AN ERA OF BIOMIMICRY: ENAMEL MATRIX DERIVATIVE

Review Article

*Nandita Jadeja, **Saket Rajput, ***Shilpi Shah, ****Mishal Shah

Abstract:

A group of leading experts were gathered to discuss what has now been 20 years of documented evidence supporting the clinical use of enamel matrix derivative (EMD). Original experiments led by Lars Hammarstrom demonstrated that enamel matrix proteins could serve as key regenerative proteins capable of promoting periodontal regeneration including new cementum, with functionally oriented inserting new periodontal ligament fibres, and new alveolar bone formation. This pioneering work and vision by Lars Hammarstrom has paved the way to an enormous amount of publications related to its biological basis and clinical use. Twenty years later, it is clear that all these studies have greatly contributed to our understanding of how biologics can act as mediators for periodontal regeneration and have provided additional clinical means to support tissue regeneration of the periodontium.

Keywords: Emdogain, Osteogain, Periodontal Regeneration.

Introduction:

Over 20 years ago, a team of researchers in Sweden including Lars Hammarstrom, Sven Lindskog and Leif Blomloff found that enamel matrix proteins (EMPs) could be utilized as a biological agent capable of periodontal regeneration (Hammarstrom *et al*. 1991, 1992, 1995). These reports originated from previous studies 15 years earlier by Lindskog *et al*. and Slavkin *et al*. reported that certain EMPs (which until then were considered enamel specific proteins) were deposited on the surface of developing tooth roots prior to cementum formation and may play a possible role in cementogenesis (Lindskog 1981a, b, Lindskog & Hammarstrom 1981, Slavkin *et al*. 1989). These observations led to the hypothesis that EMPs may play an integral role in the future differentiation of periodontal tissues prior to cementum formation, and has been the basis of a number of biological and clinical studies thereafter demonstrating that EMPs are proteins secreted by Hertwig's epithelial root sheet capable of promoting periodontal regeneration (Gestrelius *et al*. 1997 Hammarstrom *et al*. 1997, Heij 1997, Zetterstrom *et al*. 1997). The purified fraction derived from the enamel layer of developing porcine teeth was given the working name enamel matrix derivative (EMD) and has been the basis of numerous publications investigating its future use in periodontal regeneration.

The major components of EMD are amelogenins, a family of hydrophobic proteins that account for more than 90% of the total protein content derived from different splice variants and post-secretory regulation, all controlled from the expression of a single gene (Lyngstadaas *et al*. 2009). These proteins self-assemble into supramolecular aggregates that form an insoluble extracellular matrix and function to control the ultrastructural organization of the developing enamel crystallites (Lyngstadaas *et al*. 2009). Other proteins found in the enamel matrix include enamelin, ameloblastin (also called amelin or sheathlin), amelotin, apin and various proteinases (Bartlett *et al*. 2006, Margolis *et al*. 2006). Although these proteins are expressed in less quantity, further investigation has confirmed their valuable roles in various aspects of periodontal regeneration discussed later in this article. The aim of this review article is to provide the reader with four important aspects concerning integral research avenues on EMD over the past 20 years. Lastly, the article will discuss future avenues of research including the five key early studies leading to the development of Osteogain, a new product incorporating EMD with better physicochemical properties for improved protein

* PG Student, ** PG Student, ***Professor & Head, **** Reader

Department Of Periodontics And Oral Implantology,
Ahmedabad Dental College And Hospital, Gandhinagar, India

ADDRESS FOR AUTHOR CORRESPONDENCE : Dr. Nandita Jadeja, E-mail : nanditajadeja30@gmail.com, Ph. : +91 9106807044

adsorption of EMPs to bone grafting materials.

