REVIEW

The efficiency of using enamel matrix derivative in therapeutic approach of periodontal furcation defects of maxillary and mandibular molars

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Abstract

Periodontal disease is an inflammatory condition which affects the covering and support structures of the teeth and, if left untreated, leads to tooth loss. Resorption of the alveolar bone from bi- and trifurcation regions of a multirooted tooth due to the progression of a periodontal disease bears involvement furcation. The degree of furcation involvement is clinically assessed by changes in the vertical and horizontal plane at the root separation area. In the approach of the furcation treatment, the morphological and functional particularities of pluriradicular teeth must be considered and the current techniques look for solutions for the regeneration of the destroyed periodontal tissues. Enamel matrix derivative (EMD) has the role of stimulating healing processes through effects on various growth factors, cement formation, angiogenesis and are used single or in combination with bone graft substitute (BGS) or guided tissue regeneration (GTR) in the complex treatment of periodontal disease. The present study follows *PubMed* publications on the efficiency of EMD in the treatment of pluriradicular tooth furcations. The findings are that there are not many studies in the usage of EMD associated with the treatment of furcations, but it is an important choice in the complex treatment of destructive periodontal disease, and further studies are needed to be done in periodontal regeneration.

Keywords: periodontal disease, furcation defects, periodontal regeneration, enamel matrix derivative.

Introduction

Periodontal disease is considered the sixth most prevalent disease in the world, with an unchanged value in the last 25 years according to a Report published by *The Economist Intelligence Unit* in June 2021 [1]. It is a part of public health interest, related to systemic diseases such as diabetes or cardiovascular disease, and which, untreated, is one of the leading causes of tooth loss.

The destruction of the bone support which holds up the teeth is the result of the inflammatory action generated by the presence of periodontal pathogenic bacteria in the dental plaque. In the lateral maxillary and mandibular areas, which are areas of maximum masticatory stress, the presence of the pluriradicular teeth not only increases the value of implantation, but also creates favorable conditions for the development of dental plaque. The morphological and topographic complexity of the root separation area of the molars and upper first premolars make these areas difficult for instrumentation, especially when periodontal pockets appear through the resorption of interradicular septa [2].

The term of ‘furcation involvement’ is used to describe the resorption of the alveolar bone into the bi- and trifurcation area of a multirooted tooth due to progression of periodontal disease, as described by the *American Academy of Periodontology* [3].

The degree of furcation involvement is clinically

assessed by changes in vertical and horizontal plane at the root separation area, more easily accessible to the mandibular molars, but more difficult to access from the approximal side at the level of the maxillary teeth, so it is important to correlate with the radiological aspect, even cone-beam computed tomography (CBCT) imaging. Several classifications of furcation defects that have been suggested were correlated to the destruction of interradicular bone septa with changes in the position of epithelial attachment, but none is complete, the most widely used being Glickman’s [3, 4].

In the approach of the furcation treatment, the morphological and functional particularities of pluriradicular teeth must be taken into account: the presence of the thickness ($\leq 150 \mu\text{m}$ to $200 \mu\text{m}$) of a cellular mixed stratified cementum with Sharpey’s fibers, intrinsic fibers and cells [4], the continuous deposition of the cementum for compensating teeth eruption and attrition, the presence of the interradicular fibers, the communication with dental pulp through the accessory canals, the exposure of the furcation area by gingival recession, and the alveolar bone loss [4].

Radicular morphogenesis is controlled by Hertwig’s epithelial root sheath, which influences root length, number, and shape. In addition, it determines the genesis of radicular dentin, as well as intervenes in the synthesis of root cementum [5].

During the disintegration of the Hertwig’s sheath, the

root epithelial cells secrete proteins such as enamel and amelogenin on the surface of the newly formed dentin. As the sheath disintegrates, the mesenchymal cells (MCs) of the dental follicle are chemotactically attracted to this protein layer and migrate to the dentin, releasing acellular cement or cell cement where Sharpey's fibers will be inserted. Other MCs differentiate into cells that form the periodontal ligament and the alveolar bone itself (this is the origin of the deep periodontium) [6].

Several factors, such as local periodontal inflammation, dento-periodontal trauma or excessive forces leading to orthodontic tooth movement cause multiple abnormalities related to a single tooth or small groups of teeth [7, 8]. In most cases, the inflammatory reaction leads to the resorption of the root [5].

In recent years, important advances have been made in terms of tissue regeneration techniques, including periodontal tissues, an important place being occupied by enamel matrix derivatives (EMDs) and MCs.

