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Role of enamel matrix derivative in periodontal regeneration and tissue engineering

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Abstract

Numerous surgical techniques have been emerged in the direction of regenerating periodontal tissues for instance guided tissue regeneration (GTR), use of bone grafting (BG) and the use of enamel matrix derivative (EMD). EMD is an extract from enamel matrix which may include amelogenins of numerous molecular weights. Amelogenins are primarily involved in the formation of enamel and periodontal attachment formation during tooth development. The biological rationale for using of EMD is to recapitulate developmental mechanisms wherein enamel matrix proteins are proposed to play essential position in stimulating cementogenesis. Enamel matrix derivative (EMD) (Emdogain-Straumann, Basel, Switzerland) is maximum extensively studied biomaterial and commercially available as bioactive agent. EMD is incredibly smooth to apply due to the fact it is delivered in a gel carrier and does now no longer require the delicate management and adaptation related to membranes used for GTR. Emdogain might have few benefits over different methods of regenerating the tissues supporting teeth lost by gum diseases and much less post-operative complications.

Emdogain might have some advan- tages over other methods of regenerating the tissue supporting teeth lost by gum disease, such as less postoperative complications, but has not been shown to save more compromised teeth or that patients noticed any aesthetic improvement 1 year after its application.

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Keywords: Regeneration, enamel matrix derivative, growth factors, Transforming growth factor- β, amelogenin, gingival tissue regeneration, tissue engineering

Introduction

Periodontal regeneration has been an enigmatic target in spite of the development of widespread regenerative surgical techniques. In recent years, dental tissue engineering has emerged as a new model for periodontal regeneration, with the application of a new biomaterials that can support the selection of proteins and growth factors. Enamel matrix derivative (EMD) is an example of a new biomaterial. Enamel matrix proteins are secreted by Hertwig's epithelial root sheath (HERS), which might play an important role in cementogenesis as well as in the development of periodontal attachment apparatus.[1,2] A commercially available preparation of EMD, EMDOGAIN (Biora Inc.), was introduced in the year of 1997 and prepared from acid extracts of porcine enamel buds. EMD has been increasing the interests and research in the field to promote the regeneration of periodontal tissue with promising positive results. When EMD applied to denuded root surfaces and periodontal bony defects, it has been found to adsorb onto surfaces and form an insoluble scaffold complex, which promotes re-colonization of periodontal regenerative cells, that enhances periodontal regeneration.[3] There has been an improvement in clinical outcomes, including significant gain in clinical attachment level and reduced probing pocket depth, observed following treatment with EMD.[4,5]

While the effects of EMD on periodontal tissue regeneration have been well demonstrated, its mechanisms of action still remain unknown with the facts. Alongside it may provide a matrix for cell recolonization, one of the important questions is that how the vitalizing actions of EMD may be depends on the effects of growth factors in promoting the regeneration of periodontal tissue. EMD has been shown to stimulate the production and release of growth factors pivotal for periodontal tissue regeneration, for instance transforming growth factor (TGF-ß).[6] Recent studies in the literature shows the presence of TGF-ß or TGF-ß-like substances in EMD.[7,8] Some other studies by Kawase et al. showed that anti-TGF-ß antibodies completely blocked TGF-ß1-induced signalling pathway.[9]

The significant roles of Hertwig's epithelial root sheath & enamel matrix proteins

Hertwig's epithelial root sheath (HERS) consists of a double layer of epithelial cells extends apically from the enamel organ. The apical growth may occur due to proliferation of the epithelial cells of the sheath. Once the root formation completed, the continuity between the enamel organ and HERS has been lost. The apical region of the developing root contains Ectomesenchymal progenitor cells which may give rise to fibroblasts, pre-odontoblasts and pre-cementoblasts cells. The role of HERS in root development and cementogenesis has become the key element of considerable centre attention. Although the epithelial cells of the inner layer of the HERS are analogous to the pre-ameloblasts, it is postulated that they could secrete enamel matrix proteins on the dentin of newly deposited root.[10] Reports have been considered much attention that the application of hydrophobic amelogenin peptides to denuded root surfaces may promote formation of new cementum.

