured from radiographs. Bone tissue is depicted in yellow; soft tissues are not illustrated. IBD = infra-bony defect depth; IBA = infra-bony defect angle. Left: before treatment; right: after treatment. In the picture on the right side a decrease in IBD (GAIN) and an increase of the IBA is represented. These changes usually occur after a successful treatment.

Results

Twenty-four consecutive patients (11 males/13 females) were enrolled in the study. Their age ranged between 30 and 66 years (mean age: 46.5 ± 11.2 year). Twelve patients were assigned to the test group and 12 to the control group. Eight patients (5 in the test group and 3 in the control group) were smokers (10–15 cigarettes/day). Each patient was treated for a single defect.

All patients were evaluated at the 12-month follow-up. Twenty-two patients (91.7% of the initial population) completed the 24-month observation period. The two remaining patients dropped out from the study after the 12-month follow-up. The first one, belonging to the control group, was not compliant with the protocol. The other one, in the test group, was withdrawn due to pulp necrosis of the treated tooth. This adverse event showed up at the 18-month control.

In both the test and the control group, a slight decrease in FMPS, FMBS, PI and GI was observed with respect to baseline values. No significant difference was detected between the two groups with respect to these parameters.

Table 1 reports the mean values of the clinical parameters at baseline, 12 and 24 months after surgery, as well as the mean gain and gain% values.

Table 2 shows the results of the one-way ANOVA and the paired *t*-test for the assessment of intra-group differences between baseline, 12- and 24-month evaluations.

Table 3 reports the results of the unpaired *t*-test and the Mann–Whitney test for the assessment of between-group differences for gain and gain% at 12 and 24 months.

Probing pocket depth (PPD) and periodontal attachment level (PAL)

No statistical difference could be detected between the two treatment groups at baseline for either parameter ($p > 0.05$).

One-way analysis of variance evidenced in both groups a significant decrease of PPD and a significant improvement

Table 1. Mean values of the parameters at baseline and after 12 and 24 months

Parameter	Group	Baseline	12 months	Gain (mm)	Gain %	24 months	Gain (mm)	Gain %
PPD (mm)	control	6.71 ± 1.25	4.14 ± 1.86	2.57 ± 1.27	39.73	3.71 ± 1.60	3.00 ± 1.15	45.51
	EMD	7.86 ± 1.46	3.14 ± 0.90	4.71 ± 1.60	58.96	3.00 ± 0.82	4.86 ± 1.95	59.75
PAL (mm)	control	8.29 ± 1.60	6.00 ± 1.91	2.29 ± 0.95	28.81	5.57 ± 1.72	2.71 ± 0.76	34.33
	EMD	9.43 ± 1.13	5.29 ± 1.11	4.14 ± 1.35	43.52	5.14 ± 1.21	4.29 ± 1.38	45.17
IBD (mm)	control	4.81 ± 0.58	3.37 ± 0.86	1.44 ± 0.74	30.14	2.97 ± 0.62	1.84 ± 0.53	38.49
	EMD	5.93 ± 1.25	2.98 ± 0.74	2.96 ± 1.13	49.16	2.49 ± 0.76	3.44 ± 1.18	57.44
IBA (deg)	control	32.5 ± 5.6	40.2 ± 7.2	7.8 ± 3.61	24.3	41.3 ± 7.8	8.9 ± 7.3	28.8
	EMD	31.7 ± 6.9	50.2 ± 8.8	19.0 ± 9.4	66.9	51.7 ± 9.1	20.5 ± 13.1	75.2

Intra-bony defect angle is expressed in degrees. The gain% is a mean of single gains at each site. PPD, periodontal pocket depth; PAL, periodontal attachment level; IBD, infra-bony defect depth; IBA, infra-bony defect angle; EMD, enamel matrix derivative.
Depth data are reported as the mean ± 1 SD.