EMDs contain proteins, especially amelogenin, extracted from porcine dental buds and are used for the potential to initiate the transformation of blood thrombus cells into cementoblasts, fibroblasts and osteoblasts, periodontal ligament formation and even induction of angiogenesis on the ability of EMD to upregulate vascular endothelial growth factor (VEGF) production by fibroblasts, with deep periodontal regeneration [6, 9, 10].

The regenerative EMD impact on periodontal tissue

can be associated with its effect on growth factors, such as transformation of bone morphogenetic proteins, transforming growth factor-beta (TGF- β), fibroblast growth factor, VEGF, and platelet-derived growth factor [11].

Several studies have been performed with EMD (as an alternative form of regenerative treatments) to replace guided tissue regeneration (GTR) by bioresorbable membrane, because GTR has shown unpredictable results [9]. The data obtained show the efficiency/advantage of EMD in the treatment of gingival retractions and alveolar septal resorptions, as a single material or in combination with addition biomaterials, applied through the coronary displaced flaps by classical techniques or tunneling.

PubMed search

In the *PubMed* database was found 1068 articles about EMD, the oldest article dating from 1976. Of these, selecting by 'enamel matrix derivatives-periodontal' and 'enamel matrix derivatives-furcation' keywords, as it is seen in Figure 1, although most articles about EMD are related to the treatment of periodontal disease, a very small number of these, actually treat the application of EMD in furcations, the first article being published in 2003 (Figure 1). Of the 57 articles selected by entering the 'enamel matrix derivatives-furcation' keywords present in the title or abstract, only 30 articles on clinical or animal studies actually corresponded by manual search.

Figure 1 – PubMed publications including enamel matrix derivatives (EMDs).

Of 30 selected articles, 15 were clinical trials, eight review articles, and seven animal studies (dogs and monkeys). A review article was excluded due to a lack of information for the chosen topic.

Of 15 clinical trial articles, randomized or test-and-

control, all of those published between 2003–2017, 14 studying class II furcations, mostly molar mandibular defects, four maxillary proximal molar furcations, and only one article about class III molar mandibular furcations (Table 1).

Table 1 – Publications on the use of EMDs in the treatment of periodontal disease molar furcations

No.	Study reference	Study type	Method	Reevaluation	No./Defects	Comments
1.	Masaeli <i>et al.</i> (2018) [12]	Review			28 articles	
2.	Queiroz <i>et al.</i> (2017) [13]	Clinical trial; randomized controlled trial	EMD; EMD + BGS (β -TCP/HA); BGS (β -TCP/HA); bacterial DNA analysis	3 and 6 months	41 patients with mandibular class II buccal furcation; 39 samples	β -TCP/HA microbiome
3.	Soares <i>et al.</i> (2020) [14]	Systematic review and meta-analysis			Five studies qualitative synthesis, two for the quantitative analysis	
4.	Ivanovski (2009) [15]	Review				
5.	Queiroz <i>et al.</i> (2015) [16]	Clinical trial – case series	EMD + β -TCP/HA	6 months	13 patients' class II mandibular furcation (no smokers)	β -TCP/HA