Slavkin et al. have reported that HERS secretes polypeptides related to, but distinct from, enamelin and amelogenin proteins.[11] The significant role of these enamel matrix proteins on how they trigger the differentiation of cells capable of forming acellular extrinsic fibre cementum (AEFC) and cellular intrinsic fibre cementum (CIFC) is a primary question which still

remains unanswered. However, the concept of epithelial (enamel organ) proteins stimulate cementogenesis has been found in clinical application for regeneration of oral tissue.

Periodontal regeneration

The management of periodontal defects, which includes the destruction of the periodontal ligament, cementum and the formation of intrabony defects, usually was a marked challenging situation in clinical periodontics. Periodontal regeneration is defined as the restoration of lost or supporting periodontium that includes formation of new alveolar bone, cementum, and periodontal ligament.

Basic principles of periodontal regeneration

The regeneration of the periodontal tissues is depending on four fundamental components: the proper signalling molecules, cells, blood supply and scaffold (Figure 1). Each of those factors perform an essential function within side the healing process and is interconnected with the generative process of newer tissues formation. Cells offers the equipment for tissue growth and differentiation. Signalling molecules, which includes growth factors elements or morphogens, modulate the cellular activity and provide a trigger point for cells to differentiate and produce a matrix scaffold. New vascular networks promoted through an angiogenic signals which further provide a nutritional base for tissue growth and maintain a homeostasis. Finally scaffolds guide and create a template structure/shape to facilitate the above techniques which may be vital for tissue regeneration.

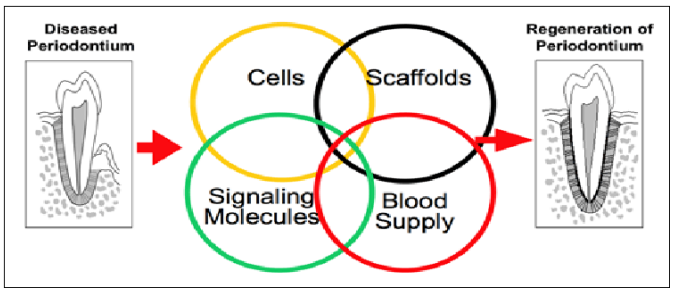

Figure 1: Critical elements required in periodontal tissue regeneration. Reconstruction of lost periodontal tissue requires the combination of cells, scaffolds, signalling molecules, and a blood supply. [Modified from TABA et al.][12]

Periodontal tissue engineering

In recent years, tissue engineering has cladded as a more modern biological model for regeneration for all medical and dental fields. Tissue engineering features a comparatively new field in constructive biology, that uses the mechanical, cellular, or biologic mediators to facilitate reconstruction or regeneration of a selected tissue. The sector of dental tissue engineering is currently success in clinical human demand.

Recent advances in molecular biological research have created unlimited quantities of recombinant growth factors accessible for requisition in tissue engineering as another therapeutic and clinical outcome for periodontal regeneration. The construct of periodontal tissue engineering had its beginnings with the concept of guided tissue regeneration (GTR).

Guided tissue regeneration (GTR)

The concept of GTR is predicted on the exclusion of gingival connective tissue cells and prevention of epithelial down-growth into the periodontal defect, thereby permitting cells with regenerative potential, that is, PDL and bone cells, to enter the wound first. GTR was supported by the presumption that the periodontal ligament contained all of the ancestor or progenitor cells

needed for the formation of bone, cementum and periodontal ligament and which selectively repopulation of the wound site by the progenitor or ancestor cells would results in improved clinical outcomes. However, long term studies and evaluations of this technique have indicated that the clinical enhancement obtained by this procedure exhibit large variability.[13,14]

Biological factors for periodontal tissue engineering

Recombinant biological factors are employed in tissue engineering as a substitute therapeutic approach for periodontal regeneration. To increase the efficiency in vivo, the incorporation of bioactive molecules, such as growth factors, into scaffolding materials will facilitate sustained release of the factor over a period of time. Various growth factors have incontestible robust effects in promoting periodontal wound repair in preclinical and clinical studies. These bioactive molecules include platelet-derived growth factors (PDGF), insulin-like growth factor (IGF-I), fibroblast growth factor (FGF-2), TGF-ß, bone morphogenetic proteins (BMPs) and enamel matrix derivatives (EMD) have shown positive results by stimulating periodontal regeneration.[15]

