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The Application of an Enamel Matrix Protein Derivative (Emdogain®) in Regenerative Periodontal Therapy: A Review

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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 review aims to present an overview of evidence-based clinical indications for regenerative therapy with EMD.

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Introduction

Results from basic research indicate the role of the different types of cementum for attaching the tooth and reparative processes in the entire periodontium. 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 [1]. Studies by Slavkin and Boyde [2] and Slavkin [3] have shown that proteins, secreted during tooth development by the Hertwig root sheath, 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 [1, 4]. They consist of a whole family of proteins, of which 90% are amelogenins, and the remaining 10% prolin-rich nonamelogenins, tuftelin and other serum proteins [4]. It has been shown that during evolution, the chemical structure of amelogenin has remained more or less constant, even among the individual animal species, exhibiting only slight differences [4]. In a series of animal experiments on root development in rats, monkeys and pigs, it was immunohistologically demonstrated that the concentration of amelogenin rises dramatically during tooth development [1]. Also, a close connection between acellular cementum and amelogenin exists [1]. These findings have also been confirmed in human teeth, where some histological sections showed a thin layer of highly mineralized enamel between dentin and root cement. These observations permit the assumption that the attachment of enamel matrix must occur on the dentin surface before the emergence of acellular cementum [1]. Based on such evidence, several in vivo experiments in animal models were conducted [1]. In an experiment, the

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lateral incisors of 2 monkeys were extracted and standardized cavities in the root surface were created, mesially and distally. The test cavities were then filled with an enamel matrix derivative (EMD), while the control cavities remained untreated. All the teeth were reimplanted into their original alveoles. Histological evaluation 8 weeks after reimplantation showed formation of acellular cementum in the defects in which EMD had been applied, but in the untreated control defects, only a reparative, cellular cementum developed [1]. On the basis of these findings, the EMD from the tooth pouches of unerupted teeth of young pigs was isolated, purified and lyophilized. Since EMD is highly hydrophobic, it was solubilized by interaction with PGA carrier, prior to using it for regenerative periodontal therapy [5]. Maycock et al. [6] identified enamel matrix proteins and proteolytic enzymes present in EMD and compared them with those extracted from developing porcine enamel. The results have shown that while developing enamel contained amelogenins, albumin, amelin and enamelin, EMD contained only amelogenins. Thus, for the time being, it may be assumed that the main component of EMD are amelogenins [4–6]. A technique or a material must, however, fulfill the following criteria in order to be classified as ‘regeneration-promoting’ [7]:

- in vitro studies, which confirm the action mechanism;
- controlled histological animal studies, which demonstrate formation of new root cementum, periodontal ligament (PDL) and alveolar bone;
- human biopsies, which show formation of root cementum, PDL and alveolar bone on a plaque-infected root surface;
- controlled clinical studies, which prove a gain of clinical attachment and radiological new bone formation.

In the following overview, the existing evidence regarding the clinical use of EMD is provided.

In vitro Studies

Several in vitro investigations have been carried out to study the mechanism of EMD on the PDL, gingival fibroblast and bone cells [8–40]. In a series of laboratory studies, the effect of EMD on migration, attaching, proliferation, biosynthesis activity and formation of mineralized nodules was examined. Immunoassays were performed to determine the possible presence of existing polypeptide factors [5, 8]. The results showed that under in vitro

conditions EMD promotes (a) the proliferation of PDL fibroblasts but not epithelial cells, (b) increased total protein synthesis of the PDL fibroblasts, and (c) the formation of mineralized nodules by PDL fibroblasts. In the above-mentioned studies no specific molecules such as IGF-1 and 2, PDGF BB, TNF, TGF- β or IL-1 β could be identified. PDL fibroblasts treated with EMD displayed an increased intracellular cAMP concentration and autocrine releasing of TGF- β 1, IL-6 and PDGF AB compared to the control group (without EMD) [12]. Although the epithelial cells showed an increased release of cAMP and PDGF AB, following the additional application of EMD, their proliferation and growth rates were inhibited [10, 12]. It was concluded that EMD simultaneously promotes the growth of mesenchymal cells by inhibiting that of the epithelial cells and the release of autocrine growth factors from PDL fibroblasts [12]. Similar findings were also reported by Okubo et al. [13], who demonstrated that EMD has no appreciable effect on osteoblastic differentiation, although it stimulates cell growth, and IGF-1 and TGF- β 1 production in PDL cells.

Palioto et al. [14] evaluated the effect of EMD, IGF-1 and the combination of these 2 factors on the proliferation, adhesion, migration and expression of type I collagen in PDL fibroblasts. The results indicated that the proliferation of PDL fibroblasts was significantly stimulated both by EMD and EMD plus IGF-1, in a dose- and time-dependent manner. However, these factors did not affect the adhesion, migration or expression of type I collagen of these cells. Other data indicate that EMD may contain additional mitogenic factors such as TGF- β and BMP-like growth factors that stimulate fibroblastic proliferation and contribute to the induction of biomineralization during periodontal regeneration [15–18].

