Enamel Matrix Derivative and Transforming Growth Factor-β1 in Class III Furcation Defects. A Histomorphometric Study in Dogs

Daniela B. Palioto[1], Guilherme de O. Macedo[2], Adriana C. Queiroz[3], Mário Taba Jr.[1], Sérgio L.S. Souza[1], Márcio F.M. Grisi[1], and Arthur B. Novaes Jr.[1]

[1]Department of Bucco-Maxillo-Facial Surgery and Traumatology and Periodontology, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, [2]School of Dentistry, Tiradentes University, Aracaju, SE, [3]School of Dentistry, Federal University of Amazonas, Manaus, AM, Brazil

Abstract

The objective of this study was to evaluate histomorphometrically, in dogs, the effect of enamel matrix derivative (EMD), with or without transforming growth factor-β1 (TGF-β1), in a periodontal Class III furcation model. Class III furcation defects were created in P3 and P4 of six dogs. The defects were allowed to stabilize for 21 days. Four experimental conditions were established: G1: control (propylene glycol alginate); G2: EMD; G3: TGF-β1 and G4: EMD + TGF-β1. After 12 weeks, the dogs were euthanized. Their jaws were removed, fixed, decalcified, dehydrated and embedded in paraffin. Semi-serial sections were obtained, stained and examined with light microscopy. The furcation defects were not completely closed in any specimen, with downgrowth of the junctional epithelium into the furcation area. The morphologic characteristics of the newly formed tissues in the test groups were similar to the control group, with slight differences in average values, but with no statistically significant differences between the groups. This study was not able to provide histological evidence that EMD, TGF-β1 and EMD + TGF-β1 present additional advantages in periodontal bone formation in a Class III furcation model in dogs.

Key words: Enamel matrix derivative; growth factors; TGF-β1; periodontal regeneration; furcation

Introduction

The complete and predictable restoration of the periodontium following trauma or infection remains a critical objective in periodontics (Reynolds *et al.*, 2003). The primary goal of regenerative periodontal therapy is to reproduce tooth-supporting tissue, including alveolar bone, periodontal ligament, and cementum (Garret, 1996). Many procedures and materials, like guided tissue regeneration, bone grafts and growth factors, have been used to achieve periodontal regeneration (Reynolds *et al.*, 2003; Murphy and Gunsolley, 2003; Giannobile and Somerman, 2003).

Correspondence to: Arthur Belém Novaes Júnior, Departamento de Cirurgia e Traumatologia Buco-maxilo-facial e Periodontia, Faculdade de Odontologia de Ribeirão Preto - Universidade de São Paulo (USP), Av. do Café s/n, 14040-904 Ribeirão Preto, SP, Brazil.
e-mail: novaesjr@forp.usp.br

The identification of the role of enamel matrix derivative (EMD) in the formation of the cementum led to the development of a biological concept for periodontal regeneration (Hammarstrom, 1997). It has been shown that inner cells from the Hertwig's epithelial root sheath have a secretory stage prior to cementum formation, suggesting that epithelial-mesenchymal interactions are essential for formation of the periodontium (Pietruska, 2001; Wennström and Lindhe, 2002). Based on this, enamel matrix proteins, derived from porcine tooth germs, are commercially available for use in periodontics, with the aim to mimic the specific events that occur in the development of supporting tissues during tooth organogenesis. Clinical trials have stated that EMD promotes CAL (clinical attachment level) gain and PD (probing depth) reduction, in addition to radiographic bone filling (Giannobile and Somerman, 2003). Histological

investigations have shown that the application of EMD leads to the formation of a new connective tissue attachment and new alveolar bone (Kalpidis and Ruben, 2002). Findings from *in vitro* studies indicate that EMD may modulate the behavior of a variety of dental and non-dental cell types by up-regulating cAMP levels, inducing synthesis and secretion of transforming growth factor beta (TGF-β) and interleukin-6 (IL-6) in periodontal ligament (PDL) cells and gingival fibroblasts and by stimulating proliferation of pre-osteoblasts and differentiation of immature osteoblasts (Schwartz *et al.*, 2000; Van der Pauw *et al.* 2000; Okubo *et al.* 2003). Moreover, it has been suggested that enamel extracts have bioactive properties, bone morphogenetic protein (BMP)-like activity (Iwata *et al.*, 2002*)* and transforming growth factor-like activity (Kawase *et al.,* 2002). Lyngstadaas *et al.* (2001) suggested that EMD can enhance the synthesis of TGF-β1 in PDL cells and that this may, in part, explain some of the activities attributed to EMD. Suzuki *et al.* (2005) reported that TGF-β-like growth factors in EMD gel contribute to the induction of mineralization during periodontal regeneration.

