J Clin Periodontol 2008; 35: 429–437 doi: 10.1111/j.1600-051X.2008.01202.x

Journal of Clinical Periodontology

A double-blind randomized clinical evaluation of enamel matrix derivative proteins for the treatment of proximal class-II furcation involvements

Renato Corrêa Viana Casarin[1], Érica Del Peloso Ribeiro[1], Francisco Humberto Nociti Jr.[1], Antônio Wilson Sallum[1], Enilson Antônio Sallum[1], Gláucia Maria Bovi Ambrosano[2] and Márcio Zaffalon Casati[1]

[1]Department of Prosthodontics and Periodontics, Division of Periodontics and [2]Department of Social Dentistry, Division of Bioestatistics, School of Dentistry at Piracicaba, Campinas State University, São Paulo, Brazil

Casarin RCV, Del Peloso Ribeiro É, Nociti FH Jr., Sallum AW, Sallum EA, Ambrosano GMB, Casati MZ. A double-blind randomized clinical evaluation of enamel matrix derivative proteins for the treatment of proximal class-II furcation involvements. J Clin Periodontol 2008; 35: 429–437. doi: 10.1111/j.1600-051X.2008.01202.x.

Abstract

Objective: The aim of the present randomized, double-blind study was to evaluate the clinical response of proximal furcations treated with enamel matrix derivative proteins (EMD).

Material and Methods: Fifteen patients, each with a pair of contralateral class-II proximal furcation involvements, presenting probing depths (PDs) $\geqslant 5$ mm and bleeding on probing (BOP) were selected. The patients were randomly assigned to: control group ($n = 15$) – open flap debridement (OFD)+24% ethylenediaminetetraacetic acid (EDTA) conditioning; test group ($n = 15$) – OFD+24% EDTA conditioning+EMD application. Plaque index (PI), BOP, PD, gingival margin position (GMP), relative vertical and horizontal clinical attachment level (RVCAL and RHCAL), vertical and horizontal bone level (VBL and HBL) and furcation closure were evaluated immediately before and 2, 4 and 6 months after the surgeries.

Results: At 6 months, the RVCAL gains of the control and test group were 0.39 ± 1.00 and 0.54 ± 0.95 mm, while the RHCAL gains were 1.21 ± 2.28 and 1.36 ± 1.26 mm ($p > 0.05$). The VBL and HBL gains of the control group were 1.04 ± 1.12 and 1.00 ± 1.79 mm, and 0.82 ± 1.82 and 1.17 ± 1.38 mm for the test group ($p > 0.05$). In addition, a statistical difference was observed in the number of the remaining class-II furcations between the test and control groups ($p < 0.05$) in this period.

Conclusion: It may be concluded that the use of EMD in proximal furcations did not promote a superior reduction in PD or a gain in clinical and osseous attachment levels, but resulted in a higher rate of class-II to class-I furcation conversion.

Key words: enamel matrix derivative; open flap debridement; proximal furcation; randomized clinical trial; regenerative procedures; split mouth

Accepted for publication 23 December 2007

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.

The present study was supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Grants 04/11757-6 and 04/12431-7.

Proximal furcation involvements represent a challenge to periodontal therapy (Jepsen et al. 2002, Murphy & Gunsolley 2003). Recently, a controlled clinical trial comparing buccal and proximal class-II furcation treated with ultrasonic non-surgical therapy showed a lower reduction of probing depth (PD) in proximal defects (Del Peloso Ribeiro et al. 2007). Guided tissue regeneration (GTR) therapy with membranes also demonstrates an unpredictable improvement. While Metzler et al. (1991) and Pontoriero & Lindhe (1995) achieved clinical benefits similar to those of open flap debridement (OFP), Avera et al.

© 2008 The Authors
Journal compilation © 2008 Blackwell Munksgaard

(1998) found a significant gain in horizontal bone level (HBL), although it was lower than that obtained in mandibular defects.

The difficulty to access, view and debridement of the furcation area, as well as in membrane adaptation, are the most common variables described by authors to justify the unpredictable results obtained with the GTR therapy to treat proximal furcation involvements (Metzler et al. 1991, Mellonig et al. 1994, Pontoriero & Lindhe 1995, Rosen et al. 1997, Avera et al. 1998, Machtei 2001). Thus, other therapeutic options should be studied to determine a predictable alternative to the treatment of these periodontal lesions.

Enamel matrix derivative proteins (EMD) proteins have been used as a periodontal regenerative procedure due to their fundamental role in cementum development (Hammarström 1997a, b, Hammarström et al. 1997). Gestrelius et al. (1997) showed that EMD proteins promote a periodontal ligament cell proliferation and increase the protein synthesis and mineral nodule formation by these cells. In addition, EMD proteins are reported to increase mineralized matrix formation, as well as the release of growth factors [fibroblast growth factor-2(FGF-2), transforming growth factor (TGF)-β, insulin-like growth factor (IGF)], to decrease the MMP1 concentration and to block osteoclast maturation (via RANKL/OPG). EMD proteins also promote a reduction in the local pathogenic flora, creating a more favourable environment for periodontal regeneration (Van der Pawn et al. 2000, Haase & Bartold 2001, Sculean et al. 2001, Arweiler et al. 2002, Mizutani et al. 2003, Keila et al. 2004, Galli et al. 2006, Walter et al. 2006).

Clinically, the application of EMD proteins in infra-osseous and mandibular furcation defects has shown similar results when compared with GTR gains in the clinical attachment level and with the reduction in PD (Mellonig 1999, Parashis & Tsiklakis 2000, Jepsen et al. 2004, Meyle et al. 2004, Parashis et al. 2004, Hoffmann et al. 2006, Sculean et al. 2006). Thus, the aim of this double-blind randomized clinical trial (RCT) was to evaluate the use of the EMD proteins in the treatment of proximal class-II furcation involvements.

Material and Methods

Study design

This study was designed as a double-blind randomized prospective controlled clinical parallel study to compare the clinical outcomes after OFD+24% ethylenediaminetetraacetic acid (EDTA) root conditioning (control group) with OFD+24% EDTA conditioning+EMD protein application (test group). The study design was approved by the ethics committee of the University of Campinas. All patients received a detailed description of the proposed treatment and gave their informed and written consent.

