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ORIGINAL ARTICLE

Enamel matrix protein derivative and/or synthetic bone substitute for the treatment of mandibular class II buccal furcation defects. A 12-month randomized clinical trial

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Abstract
Objective This study aims to clinically evaluate the treatment of mandibular class II furcation defects with enamel matrix derivative (EMD) and/or a bone substitute graft made of β-tricalcium phosphate/hydroxyapatite (βTCP/HA).
Materials and methods Forty-one patients, presenting a mandibular class II buccal furcation defect, probing pocket depth (PPD) ≥4 mm and bleeding on probing, were included. They were randomly assigned to the groups: 1—EMD (n=13); 2—βTCP/HA (n=14); 3—EMD+βTCP/HA (n=14). Plaque index (PI), gingival index (GI), relative gingival margin position (RGMP), relative vertical and horizontal attachment level (RVCAL and RHCAL), and PPD were evaluated at baseline and 6 and 12 months. The mean horizontal clinical attachment level gain was considered the primary outcome variable.
Results No significant intragroup differences were observed for RGMP, but significant changes were observed for RVCAL, RHCAL, and PPD for all groups ($p<0.05$). After 12 months, the mean horizontal clinical attachment level gain was 2.77±0.93 mm for EMD, 2.64±0.93 mm for βTCP/HA, and 2.93±0.83 mm for EMD+βTCP/HA, with no significant differences among the groups. At the end of the study, 85.3 % of the sites were partially closed; however, no complete closure was observed.
Conclusion EMD+βTCP/HA does not provide a significant advantage when compared to the isolated approaches. All three tested treatments promote significant improvements and partial closure of class II buccal furcation defects. Based on its potential to induce periodontal regeneration, EMD may be considered an attractive option for this type of defect, but complete closure remains an unrealistic goal.
Clinical relevance The partial closure of buccal furcation defects can be achieved after the three tested approaches. However, the combined treatment does not provide a significant benefit when compared to the isolated approaches.

Keywords Periodontal disease · Randomized controlled trial · Enamel matrix protein · Hydroxyapatite-beta tricalcium phosphate · Furcation defects

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Introduction

Several methods have been used to treat furcation defects, such as non-surgical scaling and root planing with manual and power-driven scalers, open flap debridement, resective treatment, and regenerative approaches [1]. Previous studies have reported good results when guided tissue regeneration (GTR) was used to treat mandibular class II furcation defects, achieving statistically greater clinical improvement when compared with non-regenerative procedures [2–4].

Enamel matrix derivative proteins (EMD) have been clinically used as an attempt to reproduce the events that occur during cementogenesis, in which amelogenin, secreted by Hertwig's epithelial root sheath (HERS), induces the formation of acellular extrinsic fiber cementum, periodontal ligament, and alveolar bone. It may promote periodontal ligament cell proliferation and increased protein synthesis and mineral nodule formation by the cells. Another interesting effect of EMD would be the reduction in the local pathogenic flora, creating a more favorable environment for periodontal regeneration [5–10].

Considering the treatment of furcation defects, the results obtained with EMD application may be comparable to those achieved with GTR [11, 12] and superior to what have been reported with open-flap debridement [13] for clinical attachment level, vertical and horizontal bone levels, and resolution of the furcation defect [14]. However, the complete closure of furcation defects does not seem to be a commonly attainable outcome with the present treatment options. Jepsen [11] showed that complete closure of mandibular furcation treated with EMD was observed in 17 % of the sites compared to 8 % with GTR.

Bone replacement grafts (bone grafts and bone graft substitutes) may provide the structural framework for clot stabilization, maturation, and remodeling that supports bone formation in osseous defects [15]. Both hydroxyapatite (HA) and β-tricalcium phosphate (βTCP) are widely employed materials in dental and periodontal surgery. They are known to be osteoconductive and to enhance bone filling in periodontal defects [16, 17].

It can be hypothesized that the association of EMD with a bone substitute might improve the clinical results in furcation lesions. Thus, the aim of this clinical trial is to evaluate the treatment of mandibular class II furcation defects with the association of enamel matrix derivative (EMD) and a bone substitute graft made of β-tricalcium phosphate/hydroxyapatite (βTCP/HA) when compared to the isolated use of each material.

