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RESEARCH ARTICLE

GAIN FOR PERIODONTAL TISSUES: EMDOGAIN®, AN ENAMEL MATRIX DERIVATIVE

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ABSTRACT

Regenerative periodontal surgeries are performed to stimulate lost periodontal tissues that were affected by periodontal disease. Surgical procedures involving root conditioning, autografts, allografts, xenografts, non-bone graft materials and even barrier membranes for guided tissue regeneration (GTR) have been used successfully in regenerative procedures. Enamel matrix derivative (EMD, Emdogain®) is one such modality, which has been designated as Osteopromotive, is used to promote periodontal regeneration, consisting of a formulation of amelogenin proteins derived from six month- old piglets. Being a xenograft and its tendency for stimulating immune reaction has led to numerous studies in-vitro and in- vivo both in animals and humans. Studies have been conducted not only focusing on the safety and effectiveness but also in terms of wound healing and in combination with other regenerative materials available. Through this paper, an attempt has been made to analyze the derivative of enamel matrix, Emdogain®.

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INTRODUCTION

Reconstructive periodontal surgery aims at predictably restoring tooth's supporting structure lost due to periodontal disease or trauma. One such modality, which has been used to promote periodontal regeneration, is an enamel matrix derivative (EMD), consisting of a formulation of amelogenin proteins from developing porcine enamel. Therapeutic approaches to the treatment of periodontitis generally fall into two major categories: those designed to halt the progression of periodontal attachment loss, and those designed to regenerate or reconstruct lost periodontal tissues (Pihlstrom and Ammons, 1997). Researchers have increased their efforts to seek procedures and materials to promote periodontal regeneration (Venezia *et al.,* 2004). Surgical procedures involving root conditioning, grafts (auto/ allo/ xeno) and/or barrier membranes for guided tissue regeneration (GTR) have been shown to contribute to a successful regenerative outcome (Garrett, 1996). Boyan *et al*., 2000 concluded that the enamel matrix derivative is not osteoinductive, but it is "osteopromotive" in that it stimulates bone formation when combined with demineralized freeze-dried bone allograft. 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. These reports originated from previous studies fifteen 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 (Miron *et al.,* 2016).

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Biologic basis that led to the advent of EMD

According to the classic theory of root formation and attachment apparatus development, Hertwig's epithelial root sheath (HERS), which is the apical extension of the enamel organ, induces the mesenchymal cells of the dental papilla to form the mantle predentin before it disintegrates and leaves the root surface. As a result of HERS apoptosis during the embryonic process, the physical barrier it forms between the mesenchymal cells of the dentinal follicle and the forming dentin disintegrates. The mesenchymal cells that have become exposed to the newly formed dentin are induced to differentiate into cementoblasts, hence responsible for cementogenesis. This process is a prerequisite for the formation of both the periodontal ligament and the alveolar bone for the completion of the attachment apparatus development (Armitage, 1991).

Table 1. Impact of EMD

Study	Objective	Outcome
Gestrelius *et al*., 1997	Rats and pigs with radio-labeled protein were observed to check the benefits of EMD	EMD adsorbs both to hydroxyapatite and collagen and to denuded dental roots and exhibited insoluble spherical complexes, and detectable amounts which remain at the treated site on the root surface for up to 2 weeks.
Iwata *et al*., 2002	Examined non-commercial fractionated enamel extracts from developing pig teeth.	Low levels of BMP were found in these enamel extracts.

