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The Role Of Enamel Matrix Protein Derivatives In Periodontal Tissue Regenetation: Revisited

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Abstract: ***Periodontitis is a chronic inflammatory disease of the supporting tissues of the teeth that often leads to the loss of attachment apparatus. Despite the development of widely available regenerative materials and techniques, complete and predictable regeneration has still been an elusive goal to achieve. In recent years, dental tissue engineering has emerged as a new tool for periodontal regeneration, to bioengineer the periodontium by targeting of proteins, such as growth factors, and regenerative cells. Enamel matrix derivative (EMD) is one example for such a biomaterial. EMD is mainly an extract of enamel matrix and containing amelogenins of various molecular weights. These amelogenins are involved in the formation of enamel and periodontal attachment during tooth development. Various clinical and histological results from animal and human have shown that treatment with EMD promotes periodontal regeneration. Moreover, clinical studies in humans have indicated that treatment with EMD has a positive effect on periodontal wound healing. This paper is aimed at reviewing the existing literature on EMD by manually and electronically searching relevant indexed journals by typing EMD, applications of EMD in periodontics, role of EMD in periodontal regeneration. The searches were limited to articles in English language and the articles describing EMD and its relation to periodontology were collected and used to prepare a concise review.***

Keywords: Periodontal regeneration, Enamel matrix protein derivative, Emdogain®

I. INTRODUCTION

Periodontitis is a chronic destructive inflammatory disease of the supporting tissues of the teeth which ultimately results in tooth loss if left untreated. Generally therapeutic approaches for treating periodontitis generally fall into two major categories, ie one to halt the progression of periodontal attachment loss, and the one designed to regenerate or reconstruct lost periodontal tissues. To achieve this ideal regeneration various surgical procedures involving root conditioning, autografts, allografts, xenografts and/or barrier membranes for guided tissue regeneration have been shown to contribute to a successful regenerative outcome.

Despite several histologic evidence of regeneration in humans complete and predictable regeneration is a goal that is still difficult to attain. In the past few decades, investigators have increased their efforts to seek procedures and materials promoting periodontal regeneration. In the past few years, growth and differentiation factors have been shown to enhance the regenerative process. Hence an alternative approach based on embryonic tooth formation using enamel matrix protein derivatives was introduced which was used for obtaining periodontal regeneration. Hence the aim of this article is to review the role of enamel matrix protein derivatives in periodontal regeneration.

II. THE ENAMEL MATRIX PROTEINS IN THE DEVELOPING ROOT

Slavkin and Boyd were the first to propose the involvement of enamel proteins in root formation. These proteins were generally believed to regulate the initiation, propagation, termination and maturation of the enamel hydroxyapatite crystallites. They are also deposited on the root

surface and provide an initial and essential step in the formation of acellular cementum.

III. COMPOSITION OF ENAMEL MATRIX DERIVATIVES

The major part of the EMD is composed of the amelogenins, a family of hydrophobic proteins that account for more than 90% of the organic constituent of the enamel matrix. The remaining 10% prolin-rich nonamelogenins which includes proline-rich enamelin, tuftelin, and ameloblastin (also called sheathlin or amelin). It also contains serum proteins.

COMPOSITION	FUNCTION
AMELOGENINS	Regulate the orientation, shape and length of the enamel crystals.[10]
NON-AMELOGENINS ✓ **Enamelin**	Play a role in enamel biomineralization.
✓ **Tuftelin**	It might serve as a nucleator of de novo crystal formation. [11]
✓ **Ameloblastin (amelin and sheathlin)**	Play a role in enamel biomineralization
✓ **OTHER FACTORS** ✓ **Enamelysin (MMP-20)** ✓ **Enamel matrix serine proteinase-1 (EMSP1)**	Processes secreted amelogenins, ameloblastin and enamelin in the extracellular matrix and subsequently degradation and removal from the mineralizing matrix during the maturation stages of amelogenesis.[12]
✓ **Tansforming growth factor β1, BMP-2 and BMP-4**	

Table 1: Composition and function of Enamel matrix proteins

The commercially available product containing EMD is called Emdogain® and is produced by Biora (Malmö, Sweden). Since 1 April 2004, the company has been incorporated into Straumann Biologics Division.

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. Hence propylene glycol was found to be effective vehicle to carry EMD. Originally the product consisted of EMD and the vehicle solution that had to be mixed before use. In order to save time and simplify the procedures a ready-to-use Emdogain gel was developed.In a large multicentre randomized controlled trial (RCT), the original EMD and the new ready-to-use Emdogain gel formulation showed no difference.The emdogain must be stored below 37°C during transport and must be refrigerated until use.Under proper refrigerated storage emdogain has a shelf life of 24 months.