No.	Study reference	Study type	Method	Reevaluation	No./Defects	Comments
6.	Koop <i>et al.</i> (2012) [17]	Systematic review			27 studies for intrabony defects and recession	One eligible study on furcations follow-up ≥ 1 year, human studies
7.	Casarin <i>et al.</i> (2010) [9]	Randomized clinical trial, double-blinded, split-mouth	OFD + EDTA, OFD + EDTA + EMD	6, 12 and 24 months	12 patients presenting bilateral class II proximal furcation, maxillary molars	
8.	Queiroz <i>et al.</i> (2016) [18]	Randomized clinical trial, blinded	EMD, β -TCP/HA, EMD + β -TCP/HA	12 months	41 patients, mandibular class II buccal furcation defect	β -TCP/HA
9.	Regazzini <i>et al.</i> (2004) [19]	Dogs, comparative study			4 dogs, 3 groups	
10.	Meyle <i>et al.</i> (2004) [20]	Randomized clinical trial	EMD, GTR	14 months reentry	48 patients, buccal class II furcation involvements, first and second molars	Influence of furcation morphology
11.	Hovey <i>et al.</i> (2006) [21]	Baboons			6 female baboons, class III furcation defects	
12.	Casarin <i>et al.</i> (2009) [22]	Pearson's and Spearman's rank correlation tests		6 months	18 class II proximal furcations (maxillary molars)	
13.	Zhu <i>et al.</i> (2009) [23]	Monkeys			3 monkeys, 6 groups, class III furcation defects	
14.	Jepsen <i>et al.</i> (2004) [24]	Randomized clinical trial, split-mouth	EMD, GTR	14 months reentry	45 patients with 90 comparable class II mandibular, first and second molars defects	Antibiotics were prescribed twice after EMD surgery and eight times after membrane surgery
15.	Fernandes <i>et al.</i> (2005) [25]	Five dogs, histologically and histometrically test-control	EMD + bioactive glass + GTR, EMD + GTR, bioglass + GTR (control)		20 teeth, 3 groups, one control	One antibiotic (Penicillin G) i.m. administration
16.	Palioto <i>et al.</i> (2012) [26]	Dogs, histomorphometrically test-control, 12 weeks	Four groups		6 dogs	TGF- β 1
17.	Peres <i>et al.</i> (2013) [27]	Randomized clinical trial, double-blind	OFD + β -TCP/HA, OFD + β -TCP/HA + EMD	6 months	30 patients, proximal class II furcation defects (maxillary molars)	
18.	Chitsazi <i>et al.</i> (2007) [28]	Clinical trial, split-mouth	OFD + EMD, EMD	6 month reentry	20 similar bilateral class II furcation defects in 10 patients	Healthy nonsmoker
19.	Jaiswal & Deo (2013) [29]	Randomized clinical trial	EMD + BGS + GTR, BGS + GTR, OFD	12 months	30 patients, mandibular class II furcation defects	Bone allograft, computerized probe, post-surgery: non-steroidal anti-inflammatory medication and systemic antibiotics
20.	Donos <i>et al.</i> (2003) [30]	Clinical trial case series	EMD	6, 12, and 36 months	10 patients, mandibular degree II furcation	0.2% Chlorhexidine digluconate three times per day, six weeks postoperative
21.	Hoffmann <i>et al.</i> (2006) [31]	Randomized clinical trial	EMD; GTR	14 months reentry	48 patients, buccal class II furcation, first and second molars	Influence of smoking, age, gender, hypertension, and oral hygiene status
22.	Casarin <i>et al.</i> (2008) [32]	A double-blind randomized clinical trial, randomized controlled trial		2, 4 and 6 months	15 patients, control group, proximal class II furcation (maxillary molars)	
23.	Donos <i>et al.</i> (2004) [33]	Clinical trial	EMD, GTR, EMD + GTR	6 and 12 months	9 patients, class III mandibular furcation	
24.	Aimetti <i>et al.</i> (2007) [34]		EMD + BGS (autologous)	24 months	11 patients, class II furcation defects	Vital molars with a minimum of 2 mm of keratinized tissue, autologous bone grafts

No.	Study reference	Study type	Method	Reevaluation	No./Defects	Comments
25.	Oliveira <i>et al.</i> (2020) [35]	Systematic review and meta-analysis, a minimum follow-up of 12 months			19 studies, 618 patients	
26.	Sculean <i>et al.</i> (2007) [36]	Review				
27.	Trombelli & Farina (2008) [37]	Review				
28.	Shirakata <i>et al.</i> (2017) [38]	Monkeys, 16 weeks, histomorphometric analysis			3 monkeys, 12 mandibular defects	
29.	Mardas <i>et al.</i> (2012) [39]	Comparative study, test-control, dogs, 5 months			9 dogs, 17 class III furcation defects	
30.	Heard & Mellonig (2000) [40]	Review			Maxillary class II furcations	Excluded

β -TCP/HA: beta-Tricalcium phosphate/Hydroxyapatite; BGS: Bone graft substitute; DNA: Deoxyribonucleic acid; EDTA: Ethylenediaminetetraacetic acid; EMD: Enamel matrix derivative; GTR: Guided tissue regeneration; i.m.: Intramuscular; OFD: Open flap debridement; TGF- β 1: Transforming growth factor-beta 1.

Clinical studies

Mandibular furcations class III

In 2004, Donos *et al.* come with a comparative study on nine patients with 14 defects, namely class III mandibular furcation involvement and the outcomes were monitored after six and 12 months through measuring the level of probing attachment in the vertical and horizontal directions at the furcation place after treatment with (i) EMD (four defects); (ii) three defects with a bioresorbable membrane (GTR); and (iii) combination of EMD and GTR (seven defects) [30, 33]. In all cases, an improvement in the values of the indices was obtained. Partial closure has occurred in mainly half of the treated furcations, where teeth presented through-and-through furcation lesion and when vertical probing followed, the level was consistently improved. GTR used single or in combination with EMD led to a lower number of partial closures, but the small number of patients included in the study did not allow a conclusion to be drawn about the efficacy and predictability of the methods used [33].