TGF-β is produced by various cells, including macrophages, as well as non-immune cells, such as gingival fibroblasts, in the periodontal tissue (Wahl *et al.*, 1993). Transforming growth factor beta 1 (TGF-β1), one of the three isoforms of TGF-β, is a multifunctional regulatory protein that appears critical for wound healing by augmenting angiogenesis and fibroblast collagen formation (Centrella *et al.*, 1987). It has been reported that TGF-β1 may play an important role in the modulation of tissue formation and development of the periodontium (Gao *et al.*, 1998). Furthermore, TGF-β1 is mitogenic to periodontal ligament cells (Brady *et al.*, 1998) and inhibits epithelial cell proliferation (Kawase *et al.*, 2002). Because of its biological effects that may provide some benefits to the regeneration process, the association between growth factors and EMD could improve periodontal regeneration. This association, however, is still little explored. Palioto *et al.* (2004) showed that the addition of IGF-I to EMD did not alter the results obtained in cell cultures. Rodrigues *et al.* (2007) observed that the combination of TGF-β1 with EMD did not positively influence PDL fibroblasts *in vitro.* Nevertheless, these interactions were not analyzed in animal models. The aim of this study was to evaluate the effects of EMD, TGF-β1 and their association in a periodontal regeneration in Class III furcation model by histomorphometric analysis in dogs.

Material and Methods

The study protocol was approved by the Institutional Ethical Committee for Animal Use (04.1.1000.53.4). Six young adult mongrel dogs, approximately 15 kg each, were included in the study. The animals presented with general good health and intact teeth, without periodontal disease and were vaccinated and treated for parasites. Before the surgeries, the animals were sedated by intramuscular (IM) dihydrotriazine injection (4 mg/kg), followed by general anesthesia with intravenous (IV) sodium thiopental (12 mg/kg). Lidocaine (2%) with 1:100,000 noradrenaline was infiltrated into the mucosa to control bleeding and simulate normal clinical routine. Intrasulcular incisions were performed on the buccal and lingual gingival mucosa of the mandibular teeth P2, P3 and P4 (*Figure 1A*), and mucoperiosteal flaps were raised to expose the alveolar bone. Class III furcation defects were surgically created in P3 and P4, using a high-speed spherical diamond surgical bur under abundant irrigation with saline solution and microchisels (#1 and #2 Fedi DE Chisel, Hu-Friedy, Chicago, IL). The dimensions of the

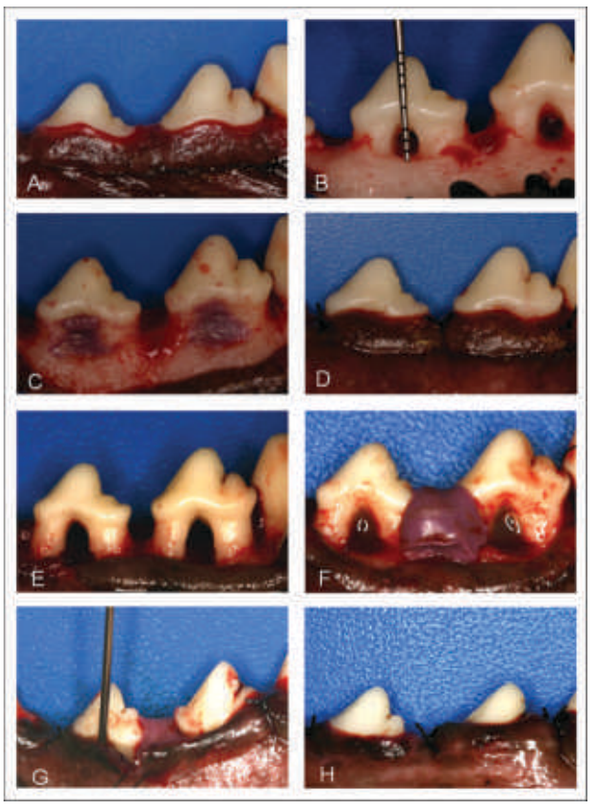

Figure 1. Clinical procedures. A. Initial appearance; B. Surgically created Class III, with 3 mm of height; C. Placement of rubber base impression material; D. Sutures; E. Class III furcation defects after a period of inflammation. Note the advanced periodontal disease, with pronounced bone loss; F. Placement of the gel-consistency materials, with a barrier made with impression material between the teeth. G. Sutures made prior to the barrier removal and additional material delivery. H. The flaps repositioned and sutured.