Population screening

Potential patients were selected from those referred to the Graduate Clinic of the Piracicaba Dental School. All patients received a complete periodontal examination, including a full-mouth periodontal probing, radiographic examination and complete anamnesis. The study inclusion criteria were (i) diagnosis of chronic periodontitis (according to the criteria of the 1999 international classification; Armitage 1999); (ii) presence of one pair of contralateral maxillary molars with proximal class-II furcations, presenting PD $\geqslant$ 5 mm and bleeding on probing (BOP), after non-surgical therapy; (iii) good general health; and (iv) age $>$ 35 years. Patients who (i) were pregnant or lactating; (ii) required antibiotic pre-medication for the performance of periodontal examination and treatment; (iii) suffered from any other systemic diseases (cardiovascular, pulmonary, liver, cerebral, diseases or diabetes); (iv) had received antibiotic treatment in the previous 3 months; (v) were taking long-term anti-inflammatory drugs; (vi) had received a course of periodontal treatment within the last 6 months; and (vii) were smokers were excluded from the study.

Non-surgical treatment

All the subjects received a full-mouth periodontal treatment 6 months before the surgical procedure. All these treatments were performed by the same operator, with an ultrasonic device (Cavitron, Dentsply, NY, USA) and specific tips for furcation debridement (PQ2N7, Hu-Friedy, Chicago, IL, USA). At the same time the subjects underwent motivation sessions, during which oral hygiene instructions were given, to ensure that the subjects could maintain a proper level of oral hygiene during the 6 months before the surgical procedure. These sessions of oral hygiene instructions were repeated until subjects showed the ability to maintain good plaque control, as evidenced by pre-treatment plaque scores $<$20%, especially at the maxillary proximal sites. During these sessions, a supragingival plaque control was performed to maintain the high status of hygiene and plaque control.

Clinical parameters

The following clinical parameters were assessed immediately before the surgical procedure. Full-Mouth Plaque Score and Full-Mouth Bleeding Score (FMBS) were calculated after assessing dichotomously the presence of plaque at site or BOP from the bottom of the pocket when probing with a manual probe and calculating the percentage of total sites that revealed the presence of plaque or BOP. The presence of plaque or BOP was also dichotomously evaluated, especially at the site denominated as the proximal furcation included in the study. These parameters were evaluated at six sites/tooth at baseline, 2, 4 and 6 months.

PD, gingival margin position (GMP) and relative vertical clinical attachment level (RVCAL) were evaluated using a PCP-15 periodontal Probe (Hu-Friedy). The relative horizontal clinical attachment level (RHCAL) was evaluated by a specific modified periodontal probe to evaluate the horizontal component of the defect (Suh et al. 2002).

Following local anaesthesia, a bone sounding was performed to avoid the necessity of re-entry surgery (Suh et al. 2002). The vertical bone level (VBL) was evaluated with a PCP-15 periodontal probe and the HBL was evaluated with a modified periodontal probe. The furcation defects were classified on a four-stage scale (Hamp et al. 1975) using a Nabers Probe (Hu-Friedy).

All these parameters were evaluated at one specific site at the proximal furcation entrance, determined by a groove made on an individually manufactured acrylic stent and recorded to the nearest 0.5 mm. The assessments of GMP, PD, RVCAL and RHCAL were performed at baseline, 2, 4 and 6 months after the surgical procedure. All other parameters were evaluated just at baseline and at the sixth month post-operatively.

Investigator calibration

Initially, a total of 15 non-study subjects presenting proximal class-II furcations were selected. The designated examiner

© 2008 The Authors
Journal compilation © 2008 Blackwell Munksgaard

(R. C. V. C.) measured the RVCAL and RHCAL of all patients, twice, within 24 h, with at least 1 h between the examinations. The examiner was judged to be reproducible, fulfilling the predetermined success criteria (the percentage of agreement within ± 1 mm between repeated measurements had to be at least 90%). The intraclass correlation was calculated to each parameter, resulting in 91% reproducibility for RVCAL and 93% for RHCAL.

Surgical procedures

Before surgery, intraoral antisepsis was performed with 0.12% chlorhexidine rinse solution and extraoral antisepsis was carried out with iodine solution. As both surgeries were performed on the same day, a coin was tossed to determine the first site to be operated. Following local anesthesia, sulcular incisions were made and mucoperiosteal flaps were raised at the buccal and palatine surfaces. Carefully, the tissue was reflected, preserving the maximum of interproximal soft tissue. Granulation tissue as well as the visible calculus were removed with hand curettes (Gracey, Hu-Friedy) and with an ultrasonic device (Cavitron, Dentsply, Tulsa, OK, USA) with specific tips for furcation scaling (UI25KFPset, Hu-Friedy). The diagnosis of the class-II furcation defect was then confirmed using a Nabers probe (Hu-Friedy) (Figs 2–4). At this point, the treatment was randomized and the procedures specific for the control or test group were chosen by a coin toss.

The test-group sites were treated as follows: the root surfaces were conditioned using 24% EDTA gel (PrefGel®, Straumann, Basel, Switzerland) for 2 min., followed by thorough rinsing with a sterile saline solution, as recommended by the manufacturer (Fig. 5). Excess fluids were removed, leaving the surgical area clear and the EMD (Emdogain®, Straumann) was then applied (Fig. 6). The protein gel was applied from the farthest end of the involved furcation until the proximal surface of the tooth was covered with EMD. The surgical flaps were then replaced at their initial position and sutured. In order to obtain a primary wound closure, modified mattress sutures (5.0 poligalactin-A; Vicryl, Johnson & Johnson, São José dos Campos, Brazil) were used. The same sequence of events was carried out for the control-group sites, excluding the EMD application.

Post-operative care

Patients were instructed to take analgesics (500 mg dipyrone) and to discontinue toothbrushing around the surgical sites during the initial 10 days after surgery. For biofilm control, patients were instructed to rinse with 0.12% clorhexidine twice a day for a month. The sutures were then removed at 10 days post-surgery and the clinical parameters were analysed at 2, 4 and 6 months (Figs 7–10).