Growth Factors

Growth factors function as signalling agents for cells and that they function as part of enormous cellular communication network that influences essential functions, such as cell division, matrix synthesis and tissue differentiation. When a growth factor starts binding to a target cell receptor, it induces an intracellular signal transduction which further reaches the nucleus and produces a biological response called as a ligand-receptor interaction. Then the receptor is activated through its modification in its conformation. Receptors has each extracellular and intracellular domains that bind to the ligand and activate the signal transduction system. A part of signal transduction system involves a so-called “transcription factor”, which is an intracellular macromolecules and is activated as part of the signaling pathways initiated by the intracellular domain of a receptor. The activated transcription factor travels to the nucleus, binds to the nuclear DNA polymer and induces the expression of a replacement factor or set of genes.

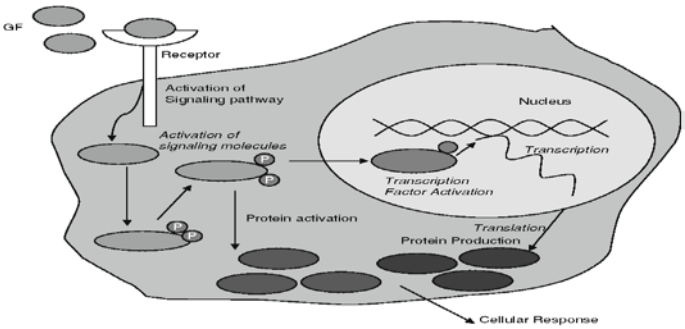

Figure 2: Diagram demonstrating the mechanism by which growth factors influence cell activity. The ligand binds to the receptor and activates the signal-transduction system. A transcription factor is activated, migrates to the nucleus, binds to the nuclear DNA and induces the expression of a new gene or protein.[16]

TGF-ß and its effects on osteoblasts

Transforming growth factor-ß (TGF-ß) is a ubiquitous peptide that is known to regulate an extensive array of cellular processes, such as proliferation, differentiation, ECM production, angiogenesis, immune responses, and cell death in many cell types including osteoblasts.[17] TGF-ß1, 2 and 3 are members of the TGF-ß superfamily. TGF-ß1, 2 and 3 stimulate mesenchymal cells to proliferate, produce ECM and induce a fibrotic response in various tissues *in vivo*. TGF-ß has been shown to be a strong promoter of ECM production in many cell types, including PDL fibroblasts. Conversely, TGF-ß1, 2 and 3 inhibit proliferation and induce the apoptosis of epithelial cells.

Bone formation by TGF-ß1 is promoted through chemotactic attraction of osteoblasts, enhancement of osteoblastic proliferation and the early stages of differentiation with the production of ECM proteins, stimulation of collagen expression and proteoglycan synthesis.

TGF-ß1 and TGF-ß2 are produced by osteoblasts and incorporated into mineralized bone matrix. TGF-ß is expressed at high levels in mature osteoblasts during bone development and growth. Bone matrix contains significant amounts of latent TGF-ß and very little active TGF-ß. Latent TGF-ß in the bone cell environment is proposed to be activated by proteolytic components of the plasminogen system.[18,19] Since osteoblasts produce plasminogen activators, these cells can mediate both the production and activation of TGF-ß in the bone cell environment. TGF-ß has been observed to both inhibit and stimulate osteoblastic cell proliferation *in vitro*, depending on TGF-ß concentration, cell density and species and the stage of osteoblastic cell differentiation. Data from many *in vitro* studies have demonstrated the role of TGF-ß1 in every stage of bone formation.[20,21]

Despite conflicting results, according to a review by JANSSENS et al,[22] most data support the following model: TGF-ß1 increases bone formation *in vitro* mainly by recruiting osteoblastic progenitors and stimulates osteoblastic proliferation, as well as promoting early stages of differentiation, such as bone matrix production.[20] However, it blocks later stages of differentiation, such as OC synthesis, and mineralization.