Table 1. Histomorphometric measurements in the study groups

	G1 (Control)	G2 (EMD)	G3 (TGF-β)	G4 (EMD + TGF-β)	*p**
Total area (mm^2)	11.79 ± 1.79	15.33 ± 1.98	13.41 ± 2.60	11.85 ± 2.23	NS
New tissue (mm^2)	7.33 ± 1.87	9.33 ± 1.89	8.50 ± 1.80	8.04 ± 2.17	NS
New bone (mm^2)	3.66 ± 1.87	2.52 ± 1.58	3.69 ± 0.94	2.48 ± 1.58	NS
New cementum (mm^2)	3.13 ± 0.91	5.36 ± 2.86	3.69 ± 1.37	4.53 ± 2.14	NS
Area supported by new bone (mm^2)	4.65 ± 1.97	4.05 ± 1.69	4.41 ± 1.07	3.56 ± 1.15	NS
Area supported by new cementum (mm^2)	5.55 ± 2.42	6.10 ± 2.01	6.09 ± 1.13	5.94 ± 1.45	NS
Furcation height (mm)	4.62 ± 0.12	5.30 ± 0.22	4.87 ± 0.39	4.76 ± 0.37	NS
Furcation extension (mm)	10.95 ± 1.01	12.58 ± 1.04	11.45 ± 0.92	11.10 ± 1.23	NS
New cementum extension (mm)	4.27 ± 0.91	4.08 ± 0.61	4.55 ± 0.70	4.19 ± 0.59	NS
New bone height (mm)	1.69 ± 0.57	1.51 ± 0.69	1.79 ± 0.45	1.36 ± 0.31	NS
New tissue height (mm)	2.99 ± 0.41	3.02 ± 0.56	3.20 ± 0.47	2.96 ± 0.48	NS
Epithelium extension (mm)	3.53 ± 0.45	3.82 ± 0.62	3.78 ± 0.63	3.65 ± 0.50	NS

*One-way ANOVA test ($p < 0.05$)

furcation defects were standardized to a depth of 3 mm in the occluso-apical direction, from the furcation fornix to the base of the defect, measured by a periodontal probe (*Figure 1B*). In the mesio-distal direction, the bone enclosing half of the mesial root and half of the distal root was removed from the buccal and lingual sides. In the bucco-lingual direction, the bone was also removed to allow communication between the buccal and lingual sides. A rubber base impression material was then placed in the defects to induce inflammation and prevent the occurrence of spontaneous repair (*Figure 1C*). Flaps were repositioned and sutured (*Figure 1D*). The surgeries were performed by a single operator (DBP). The animals were fed only water-softened dog food to prevent suture disruption and to increase plaque accumulation. After seven days, sutures were removed, but the impression material was left for 14 days. After the material removal, the animals were, subsequently, submitted to a plaque control protocol and daily topical applications of 0.12% chlorhexidine digluconate solution. After two weeks, the previously described sedation procedure was repeated. On the afternoon prior to this surgery, the dogs had IM injections of 20,000 IU penicillin and erythromycin (0.1 g/kg). Buccal and lingual intrasulcular incisions were performed on the mandibular regions of P3 and P4, bilaterally. Full-thickness mucoperiosteal flaps were raised, the defects debrided, and the roots scaled using Gracey curets, and, to facilitate histomorphometric analysis, reference notches were made in the roots at the bone crest level, with spherical 33.5 burs with abundant irrigation with saline solution. The root surfaces of all experimental teeth, irrespective of the treatment modality, were conditioned with 24% EDTA-containing gel (PrefGel, Straumann) for 2 min according to the instructions given by the manufacturer. The EDTA remnants were removed with copious rinsing with sterile saline. Four experimental conditions were established: G1: control - propylene glycol alginate (compounded by our pharmacy); G2: EMD (Emdogain, Straumann); G3: 1.2 g/ml TGF-β1 + propylene glycol alginate; and G4: EMD and 1.2 g/ml TGF-β1. The propylene glycol alginate was chosen as a vehicle to TGF-β1 because this is the one used as a carrier for EMD, so that they would be the same for both treatments. The defects were randomly assigned to one of the treatments. As the materials present a gel consistency, a barrier between P3 and P4 was made with impression material in order to avoid one material interfering in the potential action of another (*Figure 1F*). Ten minutes were waited to allow gelification. After the placement of the material, the flap around P3 was coronally repositioned and sutured (*Figure 1G*). The impression material barrier was then removed and the flap around P4 was sutured (*Figure 1H*). Sutures were removed after 14 days. Following surgery, the animals were fed only water-softened dog food and were submitted to daily applications of topical 0.12% chlorhexidine digluconate solution. In addition, every week the dogs were sedated for prophylaxis. After 12 weeks, the dogs were euthanized by sodium thiopental overdose. Their jaws were removed, sectioned in two blocks, fixed in a formalin solution and decalcified by multiple baths of EDTA. The tissue