Mandibular furcations class II

The first published clinical study using EMD for surgical therapy of furcations was found in 2003, which contained a clinical trial of 10 patients with evaluation at six, 12, and 36 months after the application of an EMD in class II mandibular furcation defect [30]. Clinical parameters were evaluated: probing attachment level in the lingual and/or buccal furcation area on a horizontal direction (PAL-H), and probing attachment level at the center of lingual/buccal furcation area on vertical direction (PAL-V) [30]. The results showed an improvement in terms of clinical parameters at six months, and the values remained unchanged at 12 and 36 months. A greater reduction of the average values of PAL-H was registered at the buccal defects compared to the lingual defects, and PAL-V had approximately equal average reductions for these two types of defects [30].

The following studies using EMD in class II mandibular furcations were performed by Jepsen *et al.* (2004) [24], Meyle *et al.* (2004) [20], and Hoffmann *et al.* (2006) [31].

They were split-mouth comparative studies that showed the efficacy of EMD compared with GTR by bioabsorbable membranes.

Jepsen *et al.* (2004) published a randomized split-mouth clinical trial, a part I study which included 45 subjects with 90 similar defects on the contralateral molars. Defects were randomly assigned (RAS) to EMD (test sites) or bioabsorbable barrier membrane (control sites) [24]. The clinical parameters measured at baseline, eight and 14 months, respectively, were: vertical attachment levels (ALs), probing depths (PDs), gingival margin (GM) levels, bleeding on probing (BOP), and vertical bone sounding (BS) related to a stent at five buccal places/tooth. Dimensions of defect were registered at surgery as well as to reentry at 14 months. The primary outcome variable was represented by change of horizontal open furcation depth, and the study showed a significantly greater reduction of horizontal furcation depth in the EMD sites (78%) than those with a membrane (67%). Also, EMD therapy had a better degree of prediction comparable to GTR, as most defects were completely closed (eight defects compare to three) and fewer defects deteriorated (one defect compared to four) [24].

After the surgery, the authors mentioned as side effects pain and swelling with the lower occurrence in the EMD-treated group.

Also, there were notable differences in antibiotics needed prescription: eight times after the membrane surgery and twice after the EMD surgery [24].

Meyle *et al.*, in 2004, included 48 patients from five centers, in a randomized trial (part II study), and made a comparison between EMD and barrier membranes for therapy of class II mandibular furcation in both contralateral lower first or second [20]. In addition, it was studied if the root morphology has implications in the modification of the post-treatment values. The clinical study was absolute after a 14 months of reentry surgery [20]. The main parameters pursued measurements and records of the hard tissue boundaries, which are describing the anatomical status of furcation defect, as well as secondary parameters: level of GM, AL, PD, and BS at five places/tooth from the buccal side: mid-mesial root, mesio-buccal, mid-distal

root, mid-furcation and disto-buccal [20]. The Wilcoxon two-sample test was used to analyze the differences between the two methods and the Hodges–Lehmann estimator. Even if similar results were obtained, a higher probability of gingival retractions was detected in the area corresponding to the center of the furcation, in the case of the treatment which used membranes ($p=0.04$) [20]. No measurable bone resorption was recorded at sites where EMD was added, but instead, slight resorptions were observed with membrane treatment [20]. In addition, the study showed that the furcation morphology was not associated with clinical outcomes at the time of surgery, irrespective of the treatment [20].

Hoffmann *et al.* (2006) studied 48 patients, including smokers with buccal degree II furcation defects in mandibular first or second molars [31]. Each patient had surgery on both left and right sides, with EMD and respectively GTR with a bioresorbable membrane and more monitoring with a reentry at 14 months after the surgery [31]. Parameters followed: changes in all anatomical dimensions of hard tissue boundaries in the furcation defect, horizontal depth of the furcation defect that is measured from the deepest point both during surgery and reentry, AL, bleeding, pocket depth, level of GM, BS and classification of lingual and buccal furcation defect. Additionally, the possible influence of the treatment results of the following factors were taken into consideration: smoking, age, gender, hypertension, and oral hygiene status [31].

Results showed a higher median reduction of the horizontal depth of defect placed at the deepest point in non-smokers for sites treated with EMD comparing with sites treated by membrane (2.75 *versus* 1.75), an inappreciable advantage of regenerative furcation treatment when it used EMD *versus* membranes therapy, in male patients of the older age group, in non-smokers, and patients with poor hygiene [31].