IV. MODE OF ACTION

EMD appears to have significant roles in regeneration by the following ways:

A. EMD AS A SCAFFOLD FOR CELL ATTACHMENT

EMD acts by adsorbing to hydroxyapatite, collagen and to denuded tooth roots. It forms insoluble spherical complexes and for more than 2 weeks detectable amounts are found to remain at the treated site of the root surface. This appears to be a sufficient period of time to permit recolonization by periodontal ligament cells or undifferentiated cells. In addition, amelogenin has been shown to have a cell-adhesive activity, which may explain partially the therapeutic effects of EMD in periodontal regeneration.

B. EMD MODULATES BACTERIAL COMPOSITION AND REDUCES PLAQUE VIABILITY

EMD is shown to have an inhibitory effect on dental plaque viability. In an invitro study Spahr A et al evaluated the effect of EMD on the growth of periodontal pathogens and found that EMD significantly inhibited the growth of the Gram-negative periodontal pathogens whereas the Gram-positive bacteria were unaffected. But further investigation revealed this inhibition is due to the alginate carrier and not the proteins in Emdogain. Hence more research is required to substantiate this proposal.

C. EMD EXERTS A BIOLOGICAL 'GUIDED TISSUE REGENERATION' EFFECT

Studies demonstrate that EMD enhances the proliferation rate, metabolism and protein synthesis, cellular attachment rate and mineral nodule formation of PDL fibroblastic cells and as well as has a similar influence on cementoblasts and mature osteoblasts. In contrast to these effects, EMD appears to exhibit a cytostatic action on epithelial cells and hinders epithelial downgrowth. Thus exhibiting the chacteristic of a biological 'guided tissue regeneration' effect.

D. EMD AS GROWTH FACTOR OR STIMULATES THE PRODUCTION OF GROWTH FACTORS

LYNGSTADAAS et al. studied various growth factor productions in EMD-cultured human PDL cells and found that growth factors (TGF-ß1, IL-6, and PDGF-AB) proliferation and metabolism of human PDL cells in culture were all significantly increased in the presence of EMD. In contrast, EMD increased cAMP and PDGF-AB secretion in epithelial cell cultures, but inhibited their growth. Similarly VAN DER PAUW et al. investigated the effects of EMD on the behavior of human PDL and gingival fibroblastic cells in vitro, with special focus on the release of TGF-ß1. It was found that both cell types released significantly higher levels of TGF-ß1 in the presence of EMD. EMD has also been suggested to have bioactive properties, such as BMP-like activity and TGF-ß-like activity. Hence it is postulated that soluble factors

contained in EMD may be responsible for the stimulating effects of EMD.

V. EVENTS FOLLOWING APPLICATION OF EMD TO ROOT SURFACE

It is critical that no saliva or blood contaminate the root surface prior to Emdogain application. Usually before emdogain application the root surface is conditioned. The manufacturer of EMD produces one root conditioner called PrefGel composed of 24% ethylenediaminetetra-acetic acid (EDTA) at neutral pH. But Mariotti in a systematic review failed to show any effectiveness of such root conditioning procedures.

The sequence of events leading to periodontal regeneration in a periodontal defect begins immediately after suturing of flaps.

Within a couple of days	Within weeks	Over time
✓ Following fibrin clot formation, during initial healing EMD facilitates migration ,attachment and proliferation of cells that have the potential to differentiate and form cementum. ✓ Marked increase in intracellular levels of cAMP and growth factors. ✓ Differentiation of cells capable of producing collagen and cementum are formed.	✓ Regeneration of cementum and the periodontal ligament begins ✓ Early growth of alveolar bone can then be observed. ✓ Subsequently mineralization starts with new bone and eventually fills the bone defect.	✓ Functional attachment develops over several months to a couple of years. ✓ There continued and marked regeneration of bone over time.