blocks were dehydrated in increasing alcohol concentrations. They were then placed into an equal parts solution of alcohol and xylol and embedded in paraffin. Five semi-serial sections, 7 μm thick, were cut from the teeth in the most central region of the furcation, in a mesio-distal direction. The sections were mounted on slides and stained with hematoxylin and eosin (H&E) or Mallory's trichrome stain (MT). A video camera (Leica DC300, Leica Microsystems Nussloch GmbH, Nussloch, Germany) attached to a microscope (Leica DMLB, Leica Microsystems Nussloch GmbH, Nussloch, Germany) allowed histomorphometric measurements. The images were processed with a Leica IM 50 (Leica IM 50, Leica Imaging Systems Ltd.). Histomorphometric analysis was then performed by a blind examiner (GMO) who did not know the group that the histological section belonged to, using Image Tool software (Image Tool for Windows, Version 3.00, UTHSCSA, San Antonio, TX, USA). The total area, new tissues, new bone, new cementum, area supported by new bone and area supported by new cementum were measured in millimeters squared. The furcation height, furcation extension, new cementum, new bone height, new tissue height and epithelium were measured in millimeters. The arithmetic average from five histologic section measurements was calculated to represent each site. The experimental unit was the animal and data were submitted to non-parametric statistical analysis ANOVA one-way test ($p < 0.05$).

Results

In two dogs, both P3 teeth (comprising G2 and G3) presented excessive mobility during the healing phase because of advanced periodontal disease and were excluded from the statistical analysis. Gingival recession was clinically observed, with no exposure of grafted materials or furcation defects. No additional adverse effects or postoperative complications were observed. It was observed that no Class III furcation defects were completely closed in all specimens, with downgrowth of the junctional epithelium into the furcation area (*Figure 2*). The morphologic characteristics of the newly formed tissues in the test groups were similar to the control group, with slight differences in average values. However, no statistically significant differences between groups were found (*Table 1*).

The histological sections are exposed in *Figures 2* and *3*. All furcations were open with epithelialized tissue in the lower portion of the defect, and new cementum with inserting collagen fibers and new bone was limited to the level of the notch. Connective tissue with subepithelial inflammatory infiltrate was present in a great portion of the defect. New attachment and new bone were observed to a varying extent. Newly formed cementum could be considered a reparative

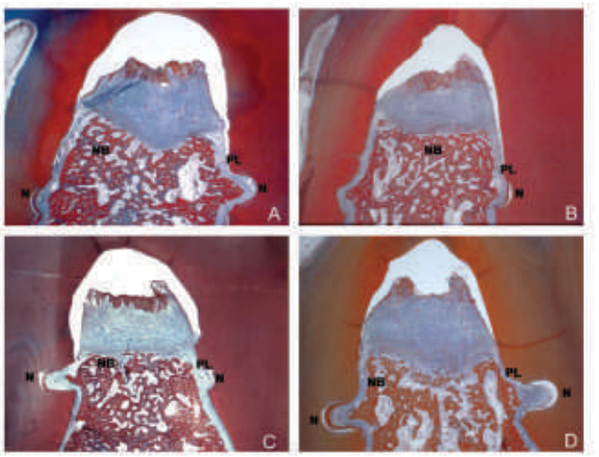

Figure 2. Mesio-distal histologic section of Class III furcation site of groups. A. EMD group (G1); B. TGF-β group (G2); C. EMD + TGF-β group (G3); D. Control group (G4). New bone (NB) and periodontal ligament (PL) above reference notch (N). (Mallory's trichrome; original magnification x1.6)