Randomization and allocation concealment

The study employed a blinded examiner with a randomized and split-mouth design. The treatment was determined for the proximal furcation during the surgical procedure. Initially, the first side to be operated was determined by a coin toss and after the debridement of the surgical site another coin toss was carried out to determine the treatment. The coin toss was performed by an operator (F. V. R.) different from the one responsible for the surgical procedure (E. D. P. R.) and different from the examiner (R. C. V. C.). The randomization code was not broken until all data had been collected. Thus, the treatment group was not revealed to the patient, to the clinical examiner or to the statistician.

Re-assessment evaluations

Re-assessment visits occurred every 15 days during the first month and monthly until the sixth month. During these appointments, the examiner recorded the clinical periodontal parameters and checked any change in the medical or health status. At the end of the appointment, a session of supragingival prophylaxis was performed as necessary.

Primary and secondary outcome measures

The primary outcome measurement of the study was RHCAL. Secondary outcomes included (i) HBL; (ii) VBL and RVCAL; (iii) PD and position of gingival margin; (iv) furcation closure at six months; (v) plaque and BOP at surgical site; (vi) full-mouth plaque index (PI) and BOP.

Power calculation

The study power value was calculated with the SAS 9.01 program (Release 9.1, 2003, SAS Institute Inc., Cary, NC, USA), considering the standard deviation of each group of the present clinical trial. A difference of 2.0 mm between the test and control groups was considered as clinically significant. The power value was evaluated for all clinical parameters in each period of evaluation. The minimum power value found was 91% (for the GMP parameter at 6 months). All other parameters presented a power value >91%. Accordingly, a sample of 15 patients was enough to determine the difference between the groups.

Data management and statistical analysis

Initially, the statistical analysis considered three populations. The safety population enrolled had received at least one treatment. From this safety population were selected the subjects who met all the inclusion criteria [intention-to-treat population (ITT)]. The subjects from the ITT population who completed the follow-up and did not violate the inclusion and exclusion criteria were considered the per-protocol (PP) population. The statistical analysis considered this PP population. The safety population was composed of 24 subjects. Of these, 16 formed the ITT population and the PP population was formed from 15 subjects (see flowchart Fig. 1). The objective of the present study was to evaluate the clinical response of proximal class-II furcations treated with EMD proteins. Thus, we tested the null hypothesis that EMD did not promote a greater improvement in the clinical parameters. To test this hypothesis, a SAS 9.01 program (SAS Institute Inc.) was used.

The homogeneity of groups at baseline (PD and RHCAL) was tested using the student *t*-test. Repeated-measures analysis of variance (ANOVA) was used to detect intra- and inter-group differences in clinical parameters (GMP, PD, RVCAL, RHCAL, VBL and HBL). When statistical difference was found, analysis of the difference was determined using the method of Tukey. The Friedman test was used to detect intra-group differences in Full-mouth plaque and bleeding indices among all periods. The number of furcation sites with or without plaque or BOP were analysed using the McNemar test (intra- and inter-group analysis). According to the Hamp et al. (1975) classification, furcation lesions were quantified and

© 2008 The Authors
Journal compilation © 2008 Blackwell Munksgaard

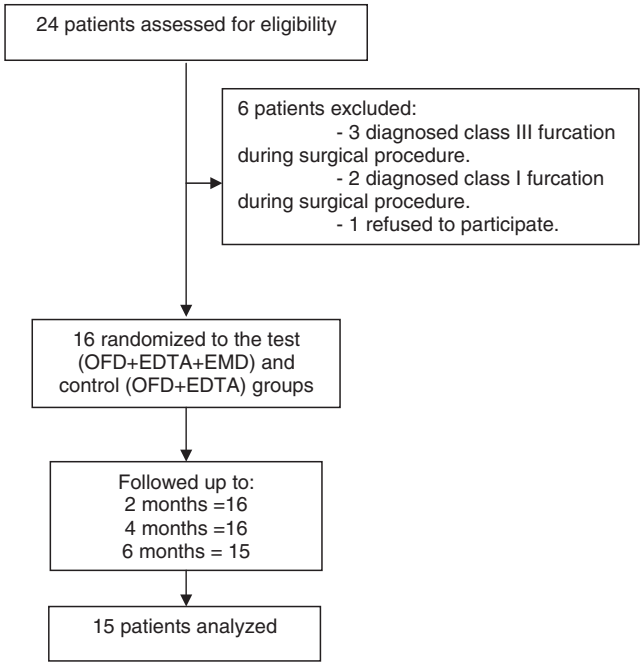

Fig. 1. Flowchart for study patients. OFD, open flap debridement; EMD, enamel matrix derivative proteins.

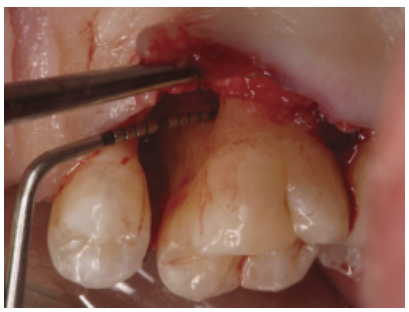

Fig. 2. View of a superior molar with diagnosis of class II proximal furcation assessed during surgical procedure.

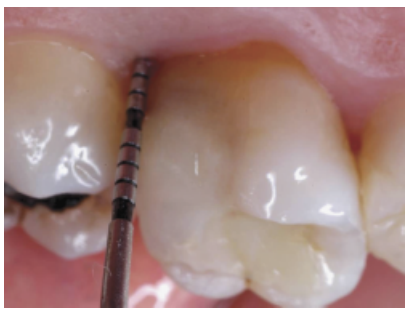

Fig. 3. Clinical appearance before surgical procedure.

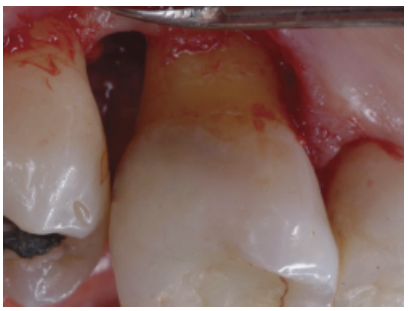

Fig. 4. Intraoperative view of surgical site presenting class II proximal furcation.

