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ENAMEL MATRIX PROTEINS: A PARADIGM SHIFT FROM REPAIR TO REGENERATION

Periodontics

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ABSTRACT

Regenerative periodontal therapy aims at reconstitution of the lost periodontal structures such as new formation of root cementum, periodontal ligament and alveolar bone. Findings from basic research indicate that enamel matrix protein derivative (EMD) has a key role in periodontal wound healing. Histological results from animal and human studies have shown that treatment with EMD promotes periodontal regeneration. Moreover, clinical studies have indicated that treatment with EMD positively influences periodontal wound healing in humans. This overview aims to provide evidence-based clinical applications of EMD for periodontal regenerative therapy.

KEYWORDS:

Enamel matrix protein derivative, Cementogenesis, Regenerative periodontal therapy, Emdogain

Introduction:

Regeneration is the reproduction or reconstitution of a lost or injured part1. Regeneration is the formation of new cementum, periodontal ligament, and alveolar bone following periodontal surgery after pathologic exposure of the root surface has occurred secondary to inflammatory periodontal diseases. Regeneration is important because it is widely assumed that the tissues generated during this process are more resistant to breakdown than tissues obtained where healing occurs by repair.

Acellular cementum is the most important tissue for the insertion of collagen fibers. It plays the largest role in attaching the tooth to the alveolar socket[2]. Studies by Slavkin and Boyde[3] and Slavkin[4] have shown that proteins, secreted during tooth development by the Hertwig's epithelial root sheath (HERS), play a crucial role in the formation of acellular root cementum. These proteins, referred to as enamel matrix proteins, constitute the largest proportion of the enamel matrix[2,5].

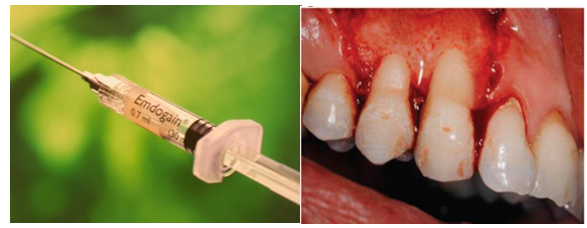
Enamel matrix proteins consist of a whole family of proteins (EMP), of which 90% are amelogenins, and the remaining 10% proline-rich non-amelogenins, tuftelin and other serum proteins5. The clinical use of an enamel matrix derivative (EMD) has been successfully proved in periodontal surgery, as promoting regeneration of periodontal tissues including new cementum, periodontal ligament (PDL) and alveolar bone[6].

EMD in the form of a purified acid extract of proteins from pig enamel matrix (Emdogain®) has been successfully employed to restore functional periodontal ligament, cementum and alveolar bone in patients with severe attachment loss. The first studies on clinical applications with EMD were published in 19977,[8] and since then a wide number of research groups have studied the mechanism of actions of EMD and the clinical potential.

The enamel matrix proteins in the developing root:

The enamel matrix was generally believed to regulate the initiation, propagation, termination, and maturation of the enamel hydroxy apatite crystallites (Simmer and Snead, 1995)9. Other findings indicate that the enamel matrix also has a function outside the developing enamel. Enamel matrix proteins are temporarily deposited onto the dentinal root surface and provide an initial and essential step in the formation of acellular cementum (Slavkin, 1976)[10].

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. Immunological (Slavkin *et al.*, 1989)[11] and immune-histochemical[2] methods both show that enamel matrix proteins are present in acellular cementum, accentuating the importance of these proteins in the cementogenesis process.



Composition:

The major fraction of the enamel matrix proteins is composed of the amelogenins, a family of hydrophobic proteins that account for more than 90% of the organic constituent of the enamel matrix[5]. The amelogenins have remained remarkably well-conserved through evolution, suggesting that they may have great functional importance[5]. The second largest component of the enamel matrix proteins is the enamelins[5]. Since the enamelins were found to contain serum proteins (Limeback *et al.*, 1989; Strawich and Glimcher, 1990), the more general term "nonamelogenin" is now commonly used to describe this high-molecular- weight fraction[2]. It includes proline-rich enamelin (Fukae and Tanabe, 1987), tuftelin (Deutsch et al., 1991), and tuft proteins (Robinson *et al.*, 1975). Three matrix proteins, corresponding to amelogenin (Hu et al., 1996), enamelin and sheathlin (also called ameloblastin or amelin) (Hu *et al.*, 1997)12, and 2 enzymes, corresponding to MMP-20 (Fukae et al., 1998) and enamel matrix serine proteinase1 (EMSP1) (Simmer et al., 1998), have been purified and the cDNA cloned from developing porcine teeth[13].