Table 2: Effect of EMD on periodontal ligament (PDL) cells in culture

Study	Objective	Outcome
Gestrelius *et al*., 1997	The mechanisms by which EMD promotes regeneration of periodontal tissues.	Enhanced proliferation of PDL cells, but not epithelial cells. It increased total protein production by PDL cells and promoted mineralized nodule formation of PDL cells. EMD had no significant effect on migration or attachment and spreading of PDL cells.
Gestrelius *et al*., 1997; Kawase *et al*., 2000	EMD and its effect upon cultured epithelial cells.	EMD seems to exhibit a cytostatic effect upon cultured epithelial cells. This may explain EMD's biological 'guided tissue regeneration', analogous to the mechanical prevention of barrier membranes.
Hoang *et al*., 2000	The specificity of the effect of EMD on human PDL cells.	When the cultured cells were exposed to EMD during a healing period of up to 9 days, an enhanced wound-fill was observed.
Haase and Bartold, 2001	The effect of EMD on matrix synthesis was investigated with the use of cultured periodontal fibroblast.	EMD significantly affected the mRNA levels for matrix proteoglycans and stimulated hyaluronic acid synthesis.
Lyngstadaas *et al*., 2001	Response of cells involved in periodontal regeneration to EMD in a comparable manner and also to check the attachment rate, growth factor production (TGF-b1, IL-6, and PDGF-AB), proliferation, and metabolism .	Not all cells involved in periodontal regeneration respond to EMD in a comparable manner. Attachment rate, growth factor production (TGF-b1, IL-6, and PDGF-AB), proliferation, and metabolism of human PDL cells in culture were all significantly increased in the presence of EMD
Hamamoto *et al*., 2002	Immunohistochemical analysis on extracted rat molars that were transplanted to the abdominal wall.	Demonstrated that EMD was still present for 4 weeks after its application
Hoang *et al*., 2002	Therapeutic effect of EMD in periodontal regeneration.	Amelogenin was shown to have a cell-adhesive activity, which may partially explain the therapeutic effect of EMD in periodontal regeneration.
Davenport *et al*., 2003	Examine the influence of EMD on the viability, proliferation, and attachment of human PDL fibroblasts to diseased root surfaces.	The viability of PDL cells was negatively affected by higher doses of EMD over time, while lower doses elicited no change when compared with control cultures.

The enamel matrix was generally believed to regulate the initiation, propagation, termination, and maturation of the enamel hydroxyapatite crystallites (Venezia *et al.,* 2004). Autoradiographic and scanning electron microscopy studies provide additional evidence that following apoptosis of HERS cells and deposition of the enamel matrix proteins onto the dentin surface, the cementogenesis process is initiated and kept modulated by these proteins (Lindskog, 1982; Slavkin *et al.,* 1989).

Composition of EMD

Commercially it is available as Emdogain (Biora AB, Malmo, Sweden), is a purified acidic extract of developing embryonal enamel derived from six month-old piglets to treat periodontal defects (Hammarstrom, 1997; Heijl, 1997). A medium or vehicle is required to deliver Enamel Matrix Proteins (which are in the form of Emdogain) at the site of periodontal defect. The amelogenins, which are the hydrophobic constituents of the enamel matrix proteins, aggregate and become almost insoluble at physiologic pH and temperature. They can be dissolved in an acidic or alkaline pH environment and at low temperature. A suitable formulation should thus have a non-neutral pH and allow for gradual reprecipitation of the matrix when physiologic conditions are re-established. PGA (propylene glycol alginate) appears to enhance EMD precipitation, thus exposing the periodontal ligament cells to the re-established protein aggregate and allowing matrix-cell interactions to take place. The other vehicles that were tested, although stable at neutral pH, appeared to prevent exposure of periodontal ligament cells to the proteins (Hammarstrom, 1997).

Clinical safety

Commercial formulation of EMD is a porcine derived xenograft, thus may stimulate an immune reaction when used in humans is of extreme importance. The enamel matrix proteins are highly conserved among mammalian species, and exposure to these proteins takes place during tooth development in early childhood. Thus, tolerance should normally be induced and the proteins recognized by the immune system as “self” proteins. Hence, they are less likely to act as antigens. In vitro studies show that EMD does not significantly modify cellular or humoral immune responses. Very high concentrations of EMD induced only a slight increase in proliferation of human lymphocytes, restricted to the CD25+ (interleukin-2 receptor) fraction of CD4+ T lymphocytes. There was a concomitant decrease in B lymphocytes, while other cell fractions (CD8+ T cells, B cells, and natural killer cells) were not affected, and immunoglobulin and cytokine (interleukin-2 and interleukin-6) production was not modified (Peteinaki *et al.,* 1988).

Studies

A. In vitro studies
B. In vivo studies
   a) Animal
   b) Human

Studies have been conducted in vitro and in vivo to know about the clinical safety, mode of action, effectiveness and also studied in combination with other regenerative procedures like GTR and Bone grafts.