Biology of Periodontal Regeneration with Enamel Matrix Proteins:

The aim of the first section of this article is to summarize that EMD exerts a significant influence on cell behaviour of many cell types by mediating cell attachment, spreading, proliferation, differentiation and survival, as well as expression of transcription factors, growth factors, cytokines, extracellular matrix constituents and other molecules involved in the regulation of bone remodelling (Bosshardt 2008). Furthermore, EMD has been shown to play a significant role in wound healing favouring soft tissue regeneration and angiogenic activity (Miron *et al*. 2014b)

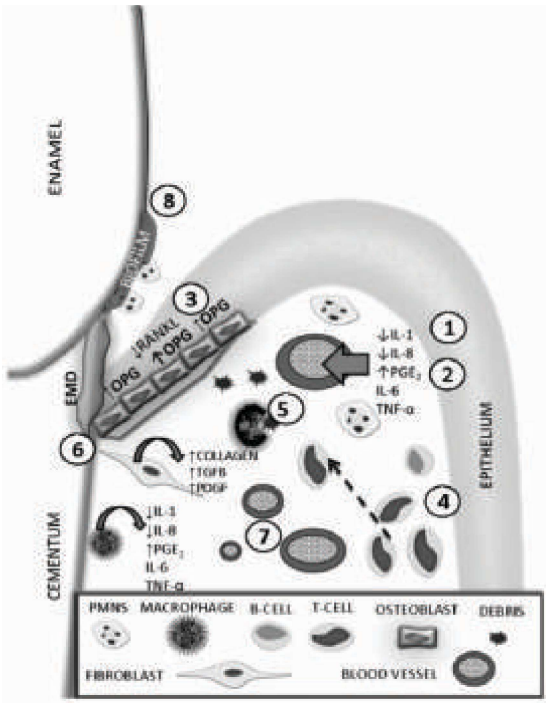

Fig. 1. Diagram depicting inflammation-modifying changes induced by enamel matrix derivative. Following application of EMD, decreased production of IL1b and IL8 (1) and increased levels of PGE2 (2) are observed with little differences in TNF-alpha expression. EMD also substantially changes the OPG/RANKL balance by increasing OPG and decreasing RANKL levels, resulting in diminished osteoclast formation/activity (3). EMD also increases the proliferation and migration of T-lymphocytes (4), which enable tissue debridement by macrophages (5). Furthermore, EMD promotes mesenchymal cell differentiation into hard tissue-forming cells and also improves PDL cell regeneration (6). Microvascular cell differentiation and angiogenesis are improved following EMD application (7) and studies demonstrate that EMD also lowers bacterial numbers (8), resulting in a reduced inflammatory state[1].

Clinical Applications of EMD:

The regeneration of lost periodontium remains the ultimate goal in periodontal regenerative therapy. A large number of techniques, including – but not limited to – root surface modification, bone and bone substitute grafting, GTR, biological mediators, and combination thereof have been employed to fulfil true periodontal regeneration. For each of the above-mentioned techniques, limitations and complications have been associated with their use, and it may thus not be surprising that the search for the ideal biomaterial capable of true periodontal regeneration continues. Over the years, the use of biologics (growth factors) has become more prominent in daily practice. A plethora of documented research from in vitro, in vivo and clinical trials is now available for enamel matrix proteins that now spans over two decades. In this section, we briefly summarize 20 years of clinical research and provide an evidenced-based flow chart for relevant clinical indications for the use of EMD either alone or in combination with a bone grafting material or barrier membrane.

Safety of EMD:

We start by describing the accumulated evidence for EMD used in a clinical setting regarding patient safety. It is important to note that amelogenins are a highly conserved gene across a variety of species including porcine and human. For these reasons, incompatibility or allergic reactions after treatment with EMD have not been reported in any clinical trial that were the direct result of EMD (Zetterstrom *et al*. 1997, Petinaki *et al*. 1998, Nikolopoulos *et al*. 2002, Froum *et al*. 2004). The results from this study further showed that treatment of intrabony defects with EMD resulted in a significant reduction in probing depths (PDs) and gain in clinical attachment level (CAL) (Froum *et al*. 2004). Following these preliminary human studies, the use of EMD has now been utilized for the treatment of a variety of defects in over 60 randomized clinical trials and over 1 million patients worldwide. No patient allergic reaction or adverse event has been reported over this 20 year period.

Clinical outcomes following non-surgical periodontal therapy:

To date, only two randomized, placebo-controlled clinical studies have evaluated the effects of EMD as adjunct to non-surgical periodontal therapy (Scaling and root planing) (Gutierrez *et al*. 2003, Mombelli *et al*. 2005). In both studies, EMD failed to show any beneficial effect. Therefore, it is recommended that EMD is combined with surgical periodontal therapy and a treatment guideline will be later provided highlighting the clinical indications supporting regenerative periodontal therapy with enamel matrix proteins.