Table 2: Events following application of EMD to root surface

VI. APPLICATION OF ENAMEL MATRIX DERIVATIVE

Application	Study	Outcome
Periodontal intrabony defect	(i)A randomized, placebo-controlled, multicenter study done in 33 patients with intrabony defects using split mouth procedure.[28] (ii) Human histological studies evaluated the effect of EMD in combination with natural bone mineral or bioactive glass.[29,30]	After 36 months, showed a greater mean gain in clinical attachment in the test group than the control group.[28] Result indicated the formation of root cementum and mineralization around the graft particles.[29,30]
Recession defects	In a controlled clinical study Miller class I and II buccal gingival recessions was examined with a coronally positioned flap alone and in combination with EMD using the split-mouth procedure.[31,32]	Clinical outcome did not show any differences between the therapies However, additional application of EMD induced statistically significant greater formation of keratinized tissue, and the root coverage maintained over a period of 2 years when compared with that using a coronally positioned flap alone.[31,32]
Furcation defects	A multicenter, randomized, controlled, split-mouth, clinical study compared treatment of mandibular class II furcation defects with 90 comparable defects on the contralateral molars.[33,34] .	Results indicated that there was a significantly greater reduction in horizontal furcation depth and a comparatively lower incidence of postoperative pain/swelling following EMD than with GTR therapy.[33,34]
Dental implants	A study evaluated the effect of the application of EMD onto the surface of dental root-shaped titanium implants.[35]	When tested with removal torque EMD application did not demonstrate any improvement in implant stability or osseous growth. [35]
Wound healing	A quantitative study examined the ultra structural changes associated with a human gingival wound 10 days after application of EMD as an adjunct to a laterally positioned flap in a patient with gingival recession[36]	A considerable difference was found in both the cellular and extracellular phases of the EMD and non-EMD sites. Fibroblasts at the EMD site were rounded with plump cytoplasm and euchromatic nuclei with well-developed rough endoplasmic reticulum and numerous mitochondria. In contrast, the fibroblasts at the non-EMD site were flattened spindle-like morphology.[36]
Avulsed teeth	A clinical study assessed the clinical outcome of 22 avulsed permanent incisors replanted with EMD. [37]	Results showed significantly less inflammation and root resorption in treated teeth compared with control group from the same region.[37]
Pulp healing and dentin regeneration	A blinded, randomized clinical study, with experimental pulpotomy and pulp capping in healthy premolars scheduled for extraction for orthodontic reasons was examined.[38]	Results demonstrated significantly more formation of pulpal secondary dentine and dentine bridging, and less inflammation in the tested teeth.[38]
Effect on tissue inflammation	A recent review study was done on the effect of EMD on tissue inflammation which particularly tested the cellular mechanism, mediators involved and soft tissue healing.[39]	The review concluded that EMD is able to affect inflammatory and healing responses by altering the expression of proinflammatory markers, also increased proliferation and migration of T lymphocytes, endothelial proliferation and increased tissue and bacterial debridement.[39]

Table 3: Application and studies on enamel matrix derivative

VII. CLINICAL SAFETY OF EMD

Although EMD (Emdogain) is a porcine-derived material, the potential of stimulating immune reactions in humans are less likely as these proteins are recognized by the immune system as "self proteins" because EMDs are quite similar and are conserved among mammalian species, as they are conserved in mammalian species. Thus exposure to these proteins takes place during tooth development in early childhood.

But very high concentrations of EMD has shown to 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 modified.

Use of Emdogain in periodontal therapy in humans had no negative impact on periodontal wound healing. Serum samples of patients treated with Emdogain in periodontal defects demonstrated low immunogenic potential even after repeated applications of Emdogain. These results were confirmed in allergy prone patients indicating that its use in humans is safe. The other concen in humans is the possibility of transfer of other infectious agents such as virus and prions, but till to date no disease transmission has been reported from the use of Emdogain.

VIII. CONCLUSION

Enamel matrix proteins are available since more than a decade which has now set a modern standard for periodontal regeneration therapy as this is one of the first product to be completely based on biomimicry. As these proteins are conserved in the mammalian species it seems to be a safe and promising product for the treatment of intrabony periodontal defects. It is evident that EMD has a modifying effect on cells and affects different cells in the healing environment in specific ways. EMD appears to have a positive effect on PDL cells, cementoblasts and osteoblasts while inhibiting epithelial cells that is favorable for the re-establishment of the periodontal tissues. Another important characteristic of this product is its inhibitory effect on the pathogenic dental plaque.

Surgical periodontal treatment of deep intrabony defects with EMD promotes periodontal regeneration. The combination of EMD and some types of bone graft/bone substitute have shown to improve soft and hard tissue parameters compared with treatment with EMD alone. When compared to coronally repositioned flaps alone, application of EMD seems to induce greater formation of keratinized tissue and provide better long-term results. In mandibular class II furcations, application of EMD may enhance periodontal regeneration, comparable with that obtained using GTR and also the treatment with EMD is preferred over GTR especially in those cases where adapting a membrane is technically challenging. In addition, membrane application is more time-consuming and technique-sensitive procedure than EMD application. But there is a lack of evidence for the use of EMD in dental implantology, especially in treating patients with peri- implantitis which require further research. Also in the future, further studies should be undertaken to evaluate the long term effect of EMD to save a teeth with a questionable prognosis.

Hence to conclude there should be additional well-controlled randomized long-term clinical trials on the effect of EMD which will provide a newer insight into the interactions of various cells and EMD and the underlying mechanisms involved that will ultimately have therapeutic relevance to periodontal regeneration.

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