In 2007, Chitsazi *et al.* evaluated the effectiveness of open flap debridement (OFD) using or not EMD in the therapy of class II furcation involvement to 10 non-smoker patients with 20 similar bilateral class II furcation defects, a split-mouth study [28].

There were investigated soft parameters of tissue: clinical probing depth (CPD) and horizontal and vertical clinical attachment level (HCAL and VCAL), as well as hard parameters of tissue, such as:

(1) Surgically exposed of the horizontal probing depths (HPDs) of the bony defect: the horizontal distance was appreciated between the deepest probed region of defects and the corresponding rubber stop contacting the root convexity, all this while the probe is relatively perpendicular to the long axis of the tooth;

(2) Vertical depth of the bone crest: appreciated by the vertical distance between lower border of the template and the alveolar crest;

(3) Vertical depth related to the base of bony defect (V-DBD): appreciated by the vertical distance between base of bony defect and lower border of template;

(4) Length corresponding to the intrabony defect (LID): the vertical distance between base of bony defect and alveolar crest [28].

The results indicated a greater improvement in the investigated indices in the EMD group, VCAL had a 13%

increase in the EMD group compared to 8.3% in OFD, but without statistically significant differences between the two groups at six months. The evaluation of soft parameters of tissue showed that addition of EMD significantly improved the horizontal attachment gain by 1.2 mm (30%) for EMD more than OFD [28].

The conclusion of the study was the fact that the use of EMD caused a substantial enhancement of the HCAL and more efficient generation of new bone both horizontally and vertically [28].

Also, in 2007, Aimetti *et al.* published a study on 11 individuals, two years in evaluation of treatment, this time with a combination of autologous bone grafts and EMD in buccal mandibular class II furcation defects of vital molars presenting at least 2 mm of keratinized tissue [34]. The results were evaluated clinically and radiologically and showed the reduction of defects from class II to class I in seven patients, four patients presenting a complete closure of the defect [34].

The next study found on *PubMed* in which the combination of EMD with bone graft substitute (BGS) was used was that of Jaiswal & Deo (2013) who followed in randomized controlled trial study effectiveness of EMD in association with freeze-dried demineralized bone allograft (BGS) but also with bioresorbable membranes (BioMesh®) [29]. Thirty systemically healthy patients were selected with the presence of a single class II buccal/lingual furcation defect on mandibular molars as determined by clinical and radiographic evaluation, GM, positioned coronally to the furcation fornix [29]. Three groups were RAS using computer software: EMD + BGS + GTR, BGS + GTR, and OFD [29]. Evaluations were performed at six and 12 months post-surgery, following several indices: HPD, vertical probing pocket depth (PPD), relative GM level (RGML) and vertical relative attachment level (V-RAL) [29]. The difference from other studies was that the recordings were made with a computerized probe (except HPD) and at the level of the furcations, the average of three determinations was considered: the mesial, the distal and the mid-buccal/mid-lingual angles.

Another difference of this study compared to others, was the postoperative attitude, in addition to oral rinsing with 0.2% Chlorhexidine gluconate, twice daily, four to six weeks, patients were prescribed a nonsteroidal anti-inflammatory medication (Ibuprofen + Paracetamol) for five days and systemic antibiotics (Amoxicillin 500 mg, three times daily) for seven days [29].

The results showed significant reduction of the parameters when EMD + BGS + GTR were used, comparing with BGS + GTR and OFD. In all groups there was a reduction in PD of 1.74 ± 1.00 mm, 0.81 ± 0.31 mm, and 0.46 ± 0.52 mm, 12 months after surgical therapy. The gain of vertical attachment to the group where EMD was used was 2.12 ± 1.07 mm compared to the other groups. Complete furcation closure was observed in 30% of cases (three) for EMD + BGS + GTR, and 0% for BGS + GTR and OFD. A partial closure (class I) for the class II furcation defects was observed in 70% of cases (seven) after EMD + BGS + GTR, 80% of defects (eight) after BGS + GTR and 20% of defects (two) after OFD. No defects remained as class II in the EMD + BGS + GTR places, 20% of defects (two) remained unchanged for BGS + GTR sites and 80% (eight) for OFD [29].

Better results by using the combination of EMD with GTR was explained by the authors by providing space for the regeneration of cells and support plus wound protection at the furcation place during healing process [29].

Conclusions of this particular study were that EMD + BGS + GTR and BGS + GTR, proved statistically significant increased results in terms of V-RAL gain, reduction of HPD, PPD and that EMD + BGS + GTR caused a statistically significant reduction of V-RAL, PPD and non-significantly important reduction of HPD, compared to BGS + GTR [29].