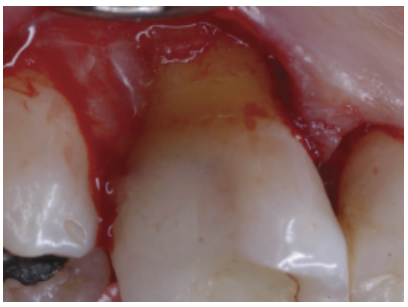

Fig. 5. EDTA 24% Gel application.

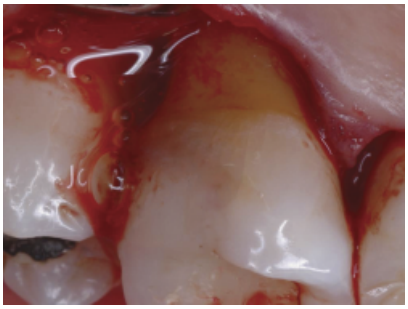

Fig. 6. EMD proteins application.

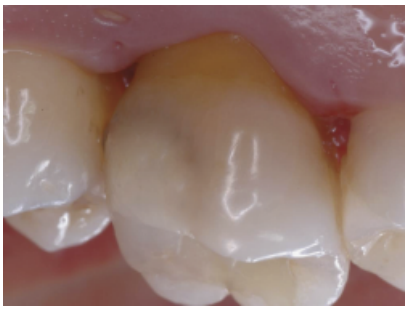

Fig. 7. Clinical appearance of 1 week post-operatively.

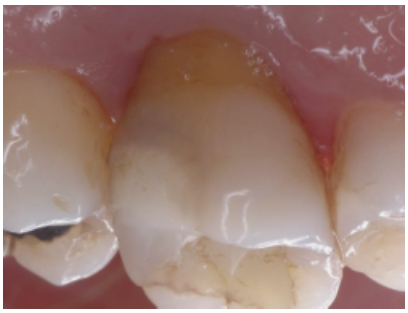

Fig. 8. Clinical appearance of 2 months post-operatively.

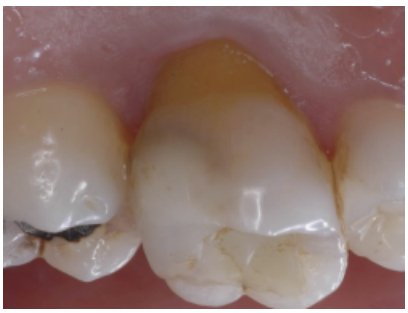

Fig. 9. Clinical appearance of 4 months post-operatively.

compared by an inter-group analysis using the McNemar test. An experimental level of significance was determined at 5% for all statistical analysis ($\alpha = 0.05$).

Results

Subject accountability

Figure 1 illustrates the study flowchart. Twenty-four patients were initially recruited at the beginning of the study. Of these, six subjects were excluded: three because they presented class-III furcations (through-to-through), confirmed at surgical procedure; two

© 2008 The Authors
Journal compilation © 2008 Blackwell Munksgaard

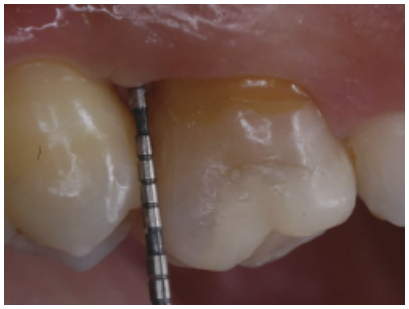

Fig. 10. Clinical appearance of 6 months post-operatively. Probing depth of 3 mm without bleeding on probing.

because they presented class-I furcations (HBL < 3 mm); and one because he refused to participate in the study (did not accept surgical treatment). None of these excluded patients had signed the consent form. As such, 16 subjects were randomly assigned to participate in the study. All participants received the allocated procedure; however, one patient was lost later during follow-up due to the administration of antibiotic medication. All other subjects were included in the statistical analysis.

Study schedule

Subject recruitment started in January 2005 and was completed by the end of June 2006. The first surgical procedure was carried out in February 2006, and all the 6-month follow-up visits were completed in December 2006. Data entry of all information and statistical analysis were performed by the end of December 2006.

Patient characteristics at baseline

Patients' characteristics at baseline were not significantly different between groups, as seen in Table 1. The mean age was 49.7 ± 6.3 years, and both groups included a majority of females (80%) and Caucasians (73%). Acceptable oral hygiene was achieved before the study, as seen from the FMBS (15.65 ± 4.91) at baseline. The mean PD at furcation and RHCAL at furcation were also not statistically different between groups at baseline (6.48 ± 1.07 and 6.40 ± 1.30; 10.18 ± 1.39 and 9.95 ± 1.69, for control and test groups, respectively). The distributions of mesial and distal furcations for each patient in the control or test groups are also shown in Table 1.

Table 1. Patient characteristics at baseline

Age	49.7 ± 6.3	
Female (%)	12 (80)	
Caucasian (%)	11 (73)	
FMBS (%)	15.65 ± 4.91	
	Control group	Test group
Furcation surface distribution		
Mesial	2	3
Distal	13	12
PD at furcation site (mm ± dp)*	6.40 ± 1.18	6.47 ± 1.39
RHCAL at furcation site (mm ± dp)*	10.23 ± 1.37	9.77 ± 1.72

*Student *t*-test ($p > 0.05$).
PD, probing depth; RHCAL, relative horizontal clinical attachment level; FMBS, full-mouth bleeding score.

Table 2. Percentage of FMPS, FMBS, plaque and bleeding values at surgical site at the different assessment times

	Baseline	2 months	4 months	6 months
FMPS (%)*	18.9 ± 1.7	18.2 ± 1.8	19.0 ± 1.0	17.0 ± 0.5
FMBS (%)*	15.6 ± 4.9	14.5 ± 1.3	13.9 ± 1.1	13.5 ± 1.0
Plaque at surgical site				
Control	0% (0)	53% (8)†	40% (6)†	20% (3)
Test	0% (0)	47% (7)†	20% (3)	13% (2)
Bleeding at surgical site				
Control	100% (15)	87% (13)	73% (11)	47% (7)†
Test	100% (15)	67% (10)†	53% (8)†	33% (5)†

*Friedman test ($p > 0.05$).
†Intra-group statistical difference from baseline, $p < 0.05$, with McNemar test.
FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score.