Materials and methods

Study design

This is a randomized, parallel, blinded, clinical trial designed to evaluate the treatment of mandibular class II furcation defects with enamel matrix derivative (EMD) along with a bone substitute graft (βTCP/HA) when compared to the isolated therapies. The study protocol has been approved by the Ethics Committee of the Piracicaba Dental School, State University of Campinas—UNICAMP (#035/2007). All patients received a detailed description of the proposed treatment and gave their written informed consent.

The patients were randomly assigned to the following treatment groups:

Group 1: During flap access surgery, the granulation tissue was removed and the root surfaces were carefully instrumented with ultrasonic and hand instruments. The furcation defects in this group received the application of enamel matrix derivative (EMD—Emdogain®; Straumann, Basel, Switzerland).

Group 2: During flap access surgery, the granulation tissue was removed and the root surfaces were carefully instrumented with ultrasonic and hand instruments. The furcation was filled with a bone substitute consisting of β-tricalcium phosphate/hydroxyapatite (βTCP/HA—Bone Ceramic®; Straumann, Basel, Switzerland)

Group 3: During flap access surgery, the granulation tissue was removed and the root surfaces were carefully instrumented with ultrasonic and hand instruments. The furcation was filled with a mixture of enamel matrix derivative proteins (EMD) (Emdogain®; Straumann, Basel, Switzerland) and bone substitute consisting of βTCP/HA (Bone Ceramic®; Straumann, Basel, Switzerland). Immediately after debridement, EMD was applied on the root surfaces. The remaining part of the material in the syringe was then mixed with the bone substitute on a sterile dappen. This mixture was used to completely fill the defect (EMD+βTCP/HA).

Randomization and allocation concealment

The treatment for each buccal furcation defect was determined during the surgical procedure (after flap elevation and root/defect debridement) using a previously made computer-generated list and opaque envelopes. The allocation concealment was conducted by a researcher (MPS) not involved in the examinations and surgical procedures. The randomization code was not broken until all data had been collected and fully statistically analyzed. Therefore, the patient, the clinical examiner, and the statistician were not aware about the treatment group during the study procedures.

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 anamnesis, a full-mouth periodontal probing, and radiographic examination. One furcation defect per patient was chosen. The furcation defects were classified on a three-stage scale [18] using a Nabers Probe (Hu-Friedy). The study inclusion criteria were (1) males and females, age ≥35 years and with the diagnosis of moderated chronic periodontitis [19]; (2) presence of a mandibular molar with buccal class II furcation defect, presenting horizontal PD ≥4 mm, bleeding on probing (BOP), minimum (<1 mm) or no gingival recession after non-surgical therapy; (3) good general health; (4) at least 2 mm of keratinized tissue; and (5) minimum interproximal bone loss (<2 mm). The patients who presented the following conditions were excluded from the study: (1) were pregnant or lactating; (2) required antibiotic premedication for the performance of periodontal examination and treatment; (3) suffered from any other systemic diseases (cardiovascular, pulmonary, liver, cerebral, diseases, or diabetes); (4) had received antibiotic treatment in the previous 3 months; (5) were taking long-term anti-inflammatory drugs; (6) had received a course of periodontal treatment within the last 6 months; (7) with non-vital/endodontically treated experimental tooth; and (8) were smokers.

Non-surgical treatment

All the subjects received a full-mouth periodontal treatment before the surgical procedure. The same operator performed the treatment at the furcation sites with an ultrasonic device (Cavitron; Dentsply, NY, USA) and specific tips for furcation debridement (PQ2N7; Hu-Friedy, Chicago, IL, USA). The subjects underwent motivation sessions, during which oral hygiene instructions were given to ensure proper level of oral hygiene before the surgical procedure. These sessions were repeated as needed, with special attention for the furcation areas, until subjects showed the ability to maintain good plaque control, as evidenced by pre-treatment plaque and bleeding scores of 20 % or less.

Clinical parameters

The following clinical parameters were assessed immediately before the surgical procedure. Full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS) were calculated after assessing dichotomously the presence of plaque and BOP (from the bottom of the pocket when probing with a manual probe) and calculating the percentage of positive sites. The presence of plaque and BOP was also dichotomously evaluated at the buccal furcation site included in the study. Probing depth (PD), relative gingival margin position (RGMP), 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 measured with the same type of probe (PCP-15 Periodontal Probe; Hu-Friedy, Chicago, IL, USA) as the distance between the deepest point reached by the probe when introduced horizontally into the