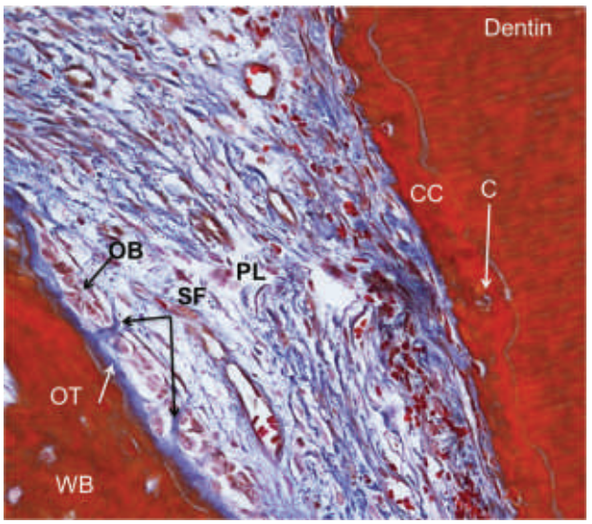

Figure 3. Histologic section showing aspects of the newly formed tissues. The arrows show cementocytes (C) inside the cellular cementum (CC), Sharpey fibers (SF) in periodontal ligament (PL), osteoblasts lining (OB) and osteoids (OT); dentin and the immaturely formed bone (WB) can also be observed (Mallory trichrome; original magnification x10).

cellular tissue that presented irregular distribution and variable thickness. It presented with predominantly intrinsic collagen fibers but also included areas with extrinsic (Sharpey) fibers. The fornix of the furcation did not present new cementum in any of the specimens. The new bone, under polarized light, seemed to be immature (woven bone), with an aspect that suggested

intense activity of osteoblasts. Cement lines were frequently observed on the newly formed bone and cementum, indicating an interface between the new bone and more recently formed bone.

Discussion

Enamel matrix derivatives (EMD) have been suggested to promote periodontal regeneration by way of mimicking the specific events that occur during the development of the periodontium. Although EMD shows clinical efficacy, the precise mechanism of tissue regeneration and which factors influence cell functions are still unclear. Some investigators have suggested that growth factors or growth factor-like activity could be involved in this biological process. It has been proposed that enamel extracts have bioactive properties, such as TGF-β-like activity (Kawase *et al.*, 2001) and that TGF-β-like growth factor is present in EMD (Suzuki *et al.*, 2005). Wada *et al.* (2008) suggested that EMD might contain TGF-β that exerts an effect on osteoblasts, participating in the bioactivity of EMD. Many properties of TGF- β1, such as regulation of the proliferation and differentiation of osteogenic cells (Linkhart *et al.*, 1996) and of the production of extracellular matrices by stimulating the synthesis of collagens, fibronectin and proteoglycans (Streuli *et al.*, 1993) support its important role in periodontal regeneration. Based on this, the TGF-β-like growth factor in EMD may have an effect similar to that of TGF- β1 in periodontal regeneration. For Kawase *et al.* (2002), it may be that EMD acted as a natural and efficient drug delivery system for exogenous TGF-β and endogenously produced cytokines. Thus, the overall effects of EMD may be to support these factors and to lengthen their biological half-lives within the limited area of application. These findings indicate that the effects of EMD are associated closely with growth factors. Therefore, we sought to determine whether the association of TGF-β1 and EMD provided additional advantages for the periodontal regeneration in furcation defects, as well as to evaluate its effects when used alone.

