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Enamel matrix derivative proteins for the treatment of proximal class II furcation involvements: a prospective 24-month randomized clinical trial

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Abstract

Objective: This study aimed to evaluate the response of proximal furcations treated with enamel matrix derivative proteins (EMD) in a 24-month follow-up.

Materials and Methods: Twelve patients presenting bilateral class II proximal furcation with vertical probing depth (PD) $\geqslant$ 5 mm and bleeding on probing were selected. The furcations were assigned to: a control group ($n = 12$), open flap debridement (OFD)+EDTA and a test group ($n = 12$) – OFD+EDTA+EMD. The gingival margin position, PD, relative vertical and horizontal clinical attachment level (RVCAL and RHCAL), vertical and horizontal bone level (VBL and HBL) and furcation closure were evaluated before treatment and after 6, 12 and 24 months.

Results: After follow-up, no statistical difference could be seen between groups. At 24 months, the test group showed 1.9 $\pm$ 1.6 mm PD reduction whereas the control group showed 1.0 $\pm$ 1.3 mm PD reduction. RHCAL gains of the control and the test group were 0.7 $\pm$ 1.3 and 1.4 $\pm$ 0.9 mm, respectively. However, at 24 months, the test group only presented five remaining class II furcations *versus* 10 furcations in the control group ($p < 0.05$).

Conclusion: It could be concluded that EMD therapy promoted a reduction in the number of proximal furcations presenting a diagnosis of class II after 24 months of treatment compared with OFD therapy.

Key words: enamel matrix derivative; open flap debridement; proximal furcation; randomized clinical trial (RCT); regenerative procedures

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Enamel matrix derivative proteins (EMD) have shown significant improvements in the regenerative treatment of periodontal defects caused by periodontal disease. These proteins show the capacity to 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 (FGF2, TGF-β, 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, EMD proteins demonstrate good results in the treatment of angular defects, reducing probing depth (PD) levels and promoting new bone formation (Mellonig 1999, Parashis & Tsiklakis 2000, Parashis et al. 2004, Sculean et al. 2006). At the same time, EMD-treated furcation has still not been

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studied completely. Recently, Trombelli & Farina (2008), in an extensive literature review, evidenced that there are only a few randomized clinical trials (RCTs) that have evaluated the clinical potential of EMD proteins in mandibular furcation regenerative therapy, in which some conferred additional benefits to open flap debridement (OFD) and guided tissue regeneration (GTR) therapies (Jepsen et al. 2004, Meyle et al. 2004, Hoffmann et al. 2006, Chitsazi et al. 2007). Moreover, the improvements achieved with EMD therapy could be maintained over the years, as it could be seen in the long-term follow-up of infra-osseous and mandibular furcation defects treated with them (Heijl et al. 1997, Sculean et al. 2003, 2004, 2006, Francetti et al. 2004, Jepsen et al. 2004, Meyle et al. 2004, Heden & Wennström 2006, Hoffmann et al. 2006, Trombelli & Farina 2008).

Regarding proximal furcation, few studies have investigated alternative forms of regenerative treatments, because GTR has shown unpredictable results (Metzler et al. 1991, Pontoriero & Lindhe 1995, Avera et al. 1998). Recently, it was demonstrated that the EMD therapy could give rise to a superior rate of furcation closure when compared with OFD in a short-term follow-up (Casarin et al. 2008). However, to date, no studies have evaluated the clinical results of EMD therapy on proximal furcation over a period longer than 6 months.

Therefore, the aim of the present randomized, controlled, double-blind study was to evaluate the benefits of the use of EMD proteins in the treatment of proximal class II furcations in a 24-month follow-up study.

Materials and Methods

Study design

The study design has been described in detail previously (Casarin et al. 2008). Briefly, the present study was designed as a double-blind, randomized split-mouth prospective controlled clinical study to compare the clinical outcomes after OFD+ 24% EDTA root conditioning (control group) with OFD+ 24% EDTA conditioning+enamel matrix derivate protein application (test group). The study design was approved by the ethics committee of the University of Campinas (protocol number 006/2005). All patients received a detailed description of the proposed treatment and gave their informed and written consent.

Initially, in the previous study (Casarin et al. 2008), a total of 15 patients presenting bilateral defects were included in the per-protocol population study (all patients presented only a pair of defects). However, only 12 patients completed the follow-up of 24 months (all three patients dropped out because they did not comply with the supportive therapy). Therefore, in the following study, only the data of the 12 remaining patients are presented (representing 12 furcation defects in each group in a split-mouth design study).