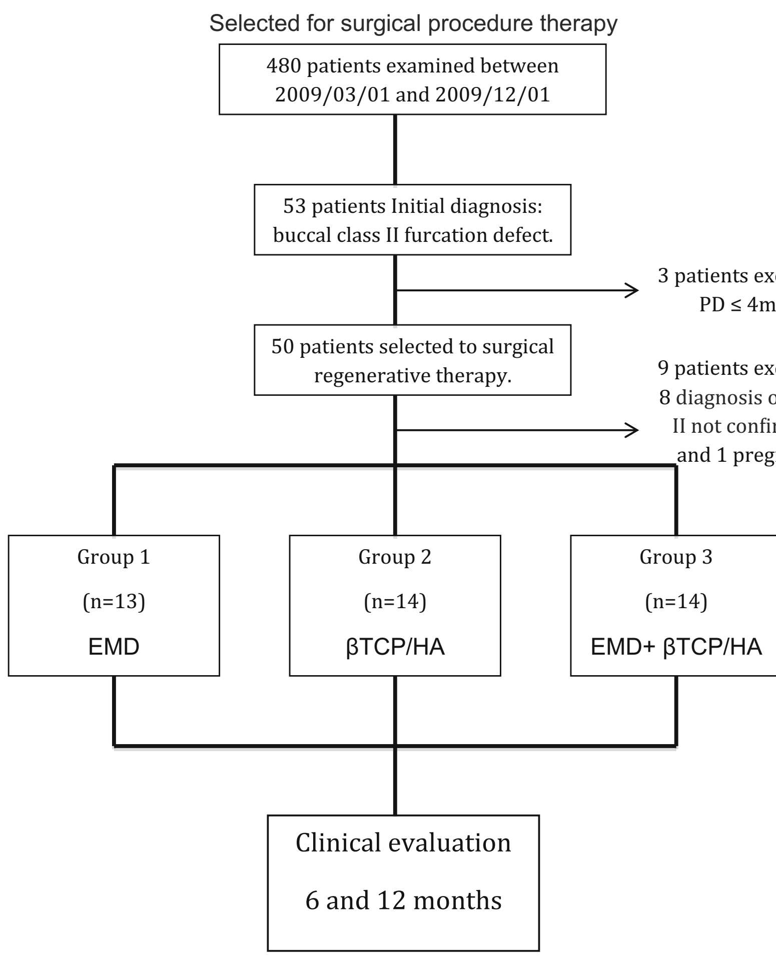

Fig. 1 Study flowchart. *HA* hydroxyapatite, *β-TCP* β-tricalcium phosphate, *EMD* enamel matrix derivative

Table 1 Patient characteristics at baseline

	Group 1	Group 2	Group 3
Age (mean±SD)[a]	53.69±6.58	53.14±5.92	54.57±5.63
Female, *n* (%)	8 (61.54)	10 (71.43)	9 (64.29)
Male, *n* (%)	5 (38.46)	4 (28.57)	5 (35.71)
PPD at furcation site, mm (±SD)[a]	4.92±0.49	4.93±0.73	4.79±0.7
RHCAL at furcation site, mm (±SD)[a]	5.08±0.49	5.64±0.93	5.5±0.76

[a] No statistically significant difference at baseline between groups (ANOVA/Tukey test, $p>0.05$)

furcation and the lower border of the stent. All these parameters were evaluated at one specific site at the buccal furcation entrance, determined by a groove made on an individually manufactured acrylic stent and recorded to the nearest 0.5 mm. The assessments of RGMP, PD, RVCAL, and RHCAL were performed at baseline and 6 and 12 months after the surgical procedure by one blinded trained examiner (L.A.Q.).

Investigator calibration

Initially, a total of 15 non-study subjects presenting buccal class II furcations were selected. The designated examiner measured the RVCAL and RHCAL of all patients, twice, within a period of 24 h (observing an interval of more than 1 h between examinations). The examiner was judged to be reproducible after fulfilling the pre-determined success criteria (the percentage of agreement within ±1 mm between repeated measurements had to be at least 90 %). The intra-class correlation was calculated to each parameter, resulting in 90 % reproducibility for RVCAL and 91 % for RHCAL.