Class III furcation defects were selected to evaluate the periodontal healing in a more challenging situation. However, no statistically significant differences in any of the investigated parameters were observed between the treatment modalities and control groups. Therefore, this study could not demonstrate that EMD, TGF-β1 or a combination of EMD and TGF-β1 are able to provide an advantage to the healing of Class III furcation defects in dogs. None of the specimens presented complete healing of the furcations, showing, as expected, that the treatment of Class III furcation defects is unpredictable. Defect configuration is an important factor in the prediction of results and the distribution of tissue resources plays an important role in the healing of any defect. In Class III furcation defects, which are extensive horizontal defects with a single bony wall, the supply of nutrients and cells is reduced, decreasing the predictability of the outcomes. As it is a challenging model, with critical size, substantial regeneration warrants clinical pursuit of the procedure in question, while limited regeneration would be less deserving (Wikesjö and Selvig, 1999). This defect configuration provides only one bony wall as an apical limit, the dental roots as proximal limits and the furcation fornix as an occlusal limit. As no membrane was used on the free surfaces, the stabilization and maintenance of the materials, which have a gel consistency and blood clots, might have been prevented, interfering negatively in the results. Moreover, after the period of dental plaque accumulation and pronounced inflammation, the periodontal disease progress was extensive, inducing an advanced bone loss. Thus, the Class III furcations treated were actually greater than the surgically created 3 mm height defect established in the first procedure, which makes the periodontal regeneration even more challenging. Perhaps acute defects could have been an alternative model because of the difficulty of obtaining similar size defects when using chronic defects because reformation potential of fibrous attachment may be considered similar on planed root surfaces previously exposed to periodontal disease and on surfaces surgically deprived of their attachment apparatus (Suaid *et al.*, 2012)

These data showed that the biomaterials used in this study were able to promote limited periodontal regeneration. Although there was no statistical significance between the groups, in terms of averages, the new cementum formation was greater in the G2 and G3, presented a greater average of new bone formation. This is in agreement with Hammarstrom (1997), who indicated that enamel matrix proteins are involved in the development of cementum and that these proteins may be used as a means to regenerate acellular extrinsic fiber cementum, and with Bonewald and Mundy (1990), who showed that TGF-β1 induces the differentiation or proliferation of osteoblastic cells while inhibiting the formation of osteoclast precursors.

Prior studies comparing the effectiveness of Class III furcation defects treatment with EMD, TGF-β1 and the association between them were not presented in the literature. Rodrigues *et al.* (2007) evaluated their effects on periodontal ligament fibroblasts and observed that EMD and TGF-β1 may play an important role in periodontal regeneration. EMD induced PDL fibroblast proliferation and migration, total protein synthesis, alkaline phosphatase activity, and mineralization. However, the combination of both factors did not positively alter PDL fibroblast behavior.

The effects of TGF-β1 in periodontal regeneration were previously investigated. The association of TGF-β1 and membranes in the treatment of Class III furcations showed no additional benefits to guided

tissue regeneration in dogs (Wikesjö *et al.*, 1998). Mohammed *et al.* (1998) observed that TGF-β1 encouraged bone regeneration in Class II furcation defects in sheep, an effect enhanced by the presence of a barrier membrane. Tatakis *et al.* (2000) implanted rhTGF-β1 in a calcium carbonate ($CaCO_3$) carrier in Class III furcation defects in dogs and obtained minimal, if any, stimulation of alveolar bone or cementum regeneration.

In the present study, the association of TGF-β1 and EMD did not warrant better histological results, as could be expected due to their biological properties. When growth factors are applied as treatment to achieve periodontal regeneration, the influence of dose and concentrations has to be considered. Many studies have shown that cells respond to growth factors in a dose-dependent manner (Matsuda *et al.*, 1992; Lucarelli *et al.*, 2003; Gruber *et al.*, 2004; Soffer *et al.*, 2004). It could explain why the association of TGF-β1 with EMD did not improved periodontal regeneration. Possibly, the ideal concentration of TGF-β1 might have been reached with the growth factors present on EMD or the endogenously produced growth factors that have their biological half-lives lengthened due to the bioactivity of EMD. Moreover, the interaction of a polypeptide growth factor with its target cell occurs at a specific cell surface receptor (Segarani *et al.*, 1992). When these receptors achieve saturation, additional levels of growth factors will no longer be effective. Thus, the TGF-β1 present in EMD could have saturated the binding receptors, which would neutralize any function of extra TGF-β1 associated with EMD.

In conclusion, the present study was not able to provide histological evidence that EMD, TGF-β1 or EMD + TGF-β1 present additional advantages in periodontal bone formation in a Class III furcation model in dogs.

References

Bonewald, L.F. and Mundy, G.R. Role of transforming growth factor-beta in bone remodeling. *Clinical Orthopaedics and Related Research* 1990; **60**:261–276.