Treatment assessments

PI and bleeding scores

The means of full-mouth plaque and bleeding scores as well the percentages of presence of plaque and bleeding at surgical site are shown in Table 2. The plaque and bleeding scores were maintained at lower than 20% throughout the study, without difference among periods. The percentage of the presence of plaque at the surgical site was 0% for both groups. However, during the second month there was an increase in this percentage, with statistical difference from baseline for both groups. During the fourth and sixth months, neither group presented statistical difference from baseline.

The values for bleeding at the surgical site demonstrated a decrease throughout the study for both groups. During the second month, the test group presented a statistical reduction in the BOP score, maintaining this reduction until the end of the evaluation. For the control group, a statistical difference from baseline was achieved only during the fourth month. No differences were observed between the groups for the PI as well for the bleeding score at the surgical site during any of the periods of assessment.

GMP and PD

PD and GMP means are shown in Table 3. An increase in the distance of the gingival margin to the stent was observed, i.e., gingival recession, and a reduction in PD was observed in both groups ($p < 0.05$). The gingival recession in the control group was 1.57 ± 1.19 mm, whereas in the test group it was 1.18 ± 1.17 mm. The PD reductions were 1.96 ± 1.03 mm and 1.71 ± 1.28 mm in the control and test group, respectively. There were no differences between the groups with regard to gingival recession and reduction in PD ($p > 0.05$).

RVCL and RHCAL

The values for RVCAL and RHCAL are depicted in Table 3. No statistical difference was observed for either parameter between groups. In both groups, a significant improvement in RHCAL

© 2008 The Authors
Journal compilation © 2008 Blackwell Munksgaard

Table 3. Means (± SD) of GMP, PD, RVCAL and RHCAL at the times of evaluation

	Baseline	2 months	4 months	6 months	0–6 months difference
GMP					
Control	2.10 ± 0.99	3.50 ± 0.65*	3.57 ± 0.84*	3.54 ± 0.91*	1.57 ± 1.19
Test	2.13 ± 1.13	3.30 ± 1.33*	3.27 ± 0.90*	3.29 ± 1.35*	1.18 ± 1.17
PD					
Control	6.40 ± 1.18	4.33 ± 0.56*	4.43 ± 0.75*	4.54 ± 0.60*	1.96 ± 1.03
Test	6.47 ± 1.39	4.63 ± 1.43*	4.53 ± 0.92*	4.82 ± 1.56*	1.71 ± 1.28
RVCAL					
Control	8.50 ± 1.10	7.83 ± 0.79*	8.00 ± 0.91*	8.07 ± 1.09*	0.39 ± 1.00
Test	8.60 ± 1.72	7.90 ± 1.80*	7.83 ± 1.22*	8.11 ± 1.79*	0.54 ± 0.95
RHCAL					
Control	10.23 ± 1.37	8.80 ± 1.03*	8.07 ± 1.09*	9.11 ± 1.77*	1.21 ± 2.28
Test	9.77 ± 1.72	8.30 ± 1.60*	8.11 ± 1.79*	8.46 ± 1.65*	1.36 ± 1.26

*Intra-group statistical difference from baseline (ANOVA/Tukey).
SD, standard deviation; GMP, gingival marginal position; PD, probing depth; RVCAL, relative vertical clinical attachment level; RHCAL, relative horizontal clinical attachment level.

Table 4. Means (± SD) of VBL and HBL at baseline and 6 months

	Baseline	6 months	0–6 months difference
VBL			
Control	10.32 ± 1.10	9.28 ± 1.25*	1.04 ± 1.12
Test	10.42 ± 1.93	9.61 ± 1.90*	0.82 ± 1.82
HBL			
Control	11.46 ± 1.82	10.42 ± 0.56*	1.00 ± 1.79
Test	10.95 ± 1.39	9.79 ± 0.15*	1.17 ± 1.38

*Intra-group statistical difference from baseline ($p < 0.05$, ANOVA/Tukey).
SD, standard deviation; VBL, vertical bone level; HBL, horizontal bone level.

($p < 0.05$) was obtained. The gain in RHCAL was 1.21 ± 2.28 mm for the control group and 1.36 ± 1.26 mm for the test group ($p < 0.05$). The RVCAL gain was 0.39 ± 1.00 mm and 0.54 ± 0.95 mm for the control and test groups, respectively ($p > 0.05$).

VBL and HBL

At baseline and 6 months, the furcation sites were anaesthetised and bone probing was performed to determine the bone level. The results of the VBL and HBL are shown in Table 4. There was an improvement in both the VBL and the HBL at 6 months ($p < 0.05$). The gain in HBL in the control group was 1.00 ± 1.79 mm, whereas in the test group the gain was 1.17 ± 1.38 mm. The VBL gain was 1.04 ± 1.12 mm and 0.82 ± 1.82 mm for the control and test groups, respectively. There was no difference between the groups for the VBL and the HBL.

Furcation closure

In the control group, 67% of the proximal furcations maintained a class-II diagnosis, while in the test group only 33% still received this classification ($p = 0.01$). Similarly, the number of class-I furcation was significantly higher in the test than in the control group ($p = 0.05$). The evaluation of the closed furcations demonstrated that, in the test group, two proximal furcations were not detectable by clinical examination. In contrast, the control group did not demonstrate any closed furcation (Table 5).

Table 5. Percentage (n) of furcation status at 6 months, according to the [18]Hamp et al. (1975) classification

	Furcation class at 6 months		
	2	1	closed
Control	67% (10)	33% (5)	0 (0)
Test	27% (4)	60% (9)	13% (2)
p*	0.01	0.05	0.16

*McNemar test.

Discussion

The treatment of proximal furcation involvements still remains a clinical challenge. This class of lesions presents a poor response to non-surgical treatment (Del Peloso Ribeiro et al. 2007) and to regenerative therapy (Metzler et al. 1991, Pontoriero & Lindhe 1995) when compared with non-proximal furcations. Thus, the present study aimed to evaluate the EMD proteins as a therapeutic alternative for the treatment of proximal furcation involvements, because EMD applications have demonstrated good clinical results in infra-osseous and non-proximal furcations (Giannobile & Somerman 2003, Jepsen et al. 2004, Chitsazi et al., 2007).