Surgical procedures

All the surgical procedures were performed by the same experienced surgeon (E.A.S.). Before surgery, intraoral antisepsis was performed with 0.12 % chlorhexidine rinse solution and extraoral antisepsis was carried out with iodine solution. Following local anesthesia, sulcular incisions were made, and full-thickness flaps were raised at the buccal surface of the experimental sites, extending to the two adjacent teeth (or ridge, if the adjacent tooth was absent). Granulation tissue as well as the visible calculus on the root surface (if present) was removed with hand curettes (Gracey; Hu-Friedy) and with an ultrasonic device (Cavitron; Dentsply, Tulsa, OK, USA) with specific tips for furcation instrumentation (UI25KFPset; Hu-Friedy). The diagnosis of the class II furcation defect was then confirmed using a Nabers probe (Hu-Friedy). At this point, the opaque envelope was open and the defect could be assigned to one of the following procedures: EMD (Emdogain®; Straumann, Basel, Switzerland) application on the root surfaces (group 1); βTCP/HA graft (Bone Ceramic®; Straumann, Basel, Switzerland) was used to fill the furcation defect (group 2); EMD (Emdogain®; Straumann, Basel, Switzerland) along with βTCP/HA (Bone Ceramic®; Straumann, Basel, Switzerland) was used following the protocol: EMD was applied on the root surfaces, then a few drops of EMD were mixed with βTCP/HA, and the combination of the two materials filled the entire defect (group 3). The surgical flaps were positioned slightly coronally and sutured using modified mattress sutures (5.0 polygalactin-A, Vicryl; Johnson & Johnson, São José dos Campos, Brazil) in order to completely cover the defects (Figs. 4, 5, and 6). No side effects were seen in any of the three groups.

Postoperative care

Patients were instructed to take analgesics (500 mg of dipyrone, four times a day) for 3 days and to discontinue tooth brushing around the surgical sites for 10 days after surgery. They were instructed to gently rinse with 0.12 % chlorhexidine, twice a day for a month. The sutures were removed at 10 days post-surgery, and the clinical parameters analyzed after 6 and 12 months. Patients were enrolled in a periodontal maintenance program during the study period. The maintenance visits occurred once a month at the first 6 months and every 3 months until the end of the study.

Primary and secondary outcome measures

The primary outcome measurement of the study was considered to be the mean horizontal clinical attachment level gain (difference between RHCAL at baseline and the values

Table 2 Full mouth plaque score and full mouth bleeding score at baseline for treatment groups (%)

	Group 1	Group 2	Group 3
FMPS % (±SD)[a]	14.57±3.83	14.36±3.1	13.54±2.99
FMBS % (±SD)[a]	9.67±3.1	9.68±2.76	8.69±1.84

FMPS full mouth plaque score, *FMBS* full mouth bleeding score, *SD* standard deviation, *Group 1* EMD, *Group 2* βTCP/HA, *Group 3* EMD+βTCP/HA

[a] No statistically significant difference at baseline between groups (ANOVA/Tukey test, $p>0.05$). No significant difference (Fisher's exact test, $p>0.05$)

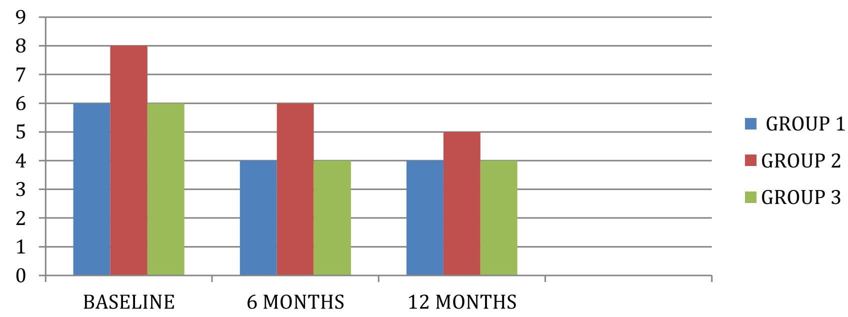

Fig. 2 Number of experimental furcation sites with detectable plaque during the study for each treatment group. No significant difference (Fisher's exact test, $p>0.05$)

observed at 6 and 12 months). Secondary outcomes included (1) PD, (2) RVCAL, (3) RPGM, (4) complete furcation closure at 12 months, (5) plaque and BOP at surgical site, and (6) full-mouth plaque index (PI) and full-mouth gingival index (GI).

Power calculation

The sample size was calculated using the software package Biostat 5.0 with parameters set to detect a difference of 2 mm between treatments and α=5 %. The 2-mm difference value was adopted in order to detect clinically significant changes on the studied variables, minimizing the chance of detecting differences that could be related to probing variations instead of actual gains in clinical attachment levels [20–26]. The estimated standard deviation was 1 mm and the size of the sample was determined to require at least 13 patients in each group [27].