Brady, T.A., Piesco, N.P., Buckley, M.J., Langkamp, H.H., Bowen, L.L. and Agarwal, S. Autoregulation of periodontal ligament cell phenotype and functions by transforming growth factor-beta1. *Journal of Dental Research* 1998; **77:**1779-1790.

Centrella, M., McCarthy, T.L. and Canalis, F. Transforming growth factor-β is a bifunctional regulator of replication and collagen synthesis in osteoblast-enriched cell cultures from fetal rat bone. *Journal of Biological Chemistry* 1987; **262**:2869-2874.

Gao, J., Symons, A.L. and Bartold, P.M. (1998) Expression of transforming growth factor-beta 1 (TGF-beta1) in the developing periodontium of rats. *Journal of Dental Research;* **77**:1708-1716.

Garrett, S. Periodontal regeneration around natural teeth. *Annals of Periodontology* 1996; **1**:621-666

Giannobile, W.V. and Somerman, M.J. Growth and amelogenin-like factors in periodontal wound healing. *Annals of Periodontology* 2003; **8**:193-204.

Gruber, R., Karreth, F., Kandler, B., *et al.* Platelet-released supernatants increase migration and proliferation, and decrease osteogenic differentiation of bone marrow-derived mesenchymal progenitor cells under *in vitro* conditions. *Platelets* 2004; **15**:29–35.

Hammarstrom, L. Enamel matrix, cementum development and regeneration. *Journal of Clinical Periodontology* 1997; **24**:658-668.

Iwata, T., Morotome, Y., Tanabe ,T., Fukae, M., Ishikawa, I. and Oida, S. Noggin blocks osteoinductive activity of porcine enamel extracts. *Journal of Dental Research* 2002; **8**:387-91.

Kalpidis, C.D. and Ruben, M.P. Treatment of intrabony periodontal defects with enamel matrix derivative: a literature review. *Journal of Periodontology* 2002; **73**:1360-76.

Kawase, T., Okuda, K., Momose, M., Kato, Y., Yoshie, H. and Burns, D.M. Enamel matrix derivative (EMDOGAIN) rapidly stimulates phosphorylation of the MAP kinase family and nuclear accumulation of smad2 in both oral epithelial and fibroblastic human cells. *Journal of Periodontal Research* 2001; **36**:367-376.

Kawase, T., Okuda, K., Yoshie, H. and Burns, D.M. Anti-TGF-beta antibody blocks enamel matrix derivative-induced upregulation of p21WAF1/cip1 and prevents its inhibition of human oral epithelial cell proliferation. *Journal of Periodontal Research* 2002; **37**:255-262.

Linkhart, T.A., Mohan, S. and Baylink, D.J. Growth factors for bone growth factor and repair: IGF, TGF-1 and BMP. *Bone* 1996; **19**:1S–2S.

Lucarelli, E., Beccheroni, A., Donati, D., *et al.* Platelet-derived growth factors enhance proliferation of human stromal stem cells. *Biomaterials* 2003; **24**:3095–3100.

Lyngstadaas, S.P., Lundberg, E., Ekdahl, H., Anderson, C. and Gestrelius, S. Autocrine growth factors in human periodontal ligament cells cultured on enamel matrix derivative. *Journal of Clinical Periodontology* 2001; **28**:181–188.

Matsuda, N., Lin, W.L., Kumar, N.M., Cho, M.I. and Genco, R.J. Mitogenic, chemotactic, and synthetic responses of rat periodontal ligament fibroblastic cells to polypeptide growth factors *in vitro*. *Journal of Periodontology* 1992; **63**:515–525.

Mohammed, S., Pack, A.R. and Kardos, T.B. The effect of transforming growth factor beta one (TGF-beta 1) on wound healing, with or without barrier membranes, in a Class II furcation defect in sheep. *Journal of Periodontal Research* 1998; **33**:335-44

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

Okubo, K., Kobaiashi, M., Takigushi, T., *et al.* Participation of endogenous IGF-I and TGF-beta 1 with enamel matrix derivative-stimulated cell growth in human periodontal ligament cells. *Journal of Periodontal Research* 2003; 38:1–9.

Palioto, D.B., Coletta, R.D., Graner, E., Joly, J.C. and Lima, A.F. The influence of enamel matrix derivative associated with insulin-like growth factor-I on periodontal ligament fibroblasts. *Journal of Periodontology* 2004; **75**:498-504.