Both groups of the present study showed improvements in the clinical parameters evaluated. Control group furcations presented a PD reduction of 1.18 ± 1.17 mm, while in the test group a reduction of 1.96 ± 1.03 mm was observed. Compared with GTR, the PD reduction was also similar to that obtained in previous studies. The distal furcations treated with GTR therapy in the study of Pontoriero & Lindhe (1995) achieved a reduction of 1.3 mm, while the mesial furcations obtained a reduction of 1.6 mm. Avera et al. (1998), using e-PTFE membranes in mesial furcations, obtained a 2.88 mm reduction in PD.

Studies evaluating GTR therapy to treat proximal furcation involvements demonstrated the possibility of horizontal bone gain in the inter-radicular area. Metzler et al. (1991) and Pontoriero & Lindhe (1995) observed minimal gains in the HBL of 0.9 and 0.3 mm, respectively, while Avera et al. (1998) showed a mean gain of 1.19 ± 0.16 mm in the HBL. In the present study, the application of EMD promoted a horizontal bone gain of 1.17 mm, which was not statistically different from that of the control group (1.00 mm gain).

As could be seen, the surgical access to periodontal debridement leads to a reduction in PD and horizontal and vertical attachment gain similar to that observed with EMD therapy. The lack of significant improvements in reduction of the PD or gain in clinical and osseous parameters after EMD therapy may be associated with the specific characteristics of proximal furcation involvements, such as anatomy and difficulty in access and hygiene at the inter-proximal faces.

Proximal furcations present a specific anatomy, which increases the incidence of their involvement in periodontal disease. The location of the furcation fornix closer to the cement–enamel

© 2008 The Authors
Journal compilation © 2008 Blackwell Munksgaard

junction (CEJ) in the upper molars than in the lower molars leads to a faster loss of attachment at the furcation entrance (Svardström & Wennström 1996). Rosenberg (1988) reported on the distance of CEJ to the fornix of the mesial and distal faces of upper molars, relating distances of 3 and 5 mm, respectively. In the presence of periodontal disease, this proximity to the oral cavity could influence the recontamination of the treated furcation surface, reducing the success of periodontal disease treatment. Furthermore, the fornix of the upper molars is inclined and presents a complex anatomy, which, beyond increasing the chance of occurrence, also impedes the access of scaling instruments (Rosenberg 1988).

The small root divergence of the proximal furcation is one of the factors that negatively influence the access of scaling instruments, because smaller furcation entrances complicate the use of the instruments in the inter-radicular region, impeding adequate decontamination (Parashis et al. 1993). Bower (1979), evaluating 114 upper and 103 lower molars, observed that 53% of furcation entrances were smaller than the size of periodontal curettes. Chiu et al. (1991) showed that 39% and 43% of mesial and distal furcations, respectively, were smaller than 0.75 mm, demonstrating the difficulty in achieving penetration with scaling instruments and reducing the effectiveness of the mechanical treatment of proximal furcation involvements.

In addition to the influence of the anatomic features of furcation lesions, the posterior location in the arch and the interproximal position of the furcation's entrance could negatively interfere in daily oral hygiene. This hygiene impedes the increase in biofilm accumulation and leads to a precocious recolonization of treated sites (Nordland et al. 1987), also reducing the effectiveness of the periodontal treatment (Svardström & Wennström 1996).

The OFD of proximal furcations, with or without EMD application, promotes the occurrence of gingival craters during initial post-operatory periods. The crater formation facilitates biofilm accumulation, as can be seen in Table 3. The presence of biofilm is a negative factor in periodontal therapy, whether regenerative or non-surgical, which can diminish the clinical response to treatment (Novaes et al. 2005). Moreover, difficulties in healing of the proximal region have already been reported in studies evaluating GTR therapy in the treatment of proximal furcations. According to the authors, the presence of the membrane in the inter-proximal space impedes a satisfactory flap adaptation, leading to problematic healing (Metzler et al. 1991, Pontoriero & Lindhe 1995, Avera et al. 1998). However, as seen in the present study, despite the use of membrane, the inter-proximal area correlated with difficulty in flap adaptation and tissue maintenance, which increased the frequency of gingival crater occurrence, leading, consequently, to a negative influence in wound healing.

It should be pointed out that the control group received conditioning with 24% EDTA. While the application of EDTA did not promote any additional benefit for the surgical access to periodontal pockets and infra-osseous defects (Mayfield et al. 1998, Blomlof et al. 2000), there are no reports in the literature that have evaluated the influence of EDTA as an adjunctive to furcation treatment. EDTA application, however, could have interfered in the clinical response, observed in the present study, because its application removes the smear layer, detoxifying the root surface and possibly enhancing the healing of the tissues (Blomlof et al. 1996, 1997). Thus, the clinical relevance of EDTA conditioning is still unknown and should be addressed in other studies.

In spite of the lack of significant difference in HBL, a higher rate of furcation class-II to class-I conversion was observed in the test group. Probably, the difference in the method of probing and characteristics of the examinations could explain this difference in the results. While the HBL is a quantitative analysis, measured with a millimetric probe, performed with a stent to determine a fixed mark and the specific site of probing, the classification of furcation status is a qualitative evaluation, performed with the Naber's probe, without stent assistance and is, therefore, a less-precise parameter. Independently, the clinical significance of this improvement in the horizontal component represents an important result to regenerative furcation therapy and could modify the prognosis of teeth. McGuire and Nunn (1996a, b) have shown that a molar with class-II furcation has a worse prognosis than a molar without furcation involvement or with class-I furcation. If regenerative therapy is successful in transforming a class-II furcation into class-I, as seen in the present study, long-term prognosis may be improved.