Table 2. Results of the statistical comparisons within the EMD and control groups

		PPD	PAL	IBD	IBA	PI	GI
EMD group							
ANOVA	F	44.33	31.11	27.18	13.15	0.54	0.59
	P	$1.1 \times 10^{-7*}$	$1.4 \times 10^{-6*}$	$3.6 \times 10^{-6*}$	$3 \times 10^{-4*}$	0.59	0.57
t-test							
0 versus 12 months	t	8.37	8.79	6.75	3.87	1	1
	P	$6.8 \times 10^{-5*}$	$5 \times 10^{-5*}$	$2.6 \times 10^{-4*}$	0.002*	0.35	0.35
0 versus 24 months	t	6.58	6.48	7.71	3.82	1	0.42
	P	$5.9 \times 10^{-4*}$	$6.4 \times 10^{-4*}$	$2.5 \times 10^{-4*}$	$8.8 \times 10^{-3*}$	0.35	0.69
12 versus 24 months	t	0.55	0.31	2.09	0.12	0	1.44
	P	0.60	0.76	0.08	0.91	1	0.20
Control group							
ANOVA	F	7.25	4.86	13.46	3.43	0.12	0.84
	P	$4.9 \times 10^{-3*}$	0.02*	$2.7 \times 10^{-4*}$	0.054	0.89	0.45
t-test							
0 versus 12 months	t	6.25	3.99	5.95	6.59	0.80	1.43
	P	$4 \times 10^{-4*}$	0.005*	$5.7 \times 10^{-4*}$	$3.1 \times 10^{-4*}$	0.45	0.20
0 versus 24 months	t	6.87	4.58	5.41	1.37	0.42	2.12
	P	$4.7 \times 10^{-4*}$	$3.8 \times 10^{-3*}$	$1.6 \times 10^{-3*}$	0.26	0.69	0.08
12 versus 24 months	t	0.70	0.42	0.74	1.69	0.42	0.55
	P	0.51	0.69	0.49	0.19	0.69	0.60

*Significantly different at $p = 0.05$. ANOVA and the *t*-test for paired samples were used. PPD = periodontal pocket depth; PAL = periodontal attachment level; IBD = infra-bony defect depth; IBW = infra-bony defect angle; PI = plaque index; GI = gingival index.

Table 3. Results of the statistical comparisons between the EMD and control groups at 12 and 24 months

Parameter		PPD	PAL	IBD	IBW
Gain					
12 months	t	3.25	2.34	2.62	3.10
	P	$5.8 \times 10^{-3*}$	0.03*	0.02*	0.008*
Mann–Whitney	*P*	*<0.05**	*<0.05**	*<0.05**	*<0.05**
24 months	t	2.16	2.65	3.67	2.80
	P	0.051	0.02*	0.003*	0.02*
Mann—Whitney	*P*	*>0.05*	*<0.05**	*<0.05**	*>0.05*
Gain%					
12 months	t	2.64	2.10	1.18	2.81
	P	0.02*	0.054	0.26	0.01*
Mann–Whitney	*P*	*<0.05**	<0.05*	*>0.05*	*<0.05**
24 months	t	1.58	1.65	3.29	1.90
	P	0.14	0.12	0.006*	0.09
Mann–Whitney	*P*	*>0.05*	*>0.05*	*<0.05**	*>0.05*

Mean gain (in mm or degrees) and mean gain% for PPD, PAL, IBD and IBA were considered for comparison. Data were analysed by both parametric (Student's *t*-test) and non-parametric (Mann–Whitney *U* test, *italic characters*) methods.
*Significantly different at $p = 0.05$.
EMD, enamel matrix derivative; PPD, probing pocket depth.

of PAL after 12 and 24 months when compared with baseline (Table 2).

By considering the individual gain and gain% in PPD with respect to baseline (Table 3), both statistical tests revealed a significant difference between the two groups at 12 months, with a better outcome for the EMD group. On the other hand, no significant difference was detected at the 24-month evaluation.

By comparing individual gain in PAL with respect to baseline, the outcome of the EMD group was significantly better than the control group at both 12 and 24 months, as shown in Table 3. Conversely, the gain% was significantly greater in the EMD group as compared with control at 12 months (by the non-parametric test), but the difference was not significant at 24 months follow-up.