Keila et al. [19] investigated the effects of EMD on rat bone marrow stromal cells and on gingival fibroblasts. EMD increased the osteogenic capacity of bone marrow and mineralized nodule formation. The presence of EMD in the initial stages (first 48 h) of the culture was crucial for this effect. In contrast, EMD did not induce osteoblastic differentiation of gingival fibroblast but increased up to 2-fold both in numbers and the amount of matrix produced. In further investigations, it was shown that the attaching, growth and metabolic rate of PDL fibroblasts increased significantly when EMD was added in cell cultures and that EMD may convert the differentiation pathway of pluripotent C2C12 mesenchymal cells into osteoblast and/or chondroblast lineage [8–10, 12, 20]. PDL fibroblasts showed a significantly increased alkaline phosphatase activity following the application of EMD

and it enhanced human PDL fibroblast proliferation [21, 22]. In the presence of EMD, human PDL fibroblasts showed some morphological changes that made them more similar to cementoblasts than to fibroblasts, suggesting a process of cellular differentiation [22]. A recent study examined the influence of EMD on the viability, proliferation and attachment of periodontal fibroblasts to diseased root surfaces [23]. The results indicated that cell viability was negatively affected for higher doses over time, while low doses displayed viability effects similar to those of the control. PDL cell proliferation appeared to be ameliorated following exposure to EMD and the scanning electron microscopy analysis suggested that cellular attachment to diseased dentin was enhanced following EMD application. Further investigations demonstrated that EMD significantly increased the mRNA synthesis of the matrix proteins versican, biglycan and decorin and led to an increased hyaluronan synthesis in the gingival and desmodontal fibroblasts [9]. It was also suggested that integrins are involved in the interaction of PDL and gingival fibroblasts with EMD [24]. However, it has to be emphasized that in most studies, EMD had a stronger effect on desmodontal than on gingival fibroblasts. Other experimental investigations have shown that the application of EMD can regulate the expression of the genes associated with cementoblasts, which, in turn crucially affects the mineralization process [25]. Inoue et al. [26] evaluated whether or not the application of EMD to different dental materials (which do not normally support cementogenesis such as gutta percha, calcium hydroxide, amalgam and super EBA cement) would alter the in vitro phenotype of PDL cells. Their findings indicated that EMD can alter the phenotype of PDL cells when cultured on these materials. However, some studies have failed to show an influence of EMD upon the proliferation of mouse fibroblasts and marrow stromal cells [27]. Very recent data have shown that neither EMD nor PGA has the ability to induce hard tissue and that enamel matrix proteins contained within EMD might aggregate on the dentin surface and inhibit the effect of the demineralized dentin matrix [28]. In a study investigating clot adhesion to protein-conditioned root surfaces, human dentin blocks were exposed to either a saturated citric acid solution or a commercial ethylenediaminetetraacetic acid (EDTA) preparation using standardized protocols [29]. Some dentin blocks were additionally conditioned with either bovine serum albumin (BSA) or EMD. Subsequently, fresh human whole blood was applied to the blocks and the blood was allowed to clot before rinsing in phosphate-buffered saline to test adhesion by means of

scanning electron microscopy. The results indicated that EDTA appeared less efficacious than citric acid in removing the smear layer and in exposing dentin tubules and collagen. Fibrin clot adhesion was best supported by the citric-acid-treated dentin surface, whereas forces produced by the rinse protocol partially removed the fibrin clot from EDTA-treated root surfaces. The results also indicated that BSA- or EMD-treated surfaces poorly retained the fibrin clot and produced a surface morphology similar to that of the smear layer.

Kawase et al. [30] examined the effect of EMD on the proliferation of oral epithelial cells (SCC25). After 3 days of treatment with EMD, cell division was prevented and at the same time the cell cycle was stopped in the G1 phase. Furthermore, it was shown that the addition of EMD significantly limited the expression of cytokeratin-18. The authors concluded that EMD does not possess a cytostatic but, rather, a cytotoxic effect on epithelial cells [30]. In an in vitro study, the combination of 4 mg EMD and active demineralized freeze-dried allogenic bone showed an increased bone induction [31]. It was concluded that EMD possesses not osteoinductive but osteopromotive characteristics when applied at certain concentrations [31]. Schwarz et al. [32] have shown that EMD stimulates the early stages of the osteoblast maturation by increasing cell proliferation. However, when applied to mature cell lines, the main effect was confined to influencing cell differentiation. A stimulatory role of EMD on mineralized tissue formation by modulating regulatory molecules, critical to bone metabolism at the RNA level, has also been reported [33].