Potential patients were selected from those referred to the Graduate Clinic of the Piracicaba Dental School. Inclusion and exclusion criteria were fully described in Casarin et al. (2008). Briefly, patients presenting (i) a diagnosis of Chronic Periodontitis (according to the criteria of the 1999 international classification; Armitage 1999); (ii) with the presence of at least one maxillary molar with proximal class II furcations, presenting PD $\geqslant$5 mm and bleeding on probing, after non-surgical therapy (Fig. 1); (iii) good general health; and (iv) age above 35 years were selected.

All patients received a full-mouth non-surgical periodontal treatment 6 months before the surgical procedure. At the same time, they underwent motivation sessions during which oral hygiene instructions were given to ensure that they could maintain a proper level of oral hygiene during the period of the study. During these sessions, a supragingival plaque control was performed to maintain the high status of hygiene and plaque control. Only patients who still presented class II furcation with a PD$\geqslant$5 mm and bleeding on probing were included.

Clinical parameters

The following clinical parameters were assessed immediately before the surgical procedure. The full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS) were calculated after dichotomously assessing the presence of plaque at the site or bleeding on probing from the bottom of the pocket when probing with a manual probe and calculating the percentage of total of sites that revealed the presence of plaque or bleeding. The presence of plaque or bleeding on probing was also dichotomously evaluated, especially at the site denominated for proximal furcation, in the study. These parameters were measured at six sites per tooth throughout 24 months.

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

Bone parameters were also evaluated in order to check the osseous level after treatment. The vertical bone level (VBL) was evaluated using a PCP-15 periodontal probe and the horizontal bone level (HBL) using the modified periodontal probe. To avoid the necessity of reentry surgery, the sites were

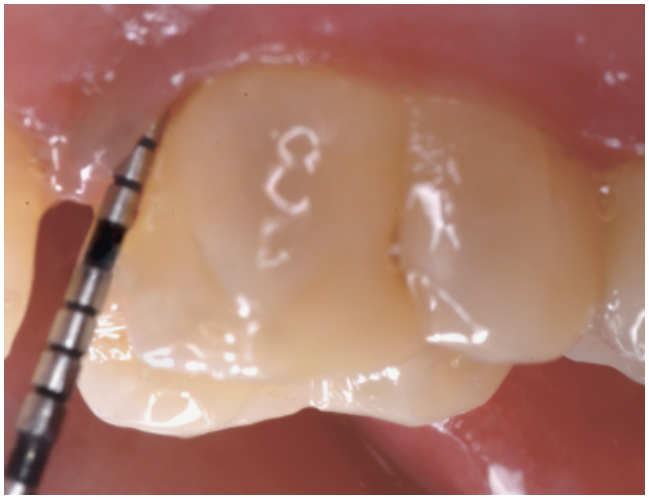

Fig. 1. Pre-operatory probing depth of proximal furcation before the surgical procedure.

anaesthetized and a bone sounding was performed (Suh et al. 2002).

Moreover, 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, by an individually determined groove made on a manufactured acrylic stent. The records consider the nearest 0.5 mm, from the stent margin, at baseline, 6, 12 and 24 months.

Investigator calibration

Initially, a total of 15 non-study patients presenting proximal class II furcations were selected. The designated examiner (R.C.V.C.) measured the RVCAL and RHCAL of all the patients twice, within 24 h, with at least 1 h between the examinations. The exam was judged to be reproducible after fulfilling the predetermined successful criteria (the percentage of agreement within $\pm$ 1 mm between repeated measurements had to be at least 90%). The intra-class correlation was calculated for each parameter, resulting in 91% reproducibility for RVCAL and 93% for RHCAL.

Surgical procedures

All surgical procedures were performed by the same clinician (E.D.P.R.). Before surgery, intra-oral antisepsis was performed with a 0.12% chlorhexidine rinse solution and extraoral antisepsis was carried out with iodine solution. Following the local anaesthesia, sulcular incisions were made and mucoperiosteal flaps were raised at buccal and palatine surfaces. Carefully, the tissue was reflected, preserving the maximum inter-proximal soft tissue. Granulation tissue, as well as the visible calculus, was removed using hand curettes (Gracey, Hu-Friedy) and an ultrasonic device (Cavitron, Dentsply, NY, USA) with specific tips for furcation scaling (UI25KFPset, Hu-Friedy). The diagnosis of the class II furcation defect was then confirmed (Fig. 2). At this point, a coin was tossed to determine the group of the site to be operated by an independent clinician (F.V.R.) not enrolled in the surgical procedures.

The test group sites were treated as follows: the root surfaces were conditioned using a 24% EDTA gel (PrefGel®, Straumann, Basel, Switzerland) for 2 min., followed by thorough rinsing with a sterile saline solution, as recommended by the manufacturer. Excess fluids were removed, leaving the surgical area clear, and the EMD (Emdogain®, Straumann, Basel, Switzerland) was then applied (Fig. 3). 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 primary wound closure, modified mattress sutures (5.0 Poligalactin-A; Vicryl, Johnson & Johnson, São José dos Campos, Brazil) were used (Fig. 4). The same sequence of events was carried out for the control group sites, excluding the EMD application.