Data management and statistical analysis

Homogeneity of treatment groups at baseline was assessed. Repeated-measures analysis of variance (ANOVA) was used to detect intra- and inter-group differences in the clinical parameters (RGMP, PD, RVCAL, RHCAL, and respective changes/gains). When a statistical difference was found, analysis of the difference was determined using the method of Tukey. The presence of BOP or plaque at furcation sites, as well as furcation re-classification at 6 months [18], was analyzed using Fisher's exact test. The level of significance was set at 5 % (SAS Institute Inc., Cary, NC, USA, release 9.1, 2003).

Results

Subject accountability

Four hundred eighty patients were examined during the screening period. Fifty-three had an initial diagnosis of buccal class II furcation defects in at least one molar, being thus eligible for the study. After hygienic therapy, three patients were excluded (did not present PPD ≥4 mm and BOP at the site). Nine of 50 patients were excluded, eight during the surgical procedure (diagnosis of class II was not confirmed) and one because of pregnancy (Fig. 1).

Study schedule

Subject recruitment started in March 2009 and was completed by December 2012. The last surgical procedure was carried out in April 2013. Data entry/statistical analyses were performed by the end of 2014.

Patient characteristics at baseline

Patients' characteristics at baseline were not significantly different among groups. Forty-one patients contributed with 41 furcation lesions. The mean PPD and RHCAL

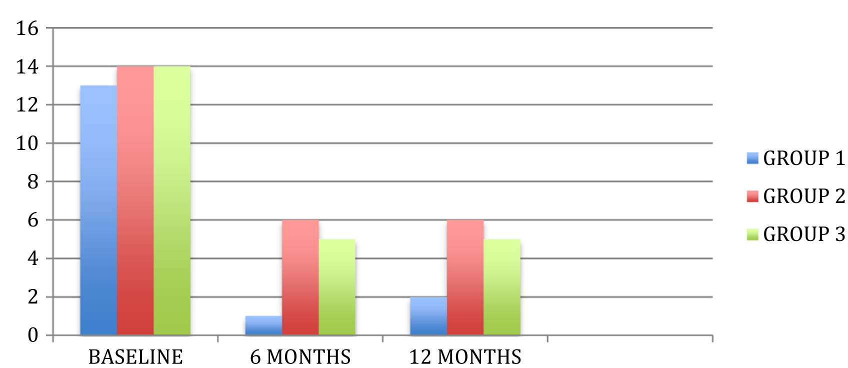

Fig. 3 Number of experimental furcation sites with bleeding on probing during the study for each treatment group. No significant difference (Fisher's exact test, $p>0.05$)

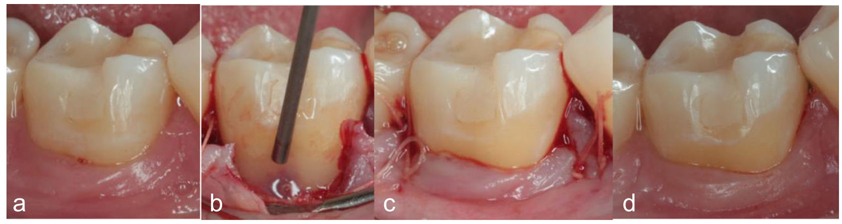

Fig. 4 **a** Initial image—group 1 (EMD). **b** Application of EMD at furcation site. **c** Flaps repositioned and sutured. **d** 12-month clinical view of surgical site

at furcation site were not statistically different among groups at baseline (Table 1).

Plaque and bleeding on probing

Acceptable oral hygiene was achieved at baseline and during the study since FMPS and FMBS remained below 15 % (Table 2). At baseline, all the treated furcations presented BOP while approximately half of the defects showed some detectable plaque. A decrease in the number of experimental sites with plaque/BOP was observed for the three groups during the study (Figs. 2 and 3). However, group 1 (EMD) showed less experimental sites with BOP after 12 months (2 out of 13) when compared with group 2 (βTCP/HA) (6 out of 14) and group 3 (EMD+βTCP/HA) (5 out of 14) (Figs. 4, 5, and 6).