Clinical outcomes in intrabony defects using EMD alone:

Heijl *et al*. published the first multicenter, randomized, placebo-controlled study evaluating the effectiveness of EMD for the treatment of intrabony defects. In that study, contra-laterally located intrabony defects were treated with either open flap debridement (OFD) alone or with additional application of EMD (Heijl *et al*. 1997). Following 36 months of healing, the results demonstrated that EMD significantly improved CAL gains and pocket depths. It was also concluded from radiographic analysis that a progressive bone gain following application with EMD amounted to 2.6 mm (66% fill) at the end of the evaluation period when compared to control defects, which showed no significant bone gain (Heijl *et al*. 1997). A subsequent controlled clinical study further showed that OFD in combination with EMD led to a three times greater defect fill when compared to OFD alone (Froum *et al*. 2001). Furthermore, additional benefits following regenerative procedures demonstrated that EMD led to significantly higher soft tissue density in three clinical studies (Trombelli *et al*. 2002, Yilmaz *et al*. 2003, Jentsch & Purschwitz 2008). Tonetti *et al*. investigated the use of EMD in regenerative therapy of deep intrabony defects in 172 patients with advanced chronic periodontitis in 12 centres (Tonetti *et al*. 2002). All patients had at least one intrabony defect of > or =3 mm. The surgical procedures included access for root instrumentation using either the simplified or the modified papilla preservation flap in order to obtain optimal tissue adaptation and primary closure.

After debridement, roots were conditioned for 2 min with a gel containing 24% EDTA followed by application of EMD in the test subjects, whereas omitted in the controls. The results of this trial indicated that regenerative periodontal surgery with EMD offers an additional benefit in terms of CAL gains, PPD reductions and predictability of outcomes with respect to papilla preservation flaps alone (Tonetti *et al*. 2002).

On the other hand, one randomized, doublemasked, placebo-controlled clinical trial failed to demonstrate any advantage for treatment of EMD when compared to placebo for the treatment of intrabony defects (Rosing *et al*. 2005). In 2009, Esposito *et al*. demonstrated in a Cochrane database systematic review that the use of EMD alone after 1 year significantly improved probing attachment levels (1.1 mm) and PPD reduction (0.9 mm) when compared to a placebo or control (Esposito *et al*. 2009). However, the high degree of heterogeneity observed among trials suggests that results should be interpreted with caution (Esposito *et al*. 2009).

Clinical outcomes in intrabony defects using EMD or GTR:

Another series of experiment focused primarily on comparing the use of EMD to GTR using either

non-resorbable or bioabsorbable membranes (Pontoriero *et al*. 1999). The results from these studies demonstrated that the use of EMD or GTR led to significantly comparable results and that both treatments led to substantially higher CAL gains and defect fill when compared to OFD alone for the treatment of single intrabony defects (Heijl *et al*. 1997, Pontoriero *et al*. 1999, Okuda *et al*. 2000, Silvestri *et al*. 2000, Froum *et al*. 2001, Sculean *et al*. 2001b, Tonetti *et al*. 2002, Zucchelli *et al*. 2002). Furthermore, the use of EMD in combination with antibiotics or root conditioning agents was investigated. It was found that the use of EMD in combination with postoperative administration of an antibiotic regimen (i.e. amoxicillin and metronidazole (Sculean *et al*. 2001a,b) or doxycycline (Eickholz *et al*. 2014)), a selective cyclooxygenase-2 inhibitor, or EDTA root conditioning did not additionally enhance periodontal regeneration (Sculean *et al*. 2001a, 2003a, 2006, Parashis *et al*. 2006, Eickholz *et al*. 2014).

Interestingly, a new series of studies have now reported that the effects of EMD may be maximized when minimally invasive surgical techniques (MIST) are applied, thus improving initial wound stability while minimizing patient morbidity (Cortellini & Tonetti 2007, Cortellini *et al*. 2008, Harrel *et al*. 2010). Although these authors show that MIST alone provides similar results to MIST plus EMD, these concepts have been the basis of more focused research in recent years and future investigation aims to predictably restore lost periodontal tissues via minimally invasive surgeries as discussed later in this article. These data indicate that although the use of EMD is generally characterized by improved periodontal regeneration with or without membrane use, the findings from a number of clinical studies have demonstrated that anatomical factors such as defect configuration seem to play an important role in EMD-induced periodontal regeneration. This concept is further discussed within the subsection on clinical indications for EMD.