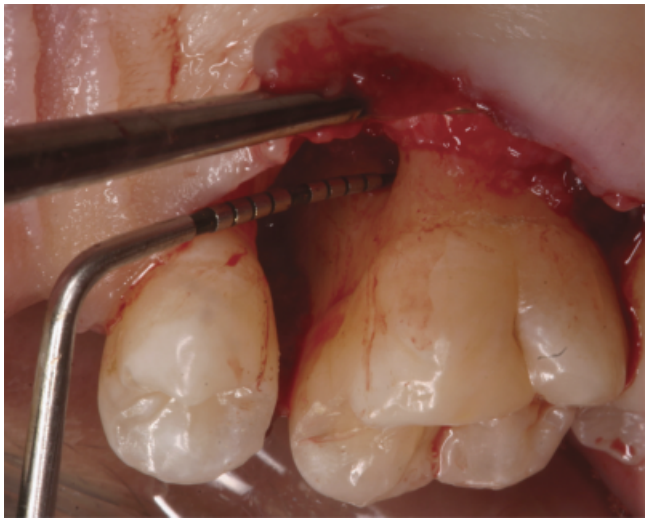

Fig. 2. Intra-operative view of the surgical site presenting class II proximal furcation.

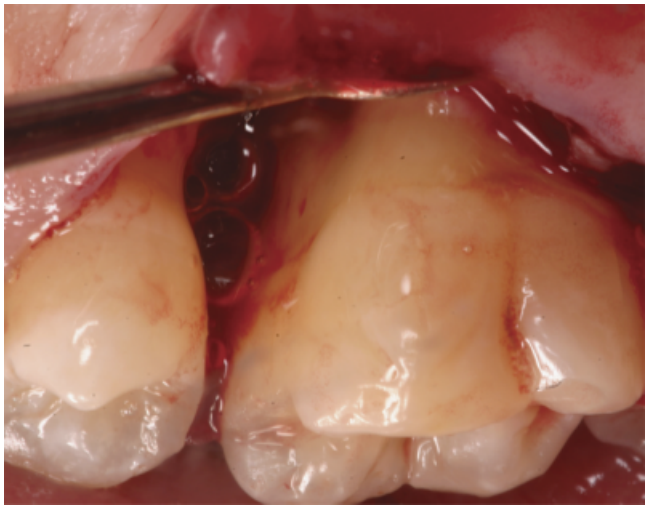

Fig. 3. Enamel matrix derivative (EMD) proteins' application.

Postoperative care

Patients were instructed to take analgesics (500 mg dipyrone, each 4 h for 2 days) 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% chlorhexidine twice a day for a month. The sutures were then removed at 10 days post-surgery.

Randomization and allocation concealment

The study followed a randomized and split-mouth design and included a blinded examiner, i.e., both treatments were used in the same patient. In order

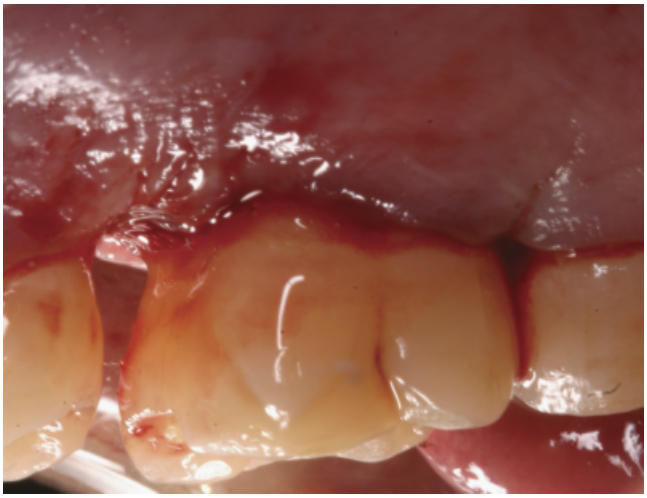

Fig. 4. Immediate post-operatory clinical appearance.

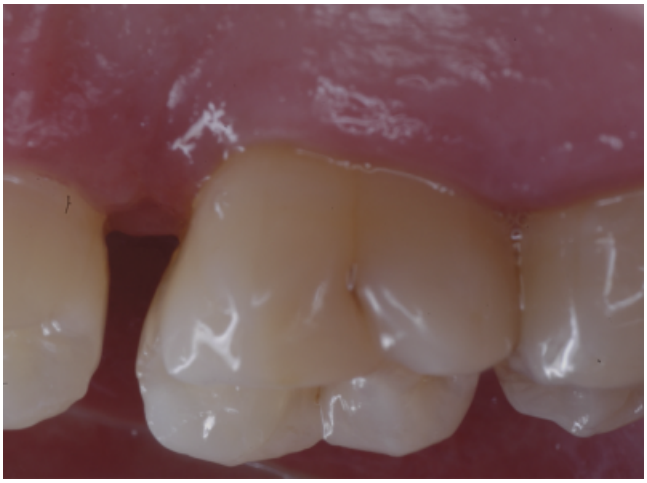

Fig. 5. Clinical appearance after 6 months.