Clinical parameters (RGMP, RVCAL, RHCAL, and PPD)

The results for the clinical parameters are shown in Table 3. The comparison between baseline and 12 months values for RGMP revealed that there was no significant increase in gingival recession in any of the groups ($p>0.05$). On the other hand, a significant reduction for PPD ($p<0.05$) was observed for the three treatments. After 12 months, the PPD reductions was 2.54±0.78 mm for group 1 (EMD), 2.36±1.01 for group 2 (βTCP/HA), and 2.43±1.02 for group 3 (EMD+βTCP/HA), with no differences among the groups. Regarding RVCAL and RHCAL, all three treatments provided statistically significant gains after 6 and 12 months. The difference between baseline and the values observed after 12 months for RVCAL (mean vertical attachment level gain) was 2.08±1.61 for group 1 (EMD), 2.29±1.27 for group 2 (βTCP/HA), and 2.14±1.29 for group 3 (EMD+βTCP/HA), with no significant differences among the groups. The mean reduction in the horizontal component of the furcation lesion, as calculated by the difference between the RHCAL values observed at baseline and after 12 months, amounted 2.77±0.93 mm for group 1 (EMD), 2.64±0.93 mm for group 2 (βTCP/HA), and 2.93±0.83 mm for group 3 (EMD+βTCP/HA), with no significant differences among the groups.

Furcation closure

Six months and 12 months after the surgeries, the furcation lesions were again evaluated according to Hamp et al.'s [18] classification (Table 4). Partial closure of the defects was observed in 33 cases, meaning that the furcations were now classified as class I, as follows: 13 furcations in group 1 (EMD), 9 furcations in group 2 (βTCP/HA group), and 11 furcations in group 3 (βTCP/HA+EMD), after 6 months of evaluation ($p>0.05$). After 12 months, two furcations improved their diagnoses to class I, one in the βTCP/HA group and another in the βTCP/HA+EMD group. Complete furcation closure was not detectable during the study period.

Discussion

The progression of periodontal disease can reach the furcation area, leading to tissue destruction and creating a formidable challenge in the treatment of this chronic disease [19]. The presence of furcation defects makes the posterior teeth more vulnerable to loss than the anterior teeth [28, 29]. Therefore, new therapeutic approaches should be continuously investigated for these areas.

Enamel matrix derivative (EMD) has been used in periodontal regenerative procedures based on its fundamental role in cementum development. Histological studies have shown the potential of this material to induce periodontal regeneration [30–32]. Clinical studies showed horizontal clinical attachment level gain, PD reduction, and the possibility of complete furcation closure after EMD treatment [1, 11, 13, 33]. The synthetic bone substitute made of HA and βTCP may

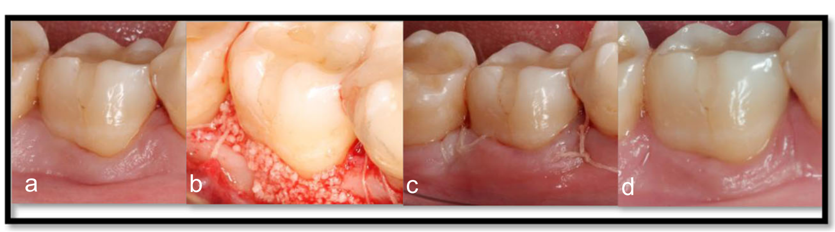

Fig. 5 **a** Initial image—group 2 (βTCP/HA). **b** Application of βTCP/HA at furcation site. **c** Flaps repositioned and sutured. **d** 12-month clinical view of surgical site

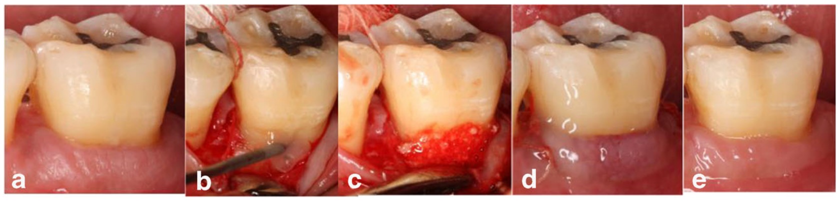

Fig. 6 **a** Initial image—group 3 (EMD+βTCP/HA). **b** Application of EMD at furcation site. **c** Mixed EMD+βTCP/HA filling the entire defect. **d** Flaps repositioned and sutured. **e** 12-month clinical view of surgical site

integrate into bone tissue, allowing osteoconductivity [34]. The combined use of different materials has been evaluated in order to find predictable approaches for periodontal regeneration in different clinical situations [35, 36]. Aiming to combine the osteoconductive and space-maintaining properties of a bone substitute with the biological properties of EMD, this study has been designed to evaluate the use of EMD+βTCP/HA. A decision was made to focus on the comparison of EMD+βTCP/HA to the isolated therapies (EMD+βTCP/HA versus βTCP/HA or EMD). Therefore, these three approaches were not compared to open flap debridement alone (OFD) or GTR in the present study. Besides the difficulties involved in finding the increased number of patients (following the inclusion/exclusion criteria) needed to conduct a study with several groups, this option was justified by the availability of historical controls, showing that both EMD [14, 37] and bone substitutes [38, 39] may provide superior clinical results to OFD and comparable results to those achieved with GTR [11, 12, 40–45].