Enamel Matrix Derivative

The implication of enamel proteins in the formation of root was first proposed by Slavkin and Boyd.[23] It has been proposed that the basement membrane contains chemotactic proteins deposited by the Hertwig root sheath (HERS) cells, that serve to direct the migration of pre-cementoblasts cells or induce cementoblasts differentiation from the dental follicle cells. Many hypotheses are suggested to clarify the function of enamel proteins in root formation: (1) They are concerned with in the attachment of cementum to root dentine; (2) they initiate cementogenesis; (3) they function as an inducer of dental follicle cells to differentiate into cementoblasts; studies with monkeys postulated that after the application of enamel matrix proteins to the clean dentin surface of the root, the formation of new acellular cementum was promoted, new alveolar bone was formed and complete attachment of the periodontal ligament was achieved.[24] These possible functions attribute to the hypothesis that enamel proteins might induce periodontium regeneration.

During a study conducted in 1997 by Hammarstrom et al. it had been showed that when porcine enamel matrix was placed in experimental cavities created in monkeys by extracting the incisors, they might initiate the formation of a tissue similar to acellular, extrinsic fibre cementum.[1] Extracted enamel proteins or purified enamel matrix derivative (EMD) produces 60–80% formation of new cementum and bone in surgically-produced periodontal defects in monkeys.[24] Studies additionally showed that EMD suspended in propylene glycol alginate (PGA) adsorbs to hydroxyapatite and collagen in the denuded dental roots forming an insoluble spherical complex, that remains on the root surface and promotes re-colonization by periodontal ligament cells.[3]

It is therefore suggested that enamel proteins have the ability to enhance complete periodontium regeneration by causation new cementum, periodontal ligament and

bone formation. This product, registered as EMDOGAIN®, has been commercially marketed by BIORA, Inc. It has received FDA approval and is available for the treatment of periodontal defects since 1997. It is derived from acidic extract which is purified form and developed in 6-month-old piglets from embryonal enamel, premixed with a propylene glycol ester of alginate (PGA) to enhance its viscosity.

Composition of enamel matrix derivatives

The major part of the EMD is consists of the amelogenin, a family of hydrophobic proteins that account for over 90% of the organic constituent of the enamel matrix. The second largest element of the enamel matrix proteins is the enamelin. The additional general term "non-amelogenin" is now currently used to describe this high-molecular-weight fraction.[24] It includes proline-rich enamelin, tuftelin and ameloblastin (also referred to as sheathlin or amelin). It additionally contains serum proteins.

Table 1: Composition of enamel matrix derivatives

90%	Amelogenin
10%	Non-amelogenin: Tuftelin Ameloblastin (sheathlin, amelin) Enamel in Enamel proteases (eg. MMP-20, EMSP1) Serum proteins (eg. Albumin) Sulphated enamel proteins

Amelogenin

These are the most abundant enamel proteins accounting for approximately 90% secreted by the ameloblast cells. They are hydrophobic proteins, rich in proline, glutamine, leucine and histidine amino acid residues and exhibit a high degree of sequence conservation. The multiple fragments of amelogenin are present in the enamel extracellular matrix which may be the products of alternative splicing of the amelogenin gene and processing of the parent molecules. It is believed that amelogenins function to regulate the orientation, shape and length of the enamel crystals. Mutations in the amelogenin gene are responsible for malformation of the enamel layer in the affected individuals, resulting in hereditary X-linked Amelogenesis Imperfecta.[25]

Tuftelin

It is an anionic non-amelogenin enamel protein first fully characterized by Deutsch et al. It is expressed as early as the bud stage of tooth development and this protein might serve as a nucleator of de novo crystal formation. The function of this protein in tooth development remains unknown, although recent studies by Paine et al. suggest that it might play a role at the level of ameloblast differentiation and/or extracellular matrix secretion.[26]

Ameloblastin (amelin and sheathlin)

It represents 5% of the non-amelogenin mRNAs and has a domain which has been identified in collagen type I as a recognition site for a2ß1integrin. Ameloblastin gene is located in the region where a family with autosomal dominant Amelogenesis Imperfecta has been linked with this protein which is important for enamel formation. Ameloblastin is present in the secretory phase of enamel formation. Its localization in the Tomes' processes of secretory ameloblasts and in the sheath spaces between rod and interrod enamel suggests a role in enamel biomineralization.