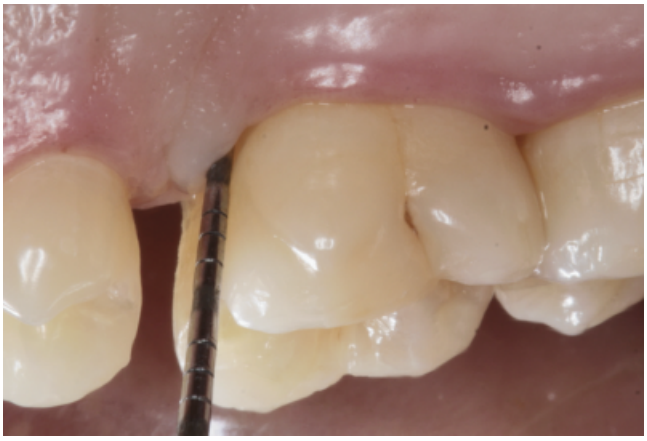

Fig. 6. Probing depth at the proximal furcation site after 24 months.

to randomize the treatment, a previous toss was carried out to determine the surgical side. After furcation debridement, a new coin toss determined the treatment to be performed. In this way, the second furcation defect received the other treatment. No patient presented more than two furcation defects. The coin toss was performed by an operator different (F.V.R.) 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, the clinical examiner or the statistician.

Re-assessment evaluations

Re-assessment visits occurred every 15 days during the first month and monthly until the 6th month. After this period, visits were scheduled every 6 months (Figs 5–7). During these appointments, the examiner recorded the clinical periodontal parameters and checked any change in the medical or the health status. At the end of the appointment, a session of supragingival prophylaxes was performed.

Primary and secondary outcome measures

The primary outcome measurement of the study was RHCAL. The secondary outcomes included (i) HBL; (ii) VBL and RVCAL; (iii) PD and position of the gingival margin; (iv) furcation closure at 6 months; (v) plaque and bleeding on probing at the surgical site; and (vi) full-mouth plaque index and bleeding on probing.

Power calculation

Using SAS 9.1 program (SAS Institute Inc., Cary, NC, USA, Release 9.1, 2003), the sample size was determined considering the literature data, to obtain a minimum power value of 0.8. After data collection, a *post hoc* power was determined, considering the standard deviation of each parameter in each period of evaluation. A difference of 2.0 mm between groups was considered as clinically significant. The power values achieved ranged between 0.71 and 0.87 (VBL and HBL parameters, respectively). The primary variable, RHCAL, presented 0.83 power value.

Data management and statistical analysis

Initially, three populations were considered. The safety population enrolled received at least one treatment. From this safety population, subjects who met all the inclusion criteria (intention-to-treat population, ITT) were selected.

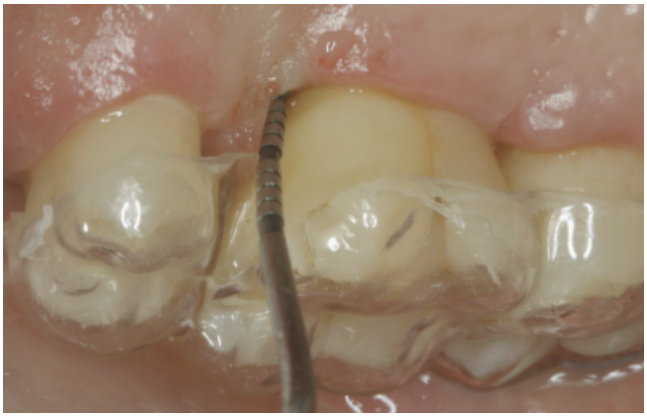

Fig. 7. Relative horizontal clinical attachment evaluation after 24 months, performed using modified probes. The acrylic stent facilitates the probe insertion.