Clinical outcomes in recession defects using EMD alone or as adjunct to soft tissue grafting:

The use of EMD has been investigated in several controlled clinical studies for the treatment of buccal Miller class I and II gingival recessions by means of coronally advanced flap (CAF). In the majority of cases, the additional use of EMD led to more formation of keratinized tissue and long-term stability of the results compared to CAF alone (Hagewald *et al*. 2002, Cueva *et al*. 2004, Spahr *et al*. 2005, Castellanos *et al*. 2006, Pilloni *et al*. 2006, Cairo *et al*. 2008, 2014) (Fig. 4). One randomized controlled clinical study comparing treatment of Miller class I and II recessions demonstrated that after a healing period of 2 years, complete root coverage could be maintained in 53% in patients treated with EMD versus 23% in the control group (Spahr *et al*. 2005). Comparable results were reported from various other groups for the treatment of either Miller class I or class 2 recession defects with topical application of EMD leading to better results (Cueva *et al*. 2004, Castellanos *et al*. 2006, Pilloni *et al*. 2006, Cairo *et al*. 2008). Another study has compared the use of EMD to a connective tissue graft (CTG) for the treatment of buccal Miller class I and II recessions with CAF (McGuire & Nunn 2003). The results from that study demonstrated very similar results after 1 year for mean root coverage.

A recent consensus conference concluded that at single recessions, the addition of autologous CTG or EMD under CAF improves complete root coverage and may be considered the procedure of choice at maxillary anterior and premolar teeth (Tonetti & Jepsen 2014). Histological evaluation of human biopsies in recession defects was then performed to analyse periodontal regeneration (Heijl 1997, McGuire & Cochran 2003). It was found that the application of EMD during conjunction with CAF resulted in enhanced formation of root cementum, periodontal ligament and alveolar bone while treatment with a CAF and a connective graft or CAF alone (McGuire & Cochran 2003) was characterized by a long junctional epithelium and even signs of root resorption. Comparable results were reported in a multicenter, controlled clinical trial (Rasperini *et al*. 2011). More recently, Roman *et al*. evaluated whether the combination of EMD with a subepithelial connective tissue graft (SCTG) plus CAF would further improve the treatment outcomes of Miller class I and II gingival recessions in 42 patients (Roman *et al*. 2013). Cordaro *et al*. (2012) compared, in a splitmouth

design, CAF with or without EMD for coverage of multiple gingival recession defects with follow-up at 6- and 24 months.

Clinical measurements (recession length, keratinized tissue, probing depth and clinical attachment level) were assessed at baseline and 6 and 24 months after surgery by a blinded examiner. Thus, the accumulated evidence from these studies suggests that the use of EMD for the treatment of gingival recessions utilized alone is capable of enhancing regeneration and improves soft tissue height/ thickness, while the combination with SCTG may further support recession coverage; however, this approach presents great variability in the clinical parameters analysed (Henriques *et al*. 2010, Rasperini *et al*. 2011).

Clinical outcomes with EMD in furcation defects:

The data on the efficacy of the use of EMD in the regenerative therapy of furcation defects are still limited (Sanz *et al*. 2015). In a multicentre, randomized, controlled, split-mouth, clinical trial of mandibular buccal class II furcation defects, a total of 45 patients with 90 comparable defects on contra-lateral molars were treated with either EMD or GTR (Jepsen *et al*. 2004, Meyle *et al*. 2004, Hoffmann *et al*. 2006). At 8 and 14 months, both treatment modalities led to significant clinical improvements. The EMD group showed significantly better results with regard to the primary outcome reduction in horizontal furcation depth as assessed during a 14 months re-entry procedure. Enamel matrix derivative demonstrated a mean reduction in horizontal probing bone level of 2.6 + /- 1.8 mm, and the guided tissue regeneration-treated sites showed a horizontal probing bone level reduction of 1.9 + /- 1.4 mm. Furthermore, with regard to patient-centred outcomes, postoperative wound healing as assessed by questionnaires on pain and swelling was superior following EMD application.