Emdogain® Formulation:

A commercial enamel matrix derivative (Emdogain®, Biora AB, Malmö, Sweden, Straumann AG, Basel, Switzerland) received FDA approval and is now available for the treatment of periodontal defects. It is a purified acidic extract of developing embryonal enamel derived from six month- old piglets. Its purpose is to act as a tissue-healing modulator that would mimic the events that occur during root development and to help stimulate periodontal regeneration[2,7]. The enamel proteins described above are present in Emdogain®.

The Emdogain® Vehicle:

The amelogenins, which are the hydrophobic constituent of the enamel matrix proteins, aggregate and become practically insoluble at physiological pH and body 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 re-precipitation of the matrix when physiological conditions are re-established. PGA appears to enhance EMD precipitation, thus exposing the periodontal ligament cells to the re-established protein

aggregate and allowing the matrix:cell interactions to take place.

The other vehicles that were tested, which were stable at neutral pH, appear to exclude the periodontal ligament cells from exposure to the proteins. The neutral pH of PGA in solution was useful for dissolving EMD, even at room temperature. Furthermore, the thixotropic rheology (i.e., the characteristics of a fluid to undergo a decrease in viscosity with time while it is subjected to constant shearing) of PGA permitted the application of EMD as a viscous formulation. The viscosity of PGA decreases under physiological conditions; thus, EMD is "released" to precipitate on the exposed root surfaces in the treated area. Thus, PGA solutions fulfill the essential requirements of a vehicle to facilitate the application of EMD during periodontal surgery.

The first marketed EMD product was supplied in a lyophilized form and was dissolved in an aqueous solution of PGA immediately prior to use. Because mixing EMD with PGA needs extra assistance and time, a new ready-to-use product, Emdogain® Gel (Biora AB, Malmö, Sweden), was developed. It is a pre-mixed formulation of EMD, where the protein has been stabilized by heat treatment prior to being mixed with the vehicle. Both formulations contain 30 mg EMD protein/mL PGA gel, with a viscosity of about 2.5 PAS (and shear-thinning rheology).

EMD Properties:
Epithelial down-growth along the root surface, is known to prevent the re-establishment of the normal periodontal architecture[14.]

Application of EMD results in limited epithelial down-growth,.

Kinetics and cell colonization after application of emdogain on root surface:

Precipitation of EMD matrix within 1 hour:
Following closure, a shift in the pH and temperature occurs that causes the Emdogain to precipitate as an aggregate on the root surface. Once it has precipitated, it becomes insoluble. Emdogain precipitates in to aggregates in the form of spheres or short rods. In pH range of 4 and 6-Emdogain is removed from an aqueous solution and adsorbed to mineral particles.

Kinetics after application onto dental roots in vivo:
Radioiodinated Emdogain in PGA solution had a dual elimination curve

1. Initial rapid washout (2-6 hours) this is due to the excess of formulation that escapes to the mouth and gets swallowed.
2. Followed by much slower phase with a half-life of 50 to 70 hours, the mean residence time is of 2 to 3 days. The extrapolation of curves beyond the 7 day measurements indicates that the last percent of Emdogain stays until 2 weeks on root surface.

Repopulation of PDL Cells:
Dentin surface to which Emdogain is applied will be covered by a thin network of protein fibers. Long slender cells progressively colonizes the surface from periphery.

After 7 days: a peripheral monolayer of cells will be observed.

After 14 days: ¾ th of the original denuded area becomes covered by such a layer of cells.

The presence of detectable amounts of Emdogain at the site of application of up to 2 weeks appears sufficiently longer period of time to permit recolonization of periodontal ligament cells.

Histologic studies in a dehiscence monkey model showed regeneration of the periodontal tissues including cementum, periodontal ligament and alveolar bone, at 8 weeks[2]. This observations suggests that enamel matrix protein can lead to true periodontal regeneration.