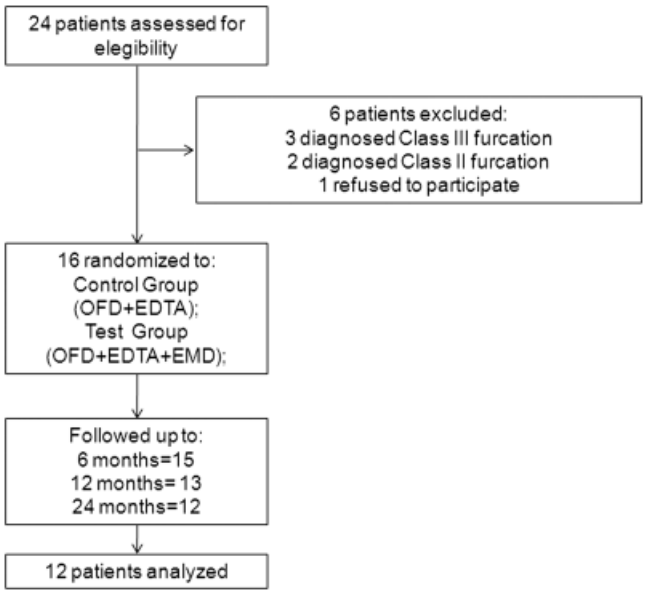

Fig. 8. Flowchart of the study.

The subjects from the ITT population who completed the follow-up and who fulfilled 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 12 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 was used.

The homogeneity of groups at baseline (PD and RHCAL) was tested using the Student's *t*-test. Repeated-measures analysis of variance (ANOVA) was used to detect intra- and inter-group differences in the clinical parameters (GMP, PD, RVCAL, RHCAL, VBL and HBL). When a statistical difference was found, analysis of the difference was determined using the method of Tukey, considering the patient as the experimental unit. The Friedman test was used to detect intra-group differences in the full-mouth plaque and bleeding index among all periods. The number of furcation sites with or without plaque or bleeding during probing was analysed using the McNemar test (intra- and inter-group analysis). According to the Hamp et al. classification (1975), furcation lesions were quantified and compared by an inter-group analysis using the McNemar test. An experimental level of significance was determined at 5% for all statistical analyses ($\alpha = 0.05$)

Results

Subject accountability

Figure 8 illustrates the study flowchart. Twenty-four patients were initially recruited at the beginning of the study. From these, six subjects were excluded: three because they presented class III furcations (through-to-through), confirmed at the surgical procedure, two because they presented class I furcations (horizontal loss < 3 mm) and one because he refused to participate in the study (did not accept the 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 the participants underwent the allocated procedure; however, one patient was lost during the 6-month follow-up due to the administration of antibiotic medication. Three other patients were lost during the 6–24-month follow-up because they did not comply with SPT. All other subjects were included in the statistical analysis. Thus, the final composition of the study population was 12 patients presenting bilateral defects.

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 24-month follow-up visits were completed in June 2008. Data entry of all information and statistical analysis were performed by the end of August 2008.

Patient characteristics at baseline

Patients' characteristics at baseline were not significantly different between groups, as can be seen in Table 1. The mean age was 51.6 ± 8.8 years, and the majority comprised females (75%) and Caucasians (75%). Acceptable oral hygiene was achieved before the study, as seen from the FMPS and FMBS (20.9 ± 2.0 and 16.4 ± 3.1, respectively) at baseline. The mean PD at furcation and RHCAL at furcation

Table 1. Patient characteristics at baseline

Age (mm ± dp)	51.6 ± 8.8
Female	75.0% ($n = 9$)
Caucasian	75.0% ($n = 9$)
Full-mouth bleeding score (%)	16.4 ± 3.1
Full-mouth plaque score (%)	20.9 ± 2.0

	Control Group	Test Group
Furcation surface distribution		
Mesial	2	2
Distal	10	10
PD at furcation site (mm ± dp)*	6.4 ± 1.2	6.4 ± 1.3
RHCAL at furcation site (mm ± dp)*	10.5 ± 1.4	10.0 ± 1.1

*Student's *t*-test ($p > 0.05$).

PD, probing depth; RHCAL, relative horizontal clinical attachment level.

Table 2. Percentage of full-mouth plaque score (FMPS), full-mouth bleeding score (FMBS), plaque and bleeding values at the surgical site at the different assessment times

	Baseline	6 months	12 months	24 months
FMPS (%)*	20.9 ± 2.0	18.1 ± 3.3	22.4 ± 6.0	21.6 ± 4.8
FMBS (%)*	16.4 ± 3.1	16.2 ± 1.9	20.6 ± 4.0	17.6 ± 4.2
Plaque at the surgical site				
Control	0% (0)	25% (3)	33% (4)†	42% (5)†
Test	0% (0)	17% (2)	25% (3)†	25% (3)†
Bleeding at the surgical site				
Control	100% (12)	50% (6)†	50% (6)†	67% (8)†
Test	100% (12)	50% (6)†	42% (5)†	33% (4)†

*Friedman test ($p > 0.05$).

†Intra-group statistical difference from baseline, $p < 0.05$, McNemar test.