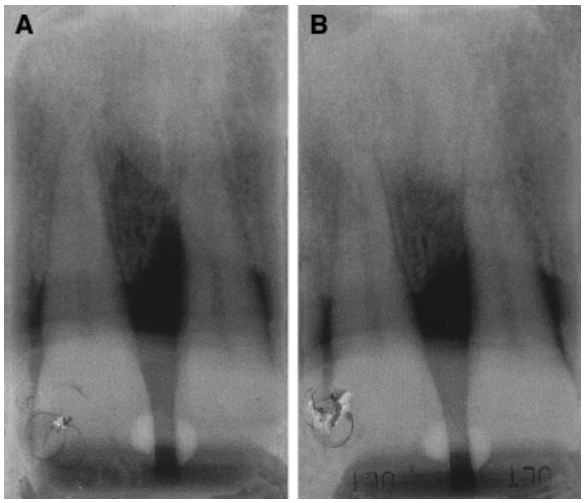

Fig. 4. Example of defect healing. (a) Pre-operative radiograph showing a 5.5-mm deep infra-bony defect. (b) Same case after 12 months; a 3-mm vertical gain has been recorded for this case. The infra-bony depth was unchanged at the 24-month follow-up (not shown).

Radiographic IBD and IBA

Fig. 4 shows two radiographs from a patient of the EMD group. Fig. 4a represents the pre-treatment condition and Fig. 4b shows the result after 12 months. The infra-bony depth was 5.5 mm at baseline and the gain after 12 months was 3 mm.

No significant difference was detected between the two groups at baseline for either parameter ($p > 0.05$).

For IBD, one-way ANOVA revealed a statistically significant difference in both groups at 12 and 24 months when compared with baseline (Table 2). In the test group, the mean gain% at 12 and 24 months when compared with baseline was 49.16% ($p < 0.01$) and 57.44% ($p < 0.01$) respectively. In the control group, the mean gain% at 12 and 24 months was 30.14% ($p < 0.01$) and 38.49% ($p < 0.01$) respectively. The IBD gain was significantly higher in the EMD when compared with the control group at either 12 or 24 months. For the gain% we found a significant difference between groups at the 24-month but not at the 12-month follow-up (Table 3).

In the EMD group, IBA at either 12 or 24 months was significantly increased ($p < 0.01$) when compared with baseline. Conversely, in the control group IBA did not show significant changes at 12 and 24 months with respect to baseline (Table 2). A significant difference between groups in gain and gain% was found for the 12-month but not for the 24-month follow-up (Table 3).

Discussion

Enamel matrix proteins have been investigated in recent years as a possible tool to enhance periodontal tissue regeneration, according to the principle of biomimicry (Gestrelius et al. 2000).

Heijl and co-workers in 1997 presented the first clinical study on the use of Emdogain® in the treatment of infra-bony defects (Heijl et al. 1997). After 3 yr of follow-up, the group treated with EMD displayed much greater periodontal regeneration when compared with control. The major difference was observed in terms of defect filling, which was assessed by radiographic technique (66% versus 0%, respectively). In that study, patients allocated to the control group were treated by conventionally modified Widman flap procedure. Indeed, the latter technique is not specifically designed for the maximal preservation of tissues. Conversely, the simplified papilla preservation flap technique that was used in the present study (Cortellini et al. 1999), aims to preserve as much as possible the regenerative potential of periodontal tissues.

Other studies with observation periods up to 12 months, using tissue-preservative surgical technique as control (Froum et al. 2001, Trombelli et al. 2002, Zucchelli et al. 2002), confirmed the clinical efficacy of Emdogain®. These investigations reported a significant reduction of probing depth together with improvement of clinical attachment level in the group treated with Emdogain®. Noticeably, these studies also revealed a good periodontal regeneration in the control group.