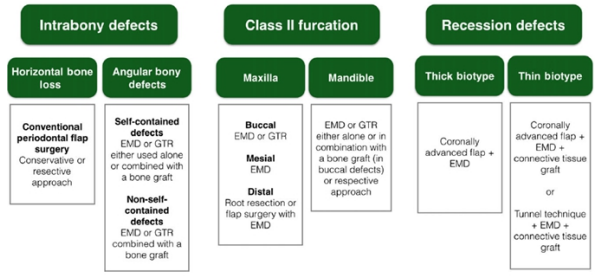
The effect of Emdogain on periodontal ligaments cells
a) Enhances proliferation of PDL cells but not epithelial cells. Cellular response to proliferation is mediated by cell surface receptors, binding to the extracellular matrix proteins.
b) Increases total protein production by PDL cells.
c) Promotes mineral nodule formation of PDL cells.
d) Have no significant effect on migration, attachment and spreading of cells

"It acts as a positive matrix for cells at a regenerative site."
The effect of EMD on bone:
a) EMD affects early stages of osteoblastic maturation by stimulating proliferation – i.e; regulates committed osteoblasts.
b) But as the cells mature in the lineage. Emdogain enhances differentiation.
It is conferred that Emdogain is an osteoconductive agent.
The effect of EMD on cementum:
It leads to the formation of acellular extrinsic fiber cementum.

Clinical safety of EMD:
EMD (Emdogain®) is a porcine-derived material (i.e., a xenograft). The enamel matrix proteins are highly conserved among mammalian species15, 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. Therefore, it is reasonable to assume that they are less likely to act as antigens. In vitro studies showed that EMD does not significantly modify cellular or humoral immune responses. Very high concentrations of EMD induced only a slight increase in the proliferation of human lymphocytes, restricted to the CD25+ (IL-2 receptor) fraction of the CD4+ T-lymphocytes. There was a concomitant decrease of B-lymphocytes, while other cell fractions (CD8+ T-cells, B-cells, and NK cells) were not affected, and immunoglobulin and cytokine (IL-2 and IL-6) production was not modified16.

Limitations:
1. The viscous nature of the material does not support the flap in large or deep defects. Thus its space making potential is limited unlike bone replacement grafts or physical barriers. When significant intraosseous defects are encountered Emdogain may be mixed with either combination of collagen or demineralized freeze dried bone allograft (DFDBA) or its combination.
2. The Manufacturer has not established safety and efficacy in patients on anticoagulant therapy.

Clinical indications for use of EMD in periodontal surgery[17]



Author and year	Conclusion
Petinaki. E et al. (1998)	Evaluated in-vitro ability of Emdogain to influence in-vitro immune system and define immunogenic properties and reported Emdogain may be safely used in clinical periodontal surgery.
S. Petter et. al. (1999)	Investigated the response of periodontal ligament cells (PDL) to Emdogain. Showed that PDL cell attachment rate and growth are stimulated by Emdogain and that cells growing on this matrix significantly increased their TGF-1 production and their ability to develop into multilayered cultures.
Schwartz. Z. et. al. (2000)	Concluded that Emdogain affects early stages of osteoblastic maturation by stimulating proliferation, but as cells mature in the lineage, EMD enhances differentiation, which may be the reason for its success in clinical use.
Kojima. T. et. al. (2000)	Suggested that EMD upregulates the ALP activity in HPDL cells by increasing transcription of the bone type mRNA of TNSALP gene. Such upregualtion may play an important role in periodontal tissue regeneration.

Hammarstrom L., Heijl L., Gastrelius S. (1997)	Showed that it was possible to obtain regeneration of 60-80% of the periodontal tissues by applying either the whole enamel matrix, the acid extract or Emdogain on the denuded root surface.
Boyan B.D., et. al. (2000)	Concluded that the clinical success of Emdogain may result from the interaction of the osteoconductive and cementoconductive properties of the EMP with osteoinductive factors present in the surrounding bone.
Heijl. (1997)	Concluded that adjunctive use of EMD could provide a regenerative technology with a potential for true periodontal regeneration.
Yukna RA, Melloning JT (2000)	Emdogain can result in a regenerative response consisting of new cementum, new alveolar bone and new PDL on a previously contaminated root surface in humans
Giulio Rasperini, et al. (2000)	Showed new connective tissue attachment, newly formed bone and formation of junctional epithelium and new cementum.
Mellonig J. (1999)	Concluded that clinical objectives of inflammation reduction, probing depth reduction and clinical attachment level gain can be attained.
Miremadi S. A. et. al. (2000)	Concluded that root coverage in EMD with coronally advanced flap is more effective

Conclusion

Enamel matrix proteins (emdogain) have produced positive clinical and histologic results. They have routinely produced 60% to 70% new bone, periodontal ligament and acellular cementum in histologic studies. Enamel matrix proteins will provide an excellent alternative to current methods and will allow the clinician to more predictably achieve true periodontal regeneration. In addition, the utility of the proteins may be expanded for other types of periodontal therapy.

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