Enamelin

It is the largest enamel protein, which concentrates along the secretory face of the ameloblast Tomes' process. It is the parental protein secreted by the ameloblasts and is then processed to produce other low molecular-weight proteins associated with progressive enamel

mineralization. It is believed to have a role in enamel biomineralization.

Other factors

Enamel proteases

Enamel proteases are required for processing secreted amelogenins, ameloblastin and enamelin in the extracellular matrix and subsequently for their degradation and removal from the mineralizing matrix during the maturation stages of amelogenesis. Enamelysin (MMP-20) and enamel matrix serine proteinase-1 (EMSP1) are such enamel proteases. Sulphated enamel

proteins are present in small amounts. Although their acidic nature suggests that they belong to the family of anionic enamel proteins, their role is unknown.

Role of EMD in periodontal tissue engineering

In vitro studies

In vitro studies have incontestable that EMD affects cellular attachment, mitogenesis, biosynthesis and differentiation. Stimulatory effects are determined in osteoblastic cells, as well as mouse osteoblast-like OCT-1, MC3T3-E1 and 2T9 cell lines.[27] Exposure to EMD increase the metabolic activity of osteoblastic cells and promoted biosynthesis of ECM molecules. Various investigations have suggested that EMD affects the expression of genes concerned with the mineralization and supports cell differentiation in osteoblastic cells, examining the specificity and maturity on cells. Exposure of EMD elicited that each ALP activity and mineralized bone nodule formation of rat bone marrow stromal cells. EMD down-regulated expression of OC and up-regulated expression of OP in MC3T3-E1 preosteoblasts and osteoblast-like MG-63 cells. Schwartz et al. focus on the reaction of osteoblasts of EMD at 3 stages of osteogenic maturation: proliferation (cell range and [3H]-thymidine incorporation), differentiation (ALP and OC), matrix synthesis (sulphate incorporation and proportion of collagen formation). Osteoblastic cell lineages of 2T9 cells (preosteoblasts), human osteoblast-like MG63 osteosarcoma cells, and normal human osteoblasts (NHOst cells) were used. EMD was found to have an effect on initial stages of osteoblastic maturation by stimulating the proliferation of cells, however as the cells moved towards the maturity stage within the lineage, EMD may starts increasing towards the differentiation.[28]

Different responses are reported according to different cell types. Studies by Hammarstrom et al. showed that EMD application resulted in additional limited epithelial down-growth, aiding periodontal healing.[24] This histologic observation was strengthened by studies done by Grestrelius et al., who found that adding EMD to cell culture media resulted in increased proliferation of PDL cells, along with increased protein and collagen production and mineralization.[3] EMD induced collagen mRNA expression in murine follicular SVF cells.

Production of proteoglycans and extracellular hyaluronan was also promoted by EMD in PDL and gingival fibroblasts. However, EMD had no significant effect on epithelial cell proliferation in vitro. EMD-applied surfaces improved attachment of PDL fibroblasts but had no effect on gingival fibroblasts and epithelial cells, indicating a selective behaviour advantageous in the early stages of healing.[15] It is going to be postulated that the biochemical environment at the root surface following the application of EMD may decrease the epithelial down-growth in such a similar type of manner in which mechanical prevention is achieved with the utilization of barrier membranes in GTR procedures.

In Vivo Studies

The efficacy of EMD to regenerate acellular extrinsic fibre cementum was first incontestable in monkeys.[1] Acellular cementum connected to the dentin was evoked eight weeks later when test cavities were treated with crude porcine enamel matrix. Another study was done by Hammarstrom et al. in monkeys' model associated with buccal dehiscence and shows that it is possible to induce regeneration of all the periodontal tissues (cementum, periodontal ligament, and alveolar bone) in order to mimics the normal development of these tissues. The specific characteristics of EMD with regard to its ability to form bone (osteoinductive, osteoconductive, or osteogenic) have been examined by a number of animal studies.[29] The results of these *in vivo* animal studies indicate that EMD has both osteo- and cemento-conductive properties.