However, other studies following up the results of regenerative therapy are necessary to clarify the real influence on the prognosis of this conversion of class-II to class-I. Although the present study evaluates just 6 months after the surgery, the application of EMD to mandibular furcation defects has previously been demonstrated to result in furcation closure in a 14-month follow-up study (Jepsen et al. 2004). Moreover, because the long-term studies evaluating the EMD as a regenerative therapy have shown an improvement in osseous parameters over the years (Heijl et al. 1997), follow-up is important to determine the benefits of regenerative therapy.

Conclusion

In conclusion, the present controlled RCT showed that the application of EMD in proximal furcations did not promote a superior reduction in PD or gain in the clinical and osseous attachment level, but allowed a higher rate of conversion of class-II to class-I furcations.

References

Armitage, G. C. (1999) Development of a classification system for periodontal diseases and conditions. *Annals of Periodontology* **4**, 1–6.

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 Investigation* **6**, 205–209.

Avera, J. B., Camargo, P. M., Klokkevold, P. R., Kenney, E. B. & Lekovic, V. (1998) Guided tissue regeneration in class II furcation involved maxillary molars: a controlled study of 8 split-mouth cases. *Journal of Periodontology* **69**, 1020–1026.

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

Blomlof, J. P., Blomlof, L. B. & Lindskog, S. F. (1997) Smear layer formed by different root planing modalities and its removal by an ethylenediaminetetraacetic acid gel preparation. *International Journal of Periodontics and Restorative Dentistry* **17**, 242–249.

Blomlof, L. B., Jonsson, B., Blomlof, J. P. & Lindskog, S. (2000) A clinical study of root surface conditioning with an EDTA gel. II. Surgical periodontal treatment. *International Journal of Periodontics and Restorative Dentistry* **20**, 566–573.

© 2008 The Authors
Journal compilation © 2008 Blackwell Munksgaard

Bower, R. C. (1979) Furcation morphology relative to periodontal treatment. Furcation entrance architecture. *Journal of Periodontology* **50**, 23–27.

Chitsazi, M. T., Mostofi Zadeh Farahani, R., Pourabbas, M. & Bahaeddin, N. (2007) Efficacy of open flap debridement with and without enamel matrix derivatives in the treatment of mandibular degree II furcation involvement. *Clinical Oral Investigation* **11**, 385–389.

Chiu, B. M., Zee, K. Y., Corbet, E. F. & Holmgren, C. J. (1991) Periodontal implications of furcation entrance dimensions in Chinese first permanent molars. *Journal of Periodontology* **62**, 308–311.

Del Peloso Ribeiro, E., Bittencourt, S., Nociti, F. H. Jr., Sallum, E. A., Sallum, A. W. & Casati, M. Z. (2007) Comparative study of ultrasonic instrumentation for the non-surgical treatment of interproximal and non-interproximal furcation involvements. *Journal of Periodontology* **78**, 224–230.

Galli, C., Macaluso, G. M., Guizzardi, S., Vescovini, R., Passeri, M. & Passeri, G. (2006) Osteoprotegerin and receptor activator of nuclear factor-kappa B ligand modulation by enamel matrix derivative in human alveolar osteoblasts. *Journal of Periodontology* **77**, 1223–1228.

Gestrelius, S., Andersson, C., Lidstrom, D., Hammarström, L. & Somerman, M. (1997) In vitro studies on periodontal ligament cells and enamel matrix derivative. *Journal of Clinical Periodontology* **24**, 685–692.

Giannobile, W. V. & Somerman, M. (2003) Growth and amelogenin-like factors in periodontal wound healing. A systematic review. *Annals of Periodontology* **1**, 193–204.

Haase, H. R. & Bartold, P. M. (2001) Enamel matrix derivative induces matrix synthesis by cultured human periodontal fibroblast cells. *Journal of Periodontology* **72**, 341–348.

Hammarström, L. (1997a) The role of enamel matrix proteins in the development of cementum and periodontal tissues. *Ciba Found Symposium* **205**, 246–260.

Hammarström, L. (1997b) Enamel matrix, cementum development and regeneration. *Journal of Clinical Periodontology* **24**, 658–659.

Hammarström, 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.

Hamp, S. E., Nyman, S. & Lindhe, J. (1975) Periodontal treatment of multirooted teeth. Results after 5 years. *Journal of Clinical Periodontology* **2**, 126–135.

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.

Hoffmann, T., Richter, S., Meyle, J., Gonzales, J. R., Heinz, B., Arjomand, M., Sculean, A., Reich, E., Jepsen, K., Jepsen, S. & Boedeker, R. H. (2006) A randomized clinical multicentre trial comparing enamel matrix derivative and membrane treatment of buccal class II furcation involvement in mandibular molars. Part III: patient factors and treatment outcome. *Journal of Clinical Periodontology* **33**, 575–583.

Jepsen, S., Eberhard, J., Herrera, D. & Needleman, I. (2002) A systematic review of guided tissue regeneration for periodontal furcation defects. What is the effect of guided tissue regeneration compared with surgical debridement in the treatment of furcation defects? *Journal of Clinical Periodontology* **29**, 103–116.

Jepsen, S., Heinz, B., Jepsen, K., Arjomand, M., Hoffmann, T., Richter, S., Reich, E., Sculean, A., Gonzales, J. R., Bödeker, R. H. & Meyle, J. (2004) A randomized clinical trial comparing enamel matrix derivative and membrane treatment of buccal class II furcation involvement in mandibular molars. Part I: study design and results for primary outcomes. *Journal of Periodontology* **75**, 1150–1160.

Keila, S., Nemcovsky, C. E., Moses, O., Artzi, Z. & Weinreb, M. (2004) In vitro effects of enamel matrix proteins on rat bone marrow cells and gingival fibroblasts. *Journal of Dental Research* **83**, 134–138.

Machtei, E. E. (2001) The effect of membrane exposure on the outcome of regenerative procedures in humans: a meta-analysis. *Journal of Periodontology* **72**, 512–516.

Mayfield, L., Soderholm, G., Norderyd, O. & Attstrom, R. (1998) Root conditioning using EDTA gel as an adjunct to surgical therapy for the treatment of intraosseous periodontal defects. *Journal of Clinical Periodontology* **25**, 707–714.

McGuire, M. K. & Nunn, M. E. (1996b) Prognosis versus actual outcome. II. The effectiveness of clinical parameters in accurately predicting tooth survival. *Journal of Periodontology* **67**, 666–674.