Table 3. Means (± SD) of gingival margin position (GMP), probing depth (PD), and relative vertical clinical attachment level (RVCAL), relative horizontal clinical attachment level (RHCAL) at the times of evaluation

	Baseline	6 months	12 months	24 months	Baseline–24-months difference
GMP					
Control	2.0 ± 0.9	3.5 ± 1.0*	3.3 ± 0.9*	3.0 ± 1.0*	*− 1.0 ± 1.2*
Test	2.5 ± 1.1	3.6 ± 1.4	3.3 ± 1.3*	3.6 ± 1.0*	*− 1.0 ± 1.1*
PD					
Control	6.4 ± 1.2	4.6 ± 0.7*	5.1 ± 0.9*	5.4 ± 0.8*	*1.0 ± 1.3*
Test	6.4 ± 1.3	4.5 ± 1.2*	5.0 ± 1.3*	4.5 ± 0.8*	*1.9 ± 1.6*
RVCAL					
Control	8.4 ± 1.1	8.0 ± 1.0*	8.4 ± 0.7*	8.4 ± 0.9*	*0.2 ± 1.0*
Test	8.9 ± 1.3	8.2 ± 1.0*	8.3 ± 1.5*	8.2 ± 1.2*	*0.8 ± 1.4*
RHCAL					
Control	10.5 ± 1.4	8.8 ± 1.3*	9.5 ± 0.7*	9.8 ± 1.4*	*0.7 ± 1.3*
Test	10.0 ± 1.1	8.9 ± 1.4*	9.4 ± 1.4*	8.7 ± 0.9*	*1.4 ± 0.9*

*Intra-group statistical difference from the baseline (ANOVA/Tukey). The numbers in italic refer to the difference between baseline and 24 months values.

were also not statistically different between groups at baseline (6.4 ± 1.2 and 6.4 ± 1.3 and 10.5 ± 1.4 and 10.0 ± 1.1 for the control and the test groups, respectively). Mesial and distal furcations for each group show an equal distribution, with no significant difference between groups.

Treatment assessments

Plaque index and bleeding scores

The means of the FMPS and FMBS, as well as the percentages of presence of plaque and bleeding at the surgical site are shown in Table 2. The plaque and bleeding scores were maintained at an average of 20% throughout the study, without a difference among periods. The percentage of the presence of plaque at the surgical site was 0% for both groups. During the long-term follow-up, the presence of plaque in the surgical site increased, and after 24 months, both groups presented a statistically higher frequency of biofilm presence in the surgical area ($p < 0.05$).

The values for bleeding at the surgical site demonstrated a decrease in its occurrence after 6 months in both groups ($p < 0.05$), which was maintained throughout the study.

Clinical parameters

The values of the clinical variables are displayed in Table 3. As can be seen, a similar gingival recession was observed ($p < 0.05$), through the months, in both groups, without a difference between them during any period of evaluation ($p > 0.05$). The values of gingival recession at 24 months were − 1.0 ± 1.2 and − 1.0 ± 1.1 mm, respectively. Regarding PD reduction, although a numerical difference could be seen, no statistical difference was observed between the control and the test group (1.0 ± 1.3 and 1.9 ± 1.6 mm in the control and the test group, respectively).

Both groups presented a slight gain in RVCAL through the periods of evaluation, without a difference between groups. Concerning RHCAL, both treatments promoted gain during 24 months of follow-up ($p < 0.05$) and no statistical difference was observed between groups (0.7 ± 1.3 and 1.4 ± 0.9 mm for the control and the test group – $p > 0.05$).

VBL and HBL

Both treatments did not promote significant vertical gain of bone (Table 4). At the same time, they promoted similar gain in the HBL. At the 24-month evaluation, the control group showed a statistical inter-radicular bone gain of 0.6 ± 1.7 mm, whereas the test group showed a 1.0 ± 1.2 mm gain. However, no difference was observed between groups.

Furcation closure

Figure 9 shows the furcation's reclassification at 6 and 24 months following the Hamp et al. classification (1975). As can be seen in the graphic, a statistical

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

	Baseline	6 months	12 months	24 months	Baseline–24-month difference
VBL					
Control	10.2 ± 1.1	9.4 ± 1.4	9.5 ± 0.7	9.5 ± 0.9	*0.5 ± 0.9*
Test	9.9 ± 1.2	9.4 ± 1.3	9.7 ± 1.9	9.5 ± 1.3	*0.6 ± 1.2*
HBL					
Control	11.6 ± 1.7	10.2 ± 1.8*	10.2 ± 0.8*	10.7 ± 1.6*	*0.6 ± 1.7*
Test	10.7 ± 1.2	10.2 ± 1.5*	9.8 ± 1.7*	9.7 ± 1.6*	*1.0 ± 1.2*

*Intra-group statistical difference from baseline ($p<0.05$) – ANOVA/Tukey. The numbers in italic refer to the difference between baseline and 24 months values.