Human clinical trials have been conducted to assess the effectiveness of EMD in terms of its ability to improve periodontal health. Comparison study was performed on EMD as placebo or with open-flap debridement in a split-mouth parallel-group study designs and shown similar results: better outcomes were achieved with EMD treatment in terms of clinical and radiographic findings.[30] Various case reports have shown favourable positive results with significant improvement in clinical and radiographic parameters following the use of EMD in the treatment of intrabony defects.[31] Many of the clinical trials and case reports in the literature used EMD as a regenerative material to treat intrabony defects associated with horizontal bone loss which are unlikely to show a positive results. There are conflicting results regarding the influence of number of defect walls. Although several studies have reported that the defect configuration significantly affected the clinical outcomes of EMD,[30] other studies did not demonstrate such an effect.[32] 3-walled defects have been associated with greater regenerative potential in both conventional and surgical procedures.[30] However, a comparable success with EMD is also observed in both 1-walled and 2-walled defects. Different mean values for gains in clinical attachment level (CAL) have been demonstrated, ranging from 1.5mm to 6mm,[30] and similar results have also been shown for radiographic bone gains.[33] There are comparatively few *in vivo* studies, which examine the effects of EMD in the treatment of furcation defects. Jepsen et al. and Meyle et al. both assessed the effectiveness of EMD in the treatment of buccal Class II furcation defects in mandibular molars. They also compared the efficacies of treatment with EMD and bioabsorbable GTR membrane barrier. Both studies reported a significant clinical improvement in EMD-treated cases as compared to untreated control. Horizontal depth reduction was greater in EMD-treated groups than in GTR membrane treatment groups.[34,35]

Mode of action

Although the effects of EMD on periodontal tissue regeneration are well documented, the mechanisms of action are largely unknown.

EMD as a scaffold for cell attachment

EMD has been shown to adsorbs both to hydroxyapatite and collagen and to denuded roots 3. It forms insoluble spherical complexes or matrix, and detectable amounts are found to remains in place treated site on the root surface over a time period of 2 weeks. This appears to be a sufficient time period to allow recolonization by periodontal ligament cells or undifferentiated cells. In addition, amelogenin have also cell-adhesive activity, which may partially demonstrate the therapeutic effects of EMD in periodontal regeneration.

EMD modulates bacterial composition and reduces plaque viability

EMD may enhance periodontal regeneration by reducing dental plaque. EMD has been found to have an inhibitory effect on dental plaque viability. The effect of EMD on the growth of periodontal pathogens was examined even *in vitro*. It was found that the Gram-negative periodontal pathogens were significantly inhibited while the Gram-positive bacteria remain unaffected. It is concluded that EMD has a positive effect on the composition of bacterial species in the post-surgical periodontal wound by selectively inhibiting the growth of periodontal-pathogens which affects wound healing and reduce the outcome of regenerative procedures.

EMD exerts a biological 'guided tissue regeneration' effect

Studies show that EMD has different responses in different types of cells. EMD improves the rate of proliferation, metabolism and protein synthesis, rate of cellular attachment and formation of mineral nodule in PDL fibroblastic cells and has a similar impact on cementoblasts and mature osteoblasts.[15,24,27] In addition, EMD improves the attachment of these cell types. In contrast to its effects on mesenchymal cells, EMD appears to exert a cytostatic action on epithelial cells.[3,15] These properties partly explain the biological effect of 'guided tissue regeneration' attributed to EMD by inhibiting the epithelial down growth.

EMD as growth factor or stimulates the production of growth factors

Because of previous studies may not be able to detect and find the presence of growth factors in EMD preparations, it had been assumed earlier that it acts as a matrix, producing a positive surrounding for cell proliferation, differentiation, matrix synthesis, and presumably the production of growth factors, that consecutively enhance tissue repair and regeneration. Therefore, this insoluble matrix enhances cells to supply growth factors, including TGF-ß, platelet-derived growth factor (PDGF) and BMPs. Lyngstadaas et al. studied numerous growth factor which are produced in EMD-cultured human PDL cells. It had been found that growth factors (TGF-ß1, IL-6, and PDGF-AB), proliferation, and metabolism of human PDL cells in culture were all considerably increased in the presence of EMD. However, EMD increase cAMP and PDGF-AB secretion in epithelial cell cultures, and inhibited their growth.[15] Results from this and previous studies further recommend that EMD favours the growth of mesenchymal cells over the growth of epithelial cells. Vander Pauw et al. investigated the results of EMP on the behaviour of human PDL and gingival fibroblastic cells *in vitro*, with special concentrate on the release of TGF-ß1. It had been found that each cell types released significantly higher levels of TGF-ß1 in the presence of EMD.[36]