Regarding the study of the association of EMD with beta-Tricalcium phosphate/Hydroxyapatite (β -TCP/HA) in the class II mandibular furcations, three studies of Queiroz *et al.* were found, conducted between 2015–2017 [13, 16, 18].

In 2015, Queiroz *et al.* published a study on 13 patients: 30 days after professional hygiene, including scaling and scaling and root planning, furcations on mandibular molars with pockets >4 mm and retraction <2 mm, used EMD (Emdogain®) and β -TCP/HA, applied full thickness flap after horizontal incision, without vertical incisions (avoid vertical realizing incision) and suture [16]. The reassessment was made six months after the intervention, for the following parameters: PD, relative VCAL (RVCAL), relative GM position (RGMP), and relative HCAL (RHCAL) [16]. Thus, an improvement was obtained in all situations, except in the position of the free GM, in 76.92% of patients the forks became class I. Only 23.08% furcation remained as class II, with especially the gain of horizontal AL [16].

In 2016, Queiroz *et al.* published a study on 41 patients when it followed the changes in the fork in three study groups: only EMD (13 patients), β -TCP/HA (14 cases), and EMD + β -TCP/HA [18]. Clinical parameters measured at baseline and after six and 12 months of treatment were as follows: plaque index (PI), PPD, RVCAL and RHCAL, gingival index (GI), and RGMP [18]. At 12 months, no differences were observed between groups in relation to the level of the free GM, but significant differences were observed between the PDs and the level of vertical and horizontal attachment [18]. Horizontal attachment gain was without significant differences between groups: 2.64 ± 0.93 mm for β -TCP/HA, 2.77 ± 0.93 mm for EMD, and 2.93 ± 0.83 mm for EMD + β -TCP/HA. At the study end, 85.3% of the places were partially closed, but no complete closure was noted [18].

In 2017, Queiroz *et al.* returned with the study of the association of EMD and β -TCP/HA from the perspective of the effect on the microbiocenosis of the furcations [13]. They performed a randomized study with baseline monitoring, three and six months [13]. In 39 patients with grade II buccal forceps in the mandible, divided into three groups, they compared the changes in microbial flora following furcation treatment with EMD alone, in association with a BGS composed of β -TCP/HA, or only bone substitute [13]. The results of the study showed that there are differences in the composition of the microbial flora in the approximate areas and furcations – the complex microbiome of the furcation, as well as greater heterogeneous sites, rather than interproximal sites [13]. More than that, a significant finding was that although species like *Porphyromonas* (*P. gingivalis*) and *Treponema* (*T. denticola*) were found

in the furcation defects, these were not even a slight part of the microbiome core of the furcations [13]. Although similar results were obtained in terms of regeneration of furcation defects, in patients with EMD in treatment a longer microbiome stability was obtained with decreasing pathogen richness and increasing commensal abundance [13].

Maxillary furcations

The treatment of maxillary furcations was less studied, in *PubMed* there were four studies, three performed by Casarin *et al.* and published in 2008 [32], 2009 [22], 2010 [9] and one by Peres *et al.* in 2013 [27].

In 2008, Casarin *et al.* published a randomized, double-blind study that evaluated the clinical outcomes of proximal furcations treated by EMD proteins in 15 patients, each with a pair of the contralateral class II proximal furcation implications [32]. The parameters followed immediately before the intervention, at two and four months after intervention were: BOP, PI, PD, RHCAL and RVCAL, GM position, furcation closure and horizontal and vertical bone level (HBL and VBL). The test-group sites: after mucoperiosteal flaps raised at the level of palatine and buccal root surfaces were conditioned using 24% Ethylenediaminetetraacetic acid (EDTA) gel + EMD compared to control group site without EMD association [32]. The results of the study do not show differences in the reduction of PD and the gain in clinical and osseous ALs between the two groups, but in the site where EMD was used, a greater transformation of furcations from class II to class I was observed, two proximal furcations were not detectable. By clinical examination, only 33% were class II furcations, compared to 67% in the control group, without total closures [32].

In 2010, Casarin *et al.* published a continuation of the study from 2008, but with follow-up at two years: a double-blind, split-mouth randomized and prospective controlled clinical study [9]. It included 12 patients (three patients were lost from the 2008 study) with class II, proximal bilateral furcation defects, patients presenting the same treatment: on one hemiarcade performed only OFD with 24% EDTA root conditioning (control group) and on the other arch also associated EMD (test group) [9]. At two years, there was a greater improvement of the bone support in the proximal furcation area in the group treated with EMD, with the transformation of a larger number of furcations in class I, only five furcations remaining class II, in two cases obtaining total closure, compared to the control group in which 10 furcations remained class II, none closing completely [9]. The other parameters investigated were not statistically changed between groups [9].