Previous histologic studies (animal models) showed that root conditioning with EDTA may significantly enhance periodontal wound healing compared to other conditioning agents such as phosphoric acid or citric acid [46–48]. The migration of PDL fibroblasts toward the root surface may be enhanced compared to control (no root conditioning) or to other conditioning agents [47]. On the other hand, results from controlled clinical studies have failed to show statistically significant differences in the treatment outcome, when comparing open flap debridement with EDTA root conditioning to a control without EDTA [49–52]. Therefore, based on the available clinical information, EDTA root conditioning was not performed in the present study.

The three tested treatments produced favorable clinical changes characterized by a significant reduction of PPD and horizontal component of the furcation defects. Specifically concerning furcation defects, the horizontal parameter is directly related to the tooth prognosis [53]. Previous studies, using EMD alone in furcations, reported that it may result in PD reduction and RVCAL and RHCAL gains [30, 54–56]. A comparison of EMD+βTCP/HA to βTCP/HA alone was performed in patients with proximal furcation defects [57]. They reported a mean gain for

Table 3 RGMP, RVCAL, RHCAL, and PPD at different evaluation periods for the groups (mean±SD in millimeters)

		Baseline	6 months	12 months	0–6 months difference#	0–12 months difference[a]	6–12 months difference[a]
RGMP	Group 1	10.15±0.9 Aa	9.92±0.95 Aa	9.92±1.12 Aa	−0.08±0.76	−0.08±0.86	0.00±0.41
	Group 2	9.57±1.09 Aa	9.71±1.07 Aa	9.79±1.12 Aa	−0.14±0.53	−0.21±0.8	−0.07±0.62
	Group 3	9.43±1.34 Aa	9.71±1.2 Aa	9.57±1.02 Aa	−0.29±0.47	−0.14±0.66	0.14±0.36
RVCAL	Group 1	14.38±1.76 Aa	12.38±1.39 Ab	12.31±1.38 Ab	2.00±1.53	2.08±1.61	0.08±0.64
	Group 2	14.64±1.22 Aa	12.29±1.68 Ab	12.36±1.5 Ab	2.36±1.22	2.29±1.27	−0.07±1.00
	Group 3	14.07±1.44 Aa	12.07±1.33 Ab	11.93±1.27 Ab	2.00±1.30	2.14±1.29	0.14±0.53
RHCAL	Group 1	5.08±0.49 Aa	2.38±0.77 Ab	2.31±0.75 Ab	2.69±0.95	2.77±0,93	−0.08±0.28
	Group 2	5.64±0.93 Aa	3.00±1.11 Ab	3.00±1.04 Ab	2.64±1.01	2.64±0,93	0.00±0.55
	Group 3	5.5±0.76 Aa	2.71±1.14 Ab	2.57±0.94 Ab	2.79±0.8	2.93±0,83	0.14±0.36
PPD	Group 1	4.92±0.49 Aa	2.46±0.78 Ab	2.38±0.65 Ab	2.46±0.78	2. 54±0,78	−0.08±0.28
	Group 2	4.93±0.73 Aa	2.57±0.94 Ab	2.57±0.76 Ab	2.36±0.93	2.36±1,01	0.00±0.55
	Group 3	4.79±0.7 Aa	2.29±0.99 Ab	2.36±1.08 Ab	2.50±1.02	2.43±1,02	−0.07±0.27

Different letters (uppercase-vertical and lowercase-horizontal for each variable) indicate statistically significant difference (ANOVA/Tukey test, $p<0.05$).
[#] No statistically significant difference ANOVA/Tukey test ($p>0.05$)

Group 1 enamel matrix derivative (EMD), *Group 2* beta tricalcium phosphate/hydroxyapatite (βTCP/HA), *Group 3* enamel matrix derivative and beta tricalcium phosphate/hydroxyapatite (EMD+βTCP/HA), *RGMP* relative gingival margin position, *RVCAL* relative vertical clinical attachment level, *RHCAL* relative horizontal clinical attachment level, *PPD* periodontal probing depth, *SD* standard deviation