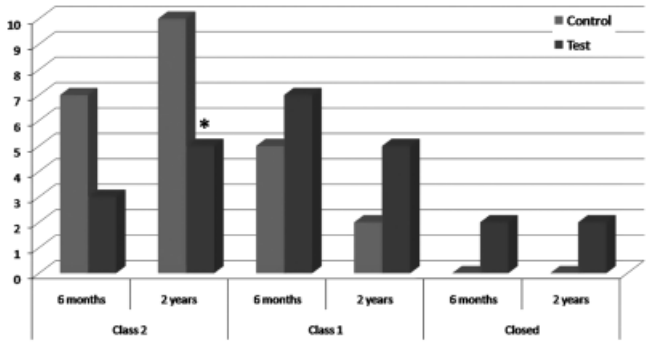

Fig. 9. Classification of furcation defects at 6 and 24 months post-treatment. *Inter-group statistical difference (Fischer's McNemar test – $p<0.05$).

difference was observed between groups in the number of remaining class II furcation at 24 months ($p<0.05$). In the control group, 10 furcations were still diagnosed as class II furcation *versus* 5 furcations in the test group. At the same time, the number of furcations that became class I or closed furcation was superior in the test group both at 6 and 24 months. The test group presented two closed furcations and five Class I lesions at 24 months, whereas only two defects showed an improvement in status (class II to class I) after follow-up in the control group. However, no statistical significance was achieved between groups.

Discussion

EMD proteins have been used as a predictable technique in order to achieve periodontal regeneration of the lost periodontal tissues due to periodontitis. Several articles were published corroborating that EMD therapy could be useful for the treatment of infra-osseous and mandibular furcation defects. However, regarding proximal furcation defects, the data were scarce. In 2008, our group showed the 6-month follow-up results of EMD therapy in the treatment of maxillary proximal furcation (Casarin et al. 2008). OFD+EMD showed a superior rate of furcation closure than OFD-treated furcations. However, different from infra-osseous and mandibular defect treatment, to date, no long-term study in the literature has evaluated the additional benefits of EMD therapy.

In the present study, at 2 years of follow-up, the application of an EMD indicated an improvement in periodontal support in proximal furcations. EMD-treated furcations presented less remaining class II furcation than the control group. Moreover, total closure was only observed in the test group (2 defects). Considering other clinical and osseous parameters, no statistical difference could be seen between the OFD and the EMD groups.

Previous studies evaluating the long-term results of EMD therapy also demonstrate a possibility of maintaining the benefits achieved in a short-term follow-up. Sculean et al. (2007, 2008), Francetti et al. (2004), Parodi et al. (2004), Heijl et al. (1997) and Heden & Wennström (2006), studying EMD therapy in the treatment of infra-osseous defects, found PD reduction, gain in clinical attachment level and new bone formation, and these remained stable during a follow-up of 2, 3, 4, 5 and 10 years.

Considering furcation defects, only a few studies have evaluated EMD therapy in a period longer than 6 months. In a series of studies, Meyle et al. (2004), Jepsen et al. (2004) and Hoffmann et al. (2006) have shown the benefit of EMD therapy in mandibular furcation defects after 14 months. The authors showed that EMD led to regenerative results (especially HBL improvement) that were superior to the membrane (GTR) procedure, besides the fact that patients preferred this treatment, because of fewer post-surgical adverse effects. Moreover, the authors investigated the patient-related factors that could modify the response of the therapy, showing that age, smoking, gender and hygiene status could interfere with the results. In a case series long-term follow-up, Donos et al. (2003) demonstrated that significant improvements could be achieved after 36 months of EMD therapy in mandibular furcations, including gain of horizontal and vertical clinical attachment. In the present study, the benefits of EMD therapy were maintained throughout the 24 months of follow-up, corroborating those previous studies. However, some specificities regarding proximal furcations should be considered.

A point that should be discussed regarding this lesion is the difficulty in maintaining proper oral hygiene and plaque control in surgical sites. As could be seen in our results, after a 6-month follow-up, when a 6-monthly plaque control session was scheduled (instead of a monthly control during the first 6 months), both groups showed accumulation of plaque in the surgical sites. It is important to note that this occurred in spite of adequate full-mouth plaque control maintenance (as can be seen in Table 2). Some factors could have contributed to this phenomenon. The anatomic features of furcation lesions, the posterior location in the arch and the inter-proximal position of the furcation's entrance could have interfered with daily oral hygiene. This location impedes adequate hygiene, increasing biofilm accumulation (Nordland et al. 1987), and also reducing the effectiveness of the periodontal treatment (Svardström & Wennström 1996) and regenerative therapy (Hoffmann et al. 2006). Thus, for proximal defects, a more strict supportive periodontal therapy might be indicated.