Casarin *et al.* studied in 2009 the influence of anatomical features and defect morphology on maxillary molars in the treatment with EMD [22]. A selected 18 patients with proximal furcations class II were treated with OFD + 24% EDTA conditioning + EMD proteins. The parameters analyzed before and six months after the intervention were PD, RHCAL and RVCAL, GM position, and horizontal and vertical bone levels were correlated with the depth defect, vertical defect, interdental distance, number of walls, furcation distance, root divergence, root measure, furcation height, root trunk, with the help of Pearson's

and Spearman's rank tests [22]. The conclusion was that the furcation and the defect of morphology influence clinical response of the EMD-based protein therapy in the involvement of class II proximal furcations [22].

Peres *et al.*, in 2013, included in a prospective double blind, controlled, parallel and randomized clinical study, thirty patients who presented at least one proximal defect of class II furcation, with PPD ≥ 5 mm and BOP [27]. The results obtained from the two types of treatment were compared: after OFD + β -TCP/HA filling (β -TCP/HA group) and OFD + β -TCP/HA + EMD filling (β -TCP/HA + EMD group). The clinical parameters were evaluated both at baseline and six months post-surgical procedure, including: GI, full-mouth PI, BOP, PPD, RGMP, RHCAL, RVCAL, relatively HBL (RHBL), relatively VBL (RVBL) [27].

The mean PPD and RHCAL were not statistically different at baseline between the groups. At six months, in both groups were observed an increase in gingival recession, and reduction in PPD with a significant improvement in both RVBL and RHBL [27]. Regarding furcation defects resolution, both β -TCP/HA-EMD and β -TCP/HA groups presented 46.6% and 26.6% of the furcation closure, 46.6% and 66.5% being converted to class I, while a single lesion in each group remained in the same class II [27].

The conclusion of the study was that both treatments might represent an option for therapeutic approach of proximal class II furcation defects, although no specific correlation of complete closure may be related to any form of the therapy [27].

Animal studies

Of the seven published studies in animals, four were in dogs and three in monkeys, six on the use of EMD in class III defects and one on class II defects.

Studies in dogs have evaluated histological and histometric effects of EMD use compared to coronally repositioned flaps or membrane association and TGF- β 1, as well as comparative studies of the association of EMD with absorbable membranes and bioactive glass (PerioGlas[®]) [19, 25, 26, 39].

The conclusions of the studies were:

- The EMD caused a significant regeneration of the periodontal defect furcations, while associating it with membranes appears to be detrimental [19];
- The use of EMD has not shown a benefit compared to a coronally advanced flap but appears to present a protective role against ankylosis in such kind of defect [39];
- EMD + absorbable membrane $\pm$ bioactive glass led to similar results to those that used bioactive glass (PerioGlas[®]) + absorbable membrane, with partial filling of the respective furcations and bone and cement regeneration that is limited to apical portion of the defects [25];
- There is not histological evidence that EMD, TGF- β 1 and EMD + TGF- β 1 present some additional advantages in periodontal bone synthesis in a dog model of class III furcation, these materials promote limited periodontal regeneration, but the new cementum and new bone formation were greater when used EMD and TGF- β 1 [26].

Factors that can influence formation of new cement are as followed: the size of the defect, the presence of remaining original cementum on the roots, the presence of gingival recession, the level of epithelial migration, the

observation time of the study and the occurrence of trauma in the site during the healing period [25]. Also, the advantages of acellular matter over the cellular cementum favoring periodontal regeneration are not yet fully known, EMD has a role in the formation of new layer of cementum.

Three studies on monkeys were included in this review. In 2006, Hovey *et al.* studied six female baboons which received 10 mm bilateral osteotomy around the first and second mandibular molars to obtain class III, subclass C furcation defects [21]. There were investigated six total sites per treatment group to one of four treatments: OFD, OFD + bioengineered tissue (Dermagraft[®] – DG), OFD + EMD, or OFD + bioengineered tissue (DG) and EMD [21]. Seven months after creation of the defect and five months after the therapy, and without oral hygiene, histometric analysis demonstrated differential regenerative responses, EMD-treated sites presenting moderate positive regenerative outcomes and no negative responses [21]. DG alone or in combination therapy led to similar or less than positive outcomes relative to OFD controls [21].