[a] No statistically significant difference ANOVA/Tukey test ($p>0.05$)

Table 4 Distribution (number and percent) of furcation defects, according to its classification (Hamp et al. 1975) in different periods

		Class according to Hamp's classification, *n* (%)		
		1	2	p
6 months	Group 1	13 (100)	0 (0)	0.0632
	Group 2	9 (64.29)	5 (35.71)	
	Group 3	11 (78.57)	3 (21.43)	
12 months	Group 1	13 (100)	0 (0)	0.1104
	Group 2	10 (71.43)	4 (28.57)	
	Group 3	12 (85.71)	2 (14.29)	

No significant difference (Fisher's exact test, $p>0.05$)

RHCAL after 6 months of 1.47 mm for bone graft alone and 1.57 mm for the association of bone graft plus EMD, with no statistical differences between groups. This is in accordance with the results observed in the present study, showing no significant differences on mean RHCAL gain after treating the furcation with the isolated approaches when compared to the combined treatment. After 12 months, the mean RHCAL was 2.7±0.9 for group 1 (EMD), 2.6±0.9 for group 2 (βTCP/HA), and 2.9±0.8 for the combined treatment (EMD+βTCP/HA). The RHCAL reduction amounted 54.5 % (EMD), 46.8 % (βTCP/HA), and 53.3 % (EMD+βTCP/HA) of the baseline value. The consequence of this reduction was that 85.3 % of all treated defects changed their diagnosis to class I after 1 year. However, no complete furcation closure could be observed during the study. This is in accordance with a previous study [13] evaluating the effects of EMD on the treatment of class II furcation defects where reduction of horizontal probing attachment level was observed after 36 months but with no complete closure of the defects. Other reports also evaluating EMD on the treatment of class II furcation showed only one site with complete closure [14]. Jepsen et al., showed complete closure of mandibular class II furcations in approximately 17 and 8 % of cases treated by EMD or GTR, respectively. Therefore, in spite of the evident reduction on the horizontal component of the furcation after treatment with current regenerative approaches, it seems also evident that the ideal outcome of complete furcation closure is not a frequent result.

In spite of the positive clinical outcomes observed when bone substitutes are used [14, 47], the results of the present study indicated that the association of βTCP/HA with EMD failed to provide a significant benefit. One point that should be considered is the difference in the histological healing pattern that may be present when comparing sites treated with EMD to sites treated exclusively with bone substitutes or the associated therapy (EMD+bone substitute). In a study conducted with the combination of EMD with biphasic calcium phosphate (BCP), in one or two wall intrabony defects around teeth schedule for extraction, it was observed that there is formation of cementum with inserting collagen fibers to a varying extent. Also, graft particles were still present and were mostly encapsulated in connective tissue, whereas formation of bone around the graft particles was observed only occasionally. Direct contact between the graft particles and the root surface (cementum or dentin) was not observed. This finding led to the conclusion that EMD may promote the formation of new cementum with an associated periodontal ligament, but there is the possibility that the presence of graft particles may interfere with the healing process, resulting in limited bone formation [58].

In conclusion, the association of the bone substitute to EMD did not provide a better clinical outcome when compared to the isolated therapies. Considering that the possibility of achieving periodontal regeneration might be increased with the use of EMD, according to previous histological studies [30–32], it seems reasonable to consider that this approach (isolated use of EMD) would be an adequate option for the treatment of class II furcation defects, similar to the ones treated in the present study. The interesting observation that bleeding at the furcation site was numerically lower in the EMD group (but not statistically significant) may suggest other properties of this material (microbiological effect?) [9], but this remains to be further evaluated. It must be recognized that there is a need for new developments in the regenerative treatments to improve the chance to promote complete closure of the defects and promote a greater positive impact on tooth prognosis.

Conclusion

EMD+βTCP/HA does not provide a significant advantage when compared to the isolated approaches. All three tested treatments promote significant improvements and partial closure of class II furcations. Based on its potential to induce periodontal regeneration, EMD may be considered an attractive option for this type of defect, but complete closure remains an unrealistic goal.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Research involving human participants All procedures performed in this study were in accordance with the ethical standards of the Ethical Committee of the Piracicaba Dental School—University of Campinas—UNICAMP and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in this study.

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