Therefore, in spite of this, some additional benefits could be attributed to EMD therapy. A higher rate of furcation closure was observed in the test group when compared with the OFD group. Studies comparing the clinical benefits of EMD *versus* OFD also found significant benefits of a regenerative therapy. Recently, Sculean et al. (2008) presented the results of 10 years of follow-up comparing EMD therapy with RTG, RTG associated with EMD and OFD for the treatment of infra-osseous defects. Their results show that all regenerative procedures led to statistically significantly higher CAL gain, without a difference between them. Similar results were also observed by other authors, in different follow-up periods, showing the benefits of EMD therapy compared with OFD alone in the treatment of infra-osseous and mandibular furcation defects (Tonetti et al. 2002, Esposito et al. 2005, Needleman et al. 2006, Chitsazi et al. 2007).

EMD has shown the capacity to promote several processes in the periodontal regeneration (cell proliferation and increased protein synthesis, mineral nodule formation, mineralized matrix formation, as well as the release of growth factors) and an antimicrobial action, reducing pathogenic flora, favouring 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, Walter et al. 2006, Bosshardt 2008). However, it should be noted that, despite these characteristics, only two of 12 furcations presented total closure after EMD application.

Studies examining the influence of anatomic factors on regenerative results could be helpful in explaining this result. Anatomic features such as the distance of root furcation and bone crest, root divergence, horizontal depth of defect and other conditions of mandibular furcations and bone anatomy could modify the regenerative response (Bowers et al. 2003, Tsao et al. 2006). Recently, the influence of bone and defect anatomy was also demonstrated in proximal furcations treated with EMD (Casarin et al. 2009). In this context, more studies should be carried out to achieve the better anatomic condition should be done aiming to increase the proximal furcation treatment predictability.

The benefits of total or partial closure of furcation have been pointed out in previous studies (McGuire & Nunn 1996a, b). This could represent an improvement in the prognosis of teeth presenting furcation lesions. However, more longitudinal follow-up studies, especially for proximal furcation, should be carried out before arriving at a conclusion. Moreover, other therapies that could improve the rate of furcation closure should be tested. A systematic review has shown that furcation lesions' treatment presented better results when bone grafting was associated with the regenerative procedure (Murphy & Gunsolley 2003). Giusto (2001) and Rosen et al. (1997) combined bone grafts with RTG therapy in the treatment of proximal furcation lesions. Both serial cases showed total closure of treated furcations. However, this possible benefit of bone graft associated with EMD should be tested in future randomized clinical studies.

The data obtained in the present study should be interpreted with caution. The major limitation of our study is the small sample size. Although other longitudinal studies also had a sample size similar to that in the present study (Francetti et al. 2004, Sculean et al. 2004, 2007, 2008), the low power value could give rise to an error when considering the data. There is a possibility of non-indication of a statistical significance when it actually exists. Moreover, the use of coin toss as a randomization method, although it is considered a simple (unrestricted) randomization method, showing unpredictability and bias prevention, presents some limitations, especially in a small sample (Schulz & Grimes 2002). Hence, other methods could be implemented in future trials. Finally, it should be considered that the present study is the first to evaluate EMD therapy in proximal furcation. Therefore, more studies with different and larger populations should be conducted in order to confirm the results of the present study.

In conclusion, although several new approaches are constantly appearing in the literature to treat the different aspects of periodontal disease, proximal furcations still remain a clinical challenge. This class of lesions presents an unpredictable 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 EMD proteins as a therapeutic alternative for the treatment of proximal furcation involvements in a 24-month follow-up, showing that this therapy could yield additional benefits in the regenerative treatment when compared with OFD alone.

Conclusion

In conclusion, the present clinical trial showed that the application of EMD proteins in proximal furcations promoted a reduction in the number of remaining class II furcations when compared with OFD associated with 24% EDTA in a 24-month follow-up.

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Clinical Relevance

Scientific rationale for the study: A recent clinical trial evaluating the benefit of the treatment of proximal furcation involvements with an EMD has presented a superior rate of furcations with some degree of closure when compared with OFD alone. However, no study has carried out a long-term follow-up. Thus, the present study evaluated the results after 24 months of treatment.

Principal findings: Clinical and osseous parameters did not demonstrate additional benefits in EMD application on proximal furcations. However, after 24 months of follow-up, the association of OFD+ 24% EDTA conditioning+EMD led to a higher rate of furcation closure than OFD+ 24% EDTA conditioning alone. Lesser number of lesions still presented a class II diagnosis in the test group compared with the control group, with a statistically significant difference between groups.

Practical implications: Within the limits of the present study, EMD therapy appeared to yield higher improvements in terms of horizontal parameters in a 24-month follow-up. It could represent an improvement in the prognosis of teeth over the years (McGuire & Nunn 1996a, b). However, caution should be exercised when considering the results. Larger and different populations and a long-term follow-up are necessary to clarify and confirm the additional benefits.