Table 3. EMD on Cementum and bone

Study	Objective	Outcome
Tokiyasu *et al*., 2000	Changes in tissues undergoing regeneration and repair.	EMD was found to regulate cementoblast and osteoblast activities.
Hakki *et al*., 2001	EMD and Epithelial-mesenchymal interactions	Epithelial-mesenchymal interactions may be important during the development of periodontal tissues, and that EMD can influence the process at multiple stages of differentiation
Ohyama *et al*., 2002	If EMD induces osteochondral progenitor cells to differentiate.	EMD may have the ability to induce osteochondral progenitor cells to differentiate. In a multipotent mesenchymal cell line, it was shown that EMD converts the differentiation pathway of the mesenchymal cells into osteoblasts and/or chondroblasts

Table 4. EMD on periodontal pathogens

Study	Objective	Outcome
Spahr *et al*., 2002	The effect of EMD on the growth of periodontal pathogens.	Marked inhibitory effect of EMD on the growth of the Gram negative periodontal pathogens was demonstrated, and the Gram-positive bacteria were unaffected.

Table 5. EMD and bone

Study	Objective	Outcome
Hammarström,1997	In monkeys, the ability of EMD to regenerate acellular extrinsic fiber cementum was first demonstrated. With surgically created buccal dehiscences of 6 mm in both sides of the monkeys' maxillae were treated either with EMD (following root conditioning with acid), with or without vehicles, or served as controls (conditioned with the acid and given no further treatment).	Acellular cementum attached to the dentin was induced after 8 weeks of healing. It was possible to obtain regeneration of 60-80% of the cementum defect by the application of either the whole enamel matrix or the acid extract of EMD to the denuded root surface. New bone formed to a slightly lesser extent.
Boyan *et al.*[4], 2000	The specific characteristic of EMD regarding its bone formation ability (osteoinductive, osteoconductive, or osteogenic) was examined by means of a nude mouse muscle implantation assay.	If EMD was implanted together with DFDBA that had limited osteoinduction ability, EMD had no detectable effect. However, active DFDBA and EMD above a threshold dose (4 mg) resulted in enhanced bone induction compared with inactive DFDBA or active DFDBA without EMD.
Kawana *et al.*[27], 2001	Locally applied EMD on bone and medullary regeneration was evaluated with the use of rat femurs in a drill-hole injury model.	Bone volume fraction of newly formed bone trabeculae on day 7 post-operatively was significantly higher in the EMD group than in the controls. EMD possesses an osteogenic effect on bone and medullary regeneration during wound healing of injured long bones

Table 6. EMD and GTR

Study	Objective	Outcome
Araujo and Lindhe, 1998	EMD was compared with a combination of EMD and GTR in the treatment of class III furcation defects in dogs.	No histological benefits in terms of periodontal regeneration were observed.
Sculean *et al*., 2000	Fenestration-type defects produced surgically in the buccal bone of monkeys were treated with EMD, GTR, or coronally repositioned flap (control). After 5 months descriptive histological evaluation of the healing was performed.	The results showed that, in the GTR group, new connective tissue attachment and new bone formation had consistently occurred, whereas, in the defects treated with EMD or with coronally repositioned flaps, new attachment and new bone formed to various extents. It was concluded that GTR treatment seems to be more predictable than EMD in terms of periodontal regeneration.

Table 7. EMD and its effect on immune reaction and healing

Study	Objective	Outcome
Zatterström *et al*., 1997	The clinical safety of EMD was first evaluated in humans that assessed the changes in IgE, IgG, IgM, and IgA.	There was no increase in these antibodies among the patients.
Heard *et al*., 2000	Multiple applications of Emdogain on periodontal wound healing, as was determined from clinical signs and symptoms reported by the treated patients	Safe product. did not have any negative impact on periodontal wound healing.
Okuda *et al*., 2001	Early wound-healing process has been evaluated by assessments of the protein levels of matrix metalloproteinases and tissue inhibitors of metalloproteinases in gingival crevicular fluid.	Emdogain treated sites showed accelerated wound healing following surgery.