McGuire, M. K. & Nunn, M. E. (1996a) Prognosis versus actual outcome. III. The effectiveness of clinical parameters in developing an accurate prognosis. *Journal of Periodontology* **67**, 658–665.

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.

Mellonig, J. T., Seamons, B. C., Gray, J. L. & Towle, H. J. (1994) Clinical evaluation of guided tissue regeneration in the treatment of grade II molar furcation invasions. *International Journal of Periodontics and Restorative Dentistry* **14**, 254–271.

Metzler, D. G., Seamons, B. C., Mellonig, J. T., Gher, M. E. & Gray, J. L. (1991) Clinical evaluation of guided tissue regeneration in the treatment of maxillary class II molar furcation invasions. *Journal of Periodontology* **62**, 353–360.

Meyle, J., Gonzales, J. R., Bodeker, R. H., Hoffmann, T., Richter, S., Heinz, B., Arjomand, M., Reich, E., Sculean, A., Jepsen, K. & Jepsen, S. (2004) A randomized clinical trial comparing enamel matrix derivative and membrane treatment of buccal class II furcation involvement in mandibular molars. Part II: secondary outcomes. *Journal of Periodontology* **75**, 1188–1195.

Mizutani, S., Tsuboi, T., Tazoe, M., Koshihara, Y., Goto, S. & Togari, A. (2003) Involvement of FGF-2 in the action of Emdogain on normal human osteoblastic activity. *Oral Diseases* **9**, 210–217.

Murphy, K. G. & Gunsolley, J. C. (2003) Guided tissue regeneration for the treatment of periodontal intrabony and furcation defects. A systematic review. *Annals of Periodontology* **8**, 266–302.

Nordland, P., Garret, S., Kiger, R., Vanooteghem, R., Huchtens, R. H. & Egelberg, L. (1987) The effect of plaque control and root debridement in molar teeth. *Journal of Clinical Periodontology* **14**, 231–236.

Novaes, A. B. Jr., Palioto, D. B., de Andrade, P. F. & Marchesan, J. T. (2005) Regeneration of class II furcation defects: determinants of increased success. *Brazilian Dental Journal* **16**, 87–97.

Parashis, A., Andronikaki-Faldami, A. & Tsiklakis, K. (2004) Clinical and radiographic comparison of three regenerative procedures in the treatment of intrabony defects. *International Journal of Periodontics and Restorative Dentistry* **24**, 81–90.

Parashis, A. & Tsiklakis, K. (2000) Clinical and radiographic findings following application of enamel matrix derivative in the treatment of intrabony defects. A series of case reports. *Journal of Clinical Periodontology* **27**, 705–713.

Parashis, A. O., Anagnou-Vareltzides, A. & Demetriou, N. (1993) Calculus removal from multirooted teeth with and without surgical access. (I). Efficacy on external and furcation surfaces in relation to probing depth. *Journal of Clinical Periodontology* **20**, 63–68.

Pontoriero, R. & Lindhe, J. (1995) Guided tissue regeneration in the treatment of degree II furcations in maxillary molars. *Journal of Clinical Periodontology* **22**, 756–763.

Rosen, P. S., Marks, M. H. & Bowers, G. M. (1997) Regenerative therapy in the treatment of maxillary molar class II furcations: case reports. *International Journal of Periodontics and Restorative Dentistry* **17**, 516–527.

Rosenberg, M. M. (1988) Furcation involvement: periodontic, endodontic and restorative interrelationships. In: Rosenberg, M. M., Kay, H. B., Keough, B. E. & Holt, R. L. (eds). *Periodontal and Prosthetic Management of Advanced Cases*, pp. 450–454. Chicago: Quintessence.

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., Berakdar, M., Willershausen, B., Arweiler, N. B., Becker, J. & Schwarz, F. (2006) Effect of EDTA root conditioning on the healing of intrabony defects treated with an enamel matrix protein derivative. *Journal of Periodontology* **77**, 1167–1172.

Suh, Y. I., Lundgren, T., Sigurdsson, T., Riggs, M. & Crigger, M. (2002) Probing bone level

© 2008 The Authors
Journal compilation © 2008 Blackwell Munksgaard

measurements for determination of the depths of class II furcation defects. *Journal of Periodontology* **73**, 637–642.

Svardström, G. & Wennström, J. L. (1996) Prevalence of furcation involvements in patients referred for periodontal treatment. *Journal of Clinical Periodontology* **23**, 1093–1099.

Van der Pawn, M. T., Van de Bos, T. & Everts, V. (2000) Enamel matrix-derived protein stimulates attachment of periodontal ligament fibroblasts and enhances alkaline phosphatase activity and transforming growth factor beta 1 release of ligament and gingival fibroblast. *Journal of Periodontology* **71**, 31–43.

Walter, C., Jawor, P., Bernimoulin, J. P. & Hagewald, S. (2006) Moderate effect of enamel matrix derivative (Emdogain Gel) on *Porphyromonas gingivalis* growth in vitro. *Archives of Oral Biology* **51**, 171–176.

Address:
Márcio Zaffalon Casati
Division of Periodontics
SP – School of Dentistry of Piracicaba
UNICAMP
Limeira Avenue, 901, Areião – Piracicaba
13414-903
São Paulo, Brazil
E-mail: casati@fop.unicamp.br

Clinical Relevance

Scientific rationale for the study: The treatment of proximal furcation involvements has presented unpredictable clinical results in both non-surgical and GTR therapy. The present study evaluates the EMD proteins as a therapeutic alternative to the treatment of proximal furcation involvements.

Principal findings: The association of OFD+24% EDTA conditioning +EMD led to similar reductions in PD, gain in vertical and horizontal attachment level and bone formation compared with the OFD. A significant rate of class-II to class-I furcation conversion was observed in the sixth month in the test group.

Practical implications: Because the only benefit achieved with the use of EMD application was the superior rate of conversion of class-II to class-I, long-term follow-up is required to confirm the better prognosis of these furcations and indicate the EMD as an alternative in the treatment of proximal furcations.

© 2008 The Authors
Journal compilation © 2008 Blackwell Munksgaard

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.