Recent research confirmed that expression of human PDL cells stimulating EMD with cDNA microarray technology revealed that most of up-regulated genes were the ones coded for growth factors and their receptors. Although it has been demonstrated that EMD functions as an insoluble matrix to activate the cells to produce growth factors, also there is another hypothesis that bioactive molecules released from EMD are also responsible for the tissue regenerative activity of EMD. The bioactive molecules might be growth factors absorbed to EMD throughout its preparation or amelogenin peptides. Numerous amelogenin gene products have been shown to actively participate in cell

signalling to stimulate matrix formation and mineralization. These multiple amelogenin gene products exist as a result of alternative splicing. The larger forms are necessary for enamel mineralization whereas small amelogenin peptides might have signal transduction function, and are shown to reinforce the expression of collagen, Sox 9 and Cbfa 1 mRNA in vitro. These small amelogenin peptides were ready to induce bone formation around implants *in vivo* by increasing the formation of ECM, matrix vascularization and mineralization.

They have comparatively similar osteogenic activities to recombinant human BMP-2. The amelogenin peptides additionally results in the formation of reparative dentin bridge and its features are corresponding to BMP-7. Protein evaluation of EMD showed the presence of proteolytic enzymes consisting of metalloendoproteases and serine proteases, in this commercial preparation. In tissue repair environment, proteolytic enzymes may produce small amelogenin peptides from EMD that may act as soluble growth-like factors to have an effect on neighbouring cells. Recent research has additionally proven the presence of growth factors in EMD.

EMD have been reported to have bioactive properties consists of BMP-like activity and TGF-ß-like activity.[9] Hence it is far postulated that soluble elements contained in EMD can be accountable for the stimulating the outcomes of EMD. Growth factors consists of TGF-ß and small amelogenin peptides, are capable candidates mediating the results of EMD.

Evidence of EMD-TGF-ß1 relationship

EMD is found to stimulate the endogenous production of TGF-ß1. EMD has also been shown to have TGF-ß1-like functions. Kawase et al. observed that during EMD preparation TGF-ß molecules are present abundantly.[9] EMD was subjected to enzyme-immunoassay for TGF-ß1 and it was found that EMD preparations contained TGF-ß1-like immunoreactivity that bound to TGF-ß receptor II. He also showed that significant levels of TGF-ß were present in EMD preparations, which led to rapid phosphorylation of the MAP kinase family and translocation of Smad2 into the nucleus in both oral epithelial and fibroblastic cells. Further study showed that anti-TGF-ß antibody absolutely blocked TGF-ß1-induced Smad2 pathway.[9] Suzuki et al. fractionated EMD and found that TGF-ß-like activity was detected in low molecular weight fractions.[7] He et al. concluded that direct contact is not required for EMD-induced cell proliferation and soluble factors such as TGF-ß1 and small amelogenin peptides may be factors mediating the effects of EMD.[8] Therefore, TGF-ß1 remains to be a candidate mediating the effects of EMD.

Conclusion

EMD has been widely used in periodontal tissue regeneration with promising results. *In vitro* studies show that EMD improve cellular activities involved in different aspects of tissue regeneration in different cell types. EMD is thought to induce endogenous production of growth factors and to sustain them at effective doses for a longer period in a local area.

Better understanding of the exact TGF-ß based signalling pathways (including Smads and MAPK pathways) is needed to provide more detailed molecular and mechanistic information on the effect of EMD. Other studies using primary osteoblastic cells and other types of cell lines, such as fibroblasts and epithelial cell types, will be useful to provide a better picture on whether such effects are cell-specific. This will offer a more recent perception into the interactions of diverse growth factors and EMD and the underlying

mechanisms involved and eventually they have therapeutic relevance to periodontal regeneration, wherein EMD may be used as a modulator of regenerative growth factors in oral tissue engineering.

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