Pietruska, M.D. A comparative study on the use of Bio-Oss and enamel matrix derivative (Emdogain) in the treatment of periodontal bone defects. *European Journal of Oral Science* 2001; **109**:178-181.

Reynolds, M.A., Aichelmann-Reidy, M.E., Branch-Mays, G.L. and Gunsolley, J.C. The efficacy of bone replacement grafts in the treatment of periodontal osseous defects. A systematic review. *Annals of Periodontology* 2003; **8**:227-265.

Rodrigues, T.L.S., Marchesan, J.T., Coletta, R.D., *et al.* Effects of enamel matrix derivative and transforming growth factor-β1 on human periodontal ligament fibroblasts. *Journal of Clinical Periodontology* 2007; **34**:514–522.

Schwartz, Z., Carnes Jr, D.L., Pulliam, R., *et al.* Porcine fetal enamel matrix derivative stimulates proliferation but not differentiation of pre-osteoblastic 2T9 cells, inhibits proliferation and stimulates differentiation of osteoblast-like MG63 cells, and increases proliferation and differentiation of normal human osteoblast NHOst cells. *Journal of Periodontology* 2000; **71**:1287-

1296.

Segarini, P.R., Ziman, J.M., Kane, C.J. and Dasch, J.R. Two novel patterns of transforming growth factor beta (TGF-beta) binding to cell surface proteins are dependent upon the binding of TGF-beta 1 and indicate a mechanism of positive cooperativity. *Journal of Biological Chemistry* 1992; **267**:1048-53.

Soffer, E., Ouhayoun, J.P., Dosquet, C., Meunier, A. and Anagnostou, F. Effects of platelet lysates on select bone cell functions. *Clinical Oral Implants Research* 2004; **15**:581–588.

Streuli, C.H., Schmidhauser, C., Kobrin, M., Bissell, M.J. and Derynck, R. Extracellular matrix regulates expression of the TGF-beta 1 gene. *Journal of Cellular Biology* 1993; **120**:253-260.

Suaid, F.F., Ribeiro, F.V., Gomes, T.R., *et al.* Autologous periodontal ligament cells in the treatment of class III furcation defects: A study in dogs. *Journal of Clinical Periodontology* 2012; **39**:377-384.

Suzuki, S., Nagano, T., Yamakoshi, Y., *et al.* Enamel matrix derivative gel stimulates signal transduction of BMP and TGF-{beta}. *Journal of Dental Research* 2005; **84**:510-514.

Tatakis, D.N., Wikesjö, U.M., Razi, S.S., *et al.* Periodontal repair in dogs: effect of transforming growth factor-beta 1 on alveolar bone and cementum regeneration. *Journal of Clinical Periodontology* 2000; **27**:698-704.

Van der Pauw, M.T., Van den Bos, T., Everts, V. and Beersten, W. Enamel matrix derived protein stimulates attachment of periodontal ligament fibroblasts and enhances alkaline phosphatase activity and transforming growth factor beta1 release of periodontal ligament and gingival fibroblasts. *Journal of Periodontology* 2000; **71**:31-43.

Wada, Y., Yamamoto, H., Nanbu, S., Mizuno, M. and Tamura, M. The suppressive effect of enamel matrix derivative on osteocalcin gene expression of osteoblasts is neutralized by an antibody against TGF-beta. *Journal of Periodontology* 2008; **79**:341-347.

Wahl, S.M., Costa, G.L., Mizel, D.E., Allen, J.B., Skaleric, U. and Mangan, D.F. Role of transforming growth factor beta in the pathophysiology of chronic inflammation. *Journal of Periodontology* 1993; **64**:450-455.

Wennström, J.L. and Lindhe, J. Some effects of enamel matrix proteins on wound healing in the dento-gingival region. *Journal of Clinical Periodontology* 2002; **29**:9-14.

Wikesjö, U.M., Razi, S.S., Sigurdsson, T.J., *et al.* Periodontal repair in dogs: effect of recombinant human transforming growth factor-beta1 on guided tissue regeneration. *Journal of Clinical Periodontology* 1998; **25**:475-481.

Wikesjö, U.M. and Selvig, K.A. (1999) Periodontal wound healing and regeneration. *Periodontology 2000;* **19**:21-23.