The results obtained in the present study demonstrated that in the treatment of infra-bony periodontal defects, excellent outcomes may be achieved with either SPP alone and SPP plus the adjunct of EMD. In fact, significant improvement with respect to the baseline condition was observed in both groups, mostly with significantly better outcomes in the test with respect to the control group. The gain in PAL level observed in the EMD group compares well with those reported in other clinical trials (Heijl et al. 1997, Heden et al. 1999, Pontoriero et al. 1999, Sculean et al. 1999a,b, Heden 2000, Bratthall et al. 2001, Froum et al. 2001, Zucchelli et al. 2002).

Anyway, one should consider that in the present study, the average size of the defects treated by EMD was more than 1 mm greater than the control at baseline, as shown in table 1. Although not statistically significant (maybe in part due to the small sample size), such a difference can be of clinical relevance. In fact, it is generally accepted that deeper defects tend to have greater regeneration (Pontoriero et al. 1999, Parodi et al. 2000, Bratthall et al. 2001). For this reason the larger gains observed in the EMD group with respect to the control group could be in part due to unequal baseline values.

Several factors may contribute to the clinical success of the surgical treatment of periodontal defects: (1) *An adequate preparation of the root surface*. This is necessary in order to reduce at the minimum level the presence of bacteria and endotoxins in the site; accurate debridement of the site followed by root conditioning with EDTA (to remove the smear layer and expose collagen fibres) is the recommended procedure in the treatment with EMD. (2) *Space maintenance*. If membranes (when used) or surrounding tissues fall into the defect site, proper regeneration may be impaired. (3) *Stability of the blood clot*. This is of fundamental importance for tissue healing. Wikesjö and co-workers in 1992 reviewed the most significant events in early periodontal wound healing, pointing out the importance of clot and wound stability in order to obtain tissue regeneration (Wikesjö et al. 1992). Mobility of the wound margin as well as tooth mobility may cause rupture of the fibrin clot, leading to failure of the treatment. (4) *Maximum*

preservation of tissues. This factor is also of fundamental importance since the interaction between periodontal tissue cells and growth factors included in the blood clot may per se stimulate tissue regeneration. It is therefore important to preserve the regenerative potential naturally present inside periodontal tissues as much as possible, by a minimally invasive surgical technique, such as the one used in the present study. (5) *Complete coverage of the wound by coronal flap repositioning*. An accurate flap closure with interproximal suture favours intra-bony healing by primary closure, avoiding bacterial contamination of the clot, and enhancing the possibility of tissue regeneration.

We did not include smoking habits among the exclusion criteria, although tobacco is certainly a negative factor for defensive and regenerative processes occurring in the oral environment (Grossi et al. 1996, Tonetti et al. 1996). This was done in order to test the efficacy of Emdogain® in the presence of one of the most common habits within the population, even though in smokers the treatment effect might be reduced. The few smokers recruited in this study did not show alterations in response to treatment with EMD but, of course, a thorough investigation would be needed to get more insight into this topic.

We cannot exclude that the adverse event that caused the patient in the EMD group to drop out from the study was related to the use of EMD. Anyway one has to make the following considerations: (1) It has been demonstrated that enamel matrix proteins remain active in situ for no more than 2 weeks (Gestrelius et al. 1997); in fact, it is during the early post-surgical period that EMD triggers the process of periodontal regeneration. After this period, EMD is progressively cleared from the surgical site. (2) The patient presented at the 12-month follow-up in good oral health condition; he had considerable attachment gain and tissue regeneration at the treated site. No signs or symptoms of tooth pulp necrosis were evidenced. (3) Since the adverse event showed up 6 months later, some other factor (such as local trauma or something else) must have caused the endodontic problem between the 12-month and the 18-month follow-up. Hence, it seems hard to believe that such an adverse event was associated with the use of EMD 18 months earlier.

In conclusion, the results of the present study reveal that the specific surgical technique used, aiming at preserving as much as possible of periodontal tissues, is per se effective and predictable. Furthermore, the adjunct of Emdogain® possibly accelerates periodontal tissue regeneration. Anyway, the small sample size of this study does not allow one to draw definite conclusions. Further studies with a larger database of patients are needed to confirm the results of the present study.

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