The other two studies were performed on three monkeys (*Macaca fascicularis*). Zhu *et al.* (2009) created six defects and wanted to show the effect of periodontal ligament cells (PDLs) (autogenous PDLs were cultured *in vitro* with Bio-Oss[®] Collagen) with or without EMD influences periodontal tissue repair in class III furcation defects [23]. The conclusion was that separately, the two materials may increase the repair of periodontal tissue around class III furcation defects, but their combination did not have a predictable result, and the treatment may be influenced by the sizes of furcal defects and the coverage of gingival flap wound [23].

Shirakata *et al.*, in 2017, evaluated the effect of a new system of liquid carrier of EMD (Osteogain[®]) that was soaked on an absorbable collagen sponge (ACS) [38]. They studied 12 class III furcation defects created in the molars of three monkeys and depending on the applied treatment they created four groups: (i) OFD + ACS, (ii) OFD + Emdogain[®]/ACS, (iii) OFD + Osteogain[®]/ACS, and (iv) OFD alone [38]. The study concluded that Osteogain[®] has favorable physicochemical characteristics favoring adsorption of amelogenin on ACS, and additionally may also enhance the periodontal wound healing (regeneration) when it is compared to Emdogain[®] [38].

Regarding the review articles, in the seven articles included, which were published between 2007 and 2020, the results are in line with those found in this review, with the mention that we also included animal studies.

In 2007, in the review of Sculean *et al.* [36] about Emdogain[®] in regenerative periodontal therapy, they included studies made by Jepsen *et al.* [24] and Meyle *et al.* [20], in 2004, and concluded that the application of EMD may improves periodontal regeneration in class II mandibular furcations, clinical results being comparable to those obtained after GTR.

Ivanovski, in 2009 [15], remarked that in periodontal regeneration, there were no randomized and controlled clinical trials to compare EMD with OFD in the therapy of furcation defects and mentions the results of Jepsen *et al.* (2004) study [24].

In the review of Trombelli & Farina, in 2008, limited evidence supports the use of bioactive agents either alone

or in specific association with graft/GTR for the treatment of furcation defects [37].

Moreover, in 2012, Koop *et al.* [17] did a meta-analysis performed for intrabony defects and recession, a randomized controlled clinical trial with a follow-up ≥ 1 year. Out of 27 studies in *PubMed*, only one was eligible about furcations, which was the one of Jepsen *et al.* (2004) [24], presented in our review.

In 2018, Masaeli *et al.* [12] finally selects 11 articles from *PubMed/MEDLINE*, *ScienceDirect*, and *EMBASE* and realized a systematization of studies according to the ways of using EMD alone or in association with BGS or GTR. In conclusions, it emphasizes the advantage of associating EMD in horizontal attachment gain, especially number of furcation conversions from class II to class I, stimulates the healing of soft tissues and accelerates tissue repair, a synergic effect, and boosts osteogenesis, when used in combination with bone graft and even pain and swelling minimization due to its antibacterial and anti-inflammatory nature [12].

In 2020, two systematic review and meta-analysis studies appeared on the use of the EMD in the therapy of class II furcation, available on *PubMed*. The one by Soares *et al.* [14] included five studies for the qualitative synthesis, as well as two for the quantitative analysis and the other by Oliveira *et al.* [35] selected 19 studies. The conclusions of both meta-analyses were that EMD does not bring additional benefits and further studies are needed.

Conclusions

Although the regenerative role of EMD in bone lesions has been extensively studied, there are few studies that have used EMD in the treatment of furcation defects. The topmost studied defects were class II mandibular furcations, except the maxillary ones, where in fact, it is significant to find alternatives to the use of membranes, which are difficult to use due to the root morphology of the maxillary molars. Besides, the analysis of the studies showed a diversity of EMD use, not only singularly, but also in association with resorbable membranes, BGS and β -TCP/HA, but few studies (three) followed the treatment results after one year. At these furcations defects, there were also changes in the microbiome compared to the one contained in the periodontal pockets. The use of EMD has led to a greater reduction in the depth of grade II furcations, compared to surgical procedures using resorbable membranes. Postoperative pain and inflammation were lower when using bone morphogenetic proteins. The clinical results obtained with the use of EMD in the treatment of grade II maxillary furcations, were inferior compared to those obtained in the treatment of mandibular furcations. Studies on animals are few, but have allowed histological analysis, so in the therapy of furcation defects grade II in dogs, a corresponding regeneration of the defects was observed in cases where only EMD was used, without the presence of membranes. The use of EMD in periodontal regenerative therapy is an important choice in the complex treatment of destructive periodontal disease and requires further studies in periodontal regeneration.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

Andreea Cristiana Didilescu has equal contributions to this paper as the first author.

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