In proximal class II furcation defects, the use of EMD led to a higher conversion rate into class I when compared to OFD alone although complete furcation closure was only rarely found (Casarin *et al*. 2010). In another trial on the treatment of proximal class II furcation defects, the effects of OFD + hydroxyapatite (HA)/b-tricalcium phosphate (b-TCP) filling, or OFD + HA/b-TCP + EMD were evaluated (Peres *et al*. 2013). No significant difference was reported between treatment modalities 6 months after therapy (Peres *et al*. 2013). In summary, the limited data on the effects of EMD in regenerative furcation therapy is encouraging, however, more evidence from further well-controlled studies is clearly needed.

Future Perspectives:

Although EMD has been utilized for a variety of clinical applications over the past 20 years, research concerning its clinical use as well as basic research to further understand its properties and biological effects are still ongoing. This section is divided into the following six subsections: (1) future use of EMD in minimally invasive surgeries, (2) use of EMD for the treatment of supra-alveolar type defects, (3) possible use of EMD for the treatment of periimplantitis and mucosal recessions around implants, (4) characteristics of various fractions of EMD, (5) development of Osteogain, a new product incorporating EMD with better physicochemical properties for bone grafting material adsorption and (6) final remarks.

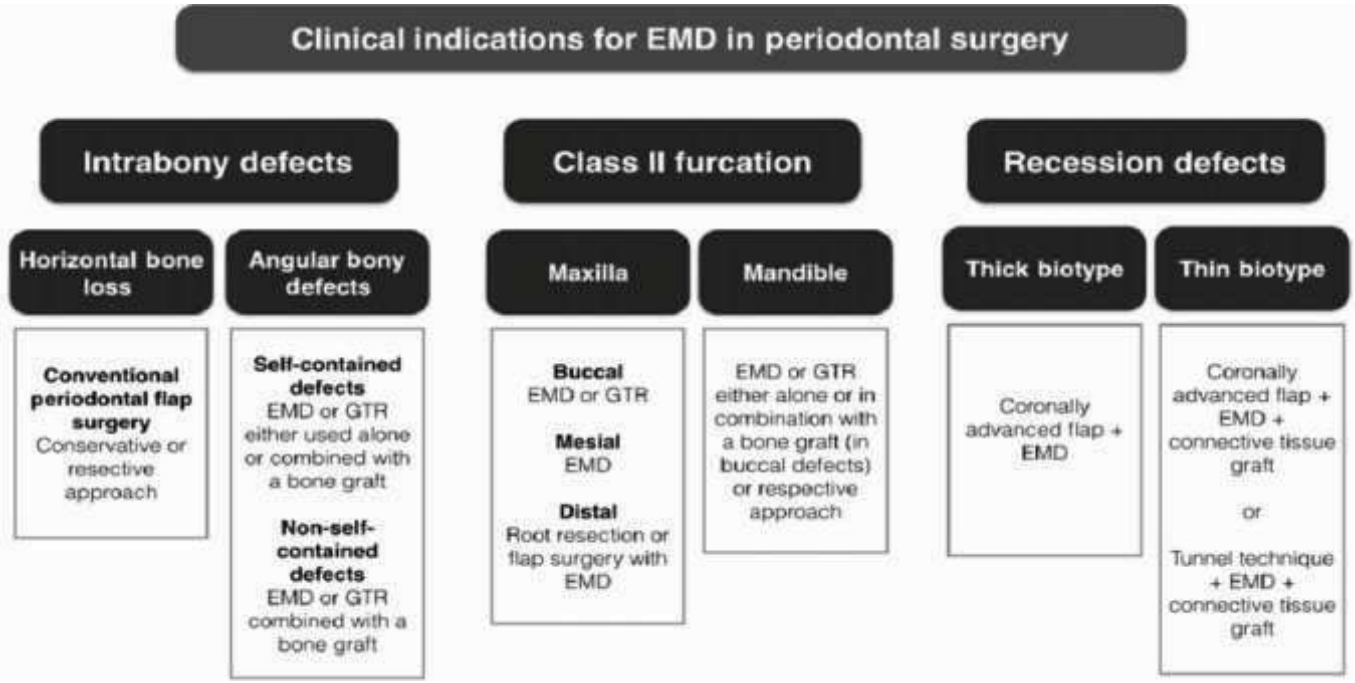

Fig. 2. Flow Chart – clinical indications for the use of EMD in periodontal surgery. Intrabony defect, furcation defect and recession defect regeneration have all demonstrated long-term clinical improvements following treatment with EMD in certain clinical indications[1].