Table 8. Effectiveness of EMD

Study	Objective	Outcome
Heijl *et al.*, 1997	One of the first human studies undertaken to compare the long term effect of EMD treatment as an adjunct to Modified Widman flap (MWF) surgery *vs.* MWF plus a placebo (PGA) Patients with test and control sites (one- or two-wall bony defects > 4 mm deep) were enrolled in the study and monitored for 36 months.	The results in the EMD group showed a gain in the clinical attachment level, probing depth reduction, and restoration of bone radiographically.
Wikesjø and Selvig,1999	Most of the clinical trials and case reports have used EMD for the treatment of intrabony defects, since horizontal bone loss defects are not likely to exhibit a successful outcome with regenerative treatment.	
Yilmaz *et al.*, 2003	EMD was also shown to achieve better clinical improvement in periodontal sites with horizontal bone loss as compared with conventional flap debridement procedures	
Sculean *et al*., 2003	Histologically, healing of advanced intrabony periodontal defects in humans following non-surgical periodontal therapy with subgingival application of EMD	Failed to demonstrate regeneration.

Table 9. Histologic reports

Study	Objective	Outcome
Heijl, 1997	The first human histological report assessing the effect of EMD on periodontal regeneration used a mandibular incisor scheduled for extraction due to orthodontic reasons.	Microscopic examination revealed formation of new acellular cementum, new periodontal ligament with inserting and functionally oriented collagen fibers, and associated alveolar bone. The new cementum covered 73% of the original defect. New bone gain was 65% of the pre-surgical bone height.
Sculean *et al*., 2002	By histological and immunochemical methods, to evaluate presence of EMD treated root surfaces following application during periodontal surgery	It was found that EMD is present on treated root surfaces for up to 4 weeks following application during periodontal surgery

Table 10. EMD and GTR

Study	Objective	Outcome
Silvestri *et al*.,2000	Effectiveness of 2 surgical treatment modalities; EMD and GTR	GTR provided better results than EMD in terms of % clinical attachment gain in patients with a baseline clinical attachment loss > 9 mm. Conversely, EMD appeared to be better than GTR in patients with a baseline clinical attachment loss < 9 mm
Pietruska, 2001	Compared EMD with GTR combined with a bovine-derived hydroxyapatite xenograft.	No significant differences in outcomes were found.

Healing pattern of periodontal tissues is influenced by the epithelial down-growth along the root surface, which is known to prevent the re-establishment of the normal periodontal architecture after any kind of surgical treatment (Nyman *et al.,* 1982). Application of EMD results in limited epithelial down-growth (Hammarstrom, 1997). In vitro studies were conducted to examine the mode of action of EMD on cells that help in periodontal regeneration. Table 1-4 briefly outlines the outcomes of studies conducted *in-vitro*.

In vivo animal studies: Table 5 and 6 highlights the studies performed in animals' in-vivo and their result.

In vivo human studies

Table 7- 10 focuses on the different human studies conducted with respect to EMD. Moreover, Mellonig in 1999 used EMD in combination with bone grafts and suggested that the EMD formulation was semi-fluid in consistency and lacked the space-maintenance ability of solid graft materials. Because space maintenance is a desirable physical characteristic of a regenerative material, particularly if bone formation is one of the treatment objectives, it was advised to use a combination of demineralized freeze-dried bone allograft (DFDBA) and EMD to overcome problems related to EMD fluidity.

DISCUSSION AND CONCLUSION

Borne graft materials are generally evaluated based on their osteogenic, osteoinductive, or osteoconductive potential. Osteogenesis refers to the formation or development of new bone by cells contained in the graft. Osteoinduction is a chemical process by which molecules contained in the graft (bone morphogenetic proteins) convert the neighboring cells into osteoblasts, which in turn form bone. Osteoconduction is a physical effect by which the matrix of the graft forms a scaffold that favors outside cells to penetrate the graft and form new hone (Carranza *et al.,* 2006). Forum *et al* have analyzed the criteria that should guide the choice of treatment technique for this osteopromotive agent. They believed that clinical results depend on (1) the dimension and morphology of the detect (deeper lesions result in greater bone fill than shallower defects), (2) the number of walls in the defect (three-wall detects have greater potential to fill than two-wall or one-wall detects), (3) the amount of root surface exposed and the ability to obtain adequate flap coverage, and (4) the angle of the defect to the long axis of the tooth (the smaller the angle, the better chance of success) (Carranza *et al.,* 2006; Froum *et al.,* 2001).

Surgical periodontal defects treated with EMD in true sense proved to be beneficial in terms of hard and soft tissue parameters but as with any other product, Emdogain® too has certain limitations; major being its availability in our country. With an anticipation of its availability in near future more studies can be conducted, evaluated and analyzed, broadening the horizons of its usage.

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