Development of Osteogain, a new carrier system for EMD:

The clinical combination of EMD with a bone grafting material has been one of the most widely used biomaterial combinations utilized for the treatment of intrabony defects. While the majority of studies combining EMD with a membrane do not lead to additional improvements, the use of EMD with a bone grafting material has demonstrated additional clinical advantages. While a recent systematic review and meta-analysis found that the combination of bone grafting material + EMD led to statistically significant better outcomes, large variability between studies were also reported (Lekovic *et al*. 2000, Velasquez-Plata *et al*. 2002, Zucchelli *et al*. 2003, Gurinsky *et al*. 2004, Kuru *et al*. 2006, Guida *et al*. 2007, Trombelli & Farina 2008).

In vitro results have also indicated variability in gene expression when primary human osteoblasts and PDL cells were cultured on various bone grafting materials in vitro with or without EMD thus raising the concern that protein function, stability or adsorption may be responsible factors in the gel-delivery system currently utilized for EMD Recently, the adsorption of amelogenins to bone grafting materials under various conditions was investigated (Miron *et al*. 2015a). These results confirm that large variability existed between the adsorption of amelogenins to different bone grafting material.

More importantly, it was found that the commercially available EMD-gel (Emdogain) adsorbed significantly less protein when compared to a liquid formulation of EMD. These preliminary findings led to a series of five subsequent studies over the past 3 years during the developmental phases of Osteogain, a new product incorporating EMD with better physicochemical properties specifically designed for combining EMD with bone grafting materials.

Conclusion:

It remains hard to believe that over 20 years have now passed since enamel matrix derivative was first introduced as a regenerative agent for periodontal tissues. Equally as surprising, it remains one of the only biomaterials still available for clinical use capable of histologically demonstrating true periodontal regeneration with new cementum formation, periodontal ligament and alveolar bone along with inserting Sharpeys fibres spanning the periodontal apparatus. It is clear that over the years, we have learned a great deal regarding the biological roles of specific

enamel matrix proteins and future investigation is constantly underway to further characterize their effects on cell and tissue behaviour.

It also becomes clinically important to further investigate the use of EMD in both carrier systems described to determine if regenerative outcomes can be even further improved by slight modifications in EMD-carrier systems or through minimally invasive surgeries. During these 20 years, over 900 publications documenting the use of EMD for a variety of in vitro and in vivo studies as well as numerous clinical trials. EMD has remained one of the gold standards for periodontal regeneration using biologics and it remains of interest to discover how the next 20 years of intensive research will further improve EMD clinical outcomes.

References:

1. Miron RJ, Sculean A, Cochran DL, Froum S, Zucchelli G, Nemcovsky C, Donos N, Lyngstadaas SP, Deschner J, Dard M, Stavropoulos A. Twenty years of enamel matrix derivative: the past, the present and the future. J Clin periodontol. 2016;43(8):668-83.

2. Carnio J, Camargo PM, Kenney EB, Schenk RK. Histological evaluation of 4 cases of root coverage following a connective tissue graft combined with an enamel matrix derivative preparation. J Periodontol. 2002;73(12):1534-43.

3. Casarin, R. C., Ribeiro Edel, P., Nociti, F. H. Jr, Sallum, A. W., Ambrosano, G. M., Sallum, E. A. & Casati, M. Z. (2010) Enamel matrix derivative proteins for the treatment of proximal class II furcation involvements: a prospective 24-month randomized clinical trial. J Periodontol 37, 1100–1109.

4. Castellanos, A., de la Rosa, M., de la Garza, M. & Caffesse, R. G. (2006) Enamel matrix derivative and coronal flaps to cover marginal tissue recessions. J Periodontol 77, 7–14.

5. Chadwick, P. & Acton, C. (2009) The use of amelogenin protein in the treatment of hard-to-heal wounds. British Journal of Nursing 18, S22.

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

7. Chong, C. H., Carnes, D. L., Moritz, A. J., Oates, T., Ryu, O. H., Simmer, J. & Cochran, D. L. (2006) Human periodontal fibroblast response to enamel matrix derivative, amelogenin, and platelet-derived growth factor-BB. J Periodontol 77, 1242–1252.