Schwarz et al. [34] investigated the effects of EMD on attachment, proliferation and viability of human SaOs₂ osteoblasts on titanium implants. The results indicated that EMD-enhanced cell proliferation and viability of human SaOs₂ osteoblasts on sandblasted/acid-etched (SLA) titanium implants are concentration-dependent. Treatment of osteoblasts with EMD significantly stimulated cell proliferation and fibroblast growth factor-2 expression but decreased alkaline phosphatase expression [35]. It was also suggested that EMD may elicit its mitogenic signal through an EMD-specific receptor tyrosine kinase towards extracellular signal-regulated kinase 1/2 [36]. It seems that EMD treatment may enhance the cellular activities of osteoblasts and osteoclasts, which in turn might support the regeneration of periodontal bony defects [37]. Since soluble peptides released from EMD may contribute to the stimulating effects on cell proliferation, a direct contact between EMD and osteoblasts might not be required to induce cell proliferation [38]. Shimizu et al. [39]

examined the ability of EMD to regulate bone sialoprotein gene transcription in osteoblast-like cells. The findings identified EMD response elements in the rat bone sialoprotein gene promoter that may mediate the effects of EMD on bone sialoprotein gene transcription.

A very recent study evaluated the effect of a combination of a bioactive glass and EMD upon the proliferation and differentiation of the mouse preosteoblastic cell line MC3T3-E1 [40]. Cells were cultured up to 28 days in contact with 3 types of granule: Bioglass 45S5 granules, 45S5 granules coated with EMD and a less reactive glass used as a control (60S). The results indicated that both Bioglass 45S5 granules alone or coated with EMD have the ability to support the growth of osteoblast-like cells in vitro and promote osteoblast differentiation by stimulating the expression of major phenotypic markers. However, the bioactive granules coated with EMD revealed significantly higher protein production than the bioactive granules alone.

Parkar and Tonetti [41] evaluated the selective effects of EMD on the activities of 268 cytokine, growth factor and receptor genes in PDL. The results indicated that 46% (125 of 268) of the tested genes were found to be expressed by the PDL cells. Of these 125 genes, 38 were differentially expressed by PDL cells cultured in the presence of EMD. Of the 38 genes, 12 most notably inflammatory genes were found to be downregulated, whereas 26 genes, many coded for growth factors and growth factor receptors, demonstrated upregulation. The results indicated that EMD downregulates the expression of genes involved in the early inflammatory phases of wound healing while simultaneously upregulating genes encoding growth- and repair-promoting molecules.

It is important to note that certain antibacterial effects and disturbances of bacterial adherence were also found to be influenced by EMD [42–47]. Plaque samples (from 24 patients with periodontitis) that were allowed to accumulate for 4 days were taken and divided into 5 equal parts afterwards [42]. Each part was mixed with 5 μ l of one of the following solutions: NaCl, EMD in water, EMD in PGA vehicle, PGA vehicle and chlorhexidine digluconate. Subsequently, vital fluorescent microscopy was used to evaluate the vitality of the plaque flora. The results showed that EMD in the PGA vehicle had a very strong antibacterial effect. It was concluded that the antibacterial effect of EMD is mainly due to the effect of the PGA carrier. These findings were later confirmed in an observer-blind, randomized 5-cell crossover study, demonstrating for the first time a direct influence of EMD on the vitality of supragingival dental plaque in vivo [43]. In a

further investigation, it was shown that EMD inhibits the growth of the periodontal pathogenic bacteria *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*. Twenty-four hours following the application of EMD no living colonies of these pathogenic bacteria could be observed. Moreover, EMD demonstrated no negative effect on Gram-positive bacteria [44]. The inhibitory effect of EMD on periodontal pathogenic bacteria was also confirmed by others [45, 46]. Recent data also suggest that *P. gingivalis* diminishes the effect of EMD on PDL cells in vitro through a cooperative action of gingipains [47]. Rincon et al. [48] evaluated the influence of EMD on cultured gingival, PDL and dermal fibroblasts, using an in vitro model of wound healing. It demonstrated that cells in vitro fill an empty space by a combination of proliferation and cell migration, thus indicating that EMD may exert an influence on cells involved in wound healing. In a study in rabbits Mirastschijski et al. [49] primarily investigated the in vivo effects of EMD on skin wound healing. Secondly, they examined the in vitro effects of EMD on dermal fibroblasts and microvascular endothelial cells. Full-thickness, circular 2-cm skin wounds in white 16-week-old rabbits were treated 3 times a week with EMD (30 mg/ml) in the vehicle PGA and the vehicle alone acted as control. EMD treatment increased the amount of granulation tissue and accelerated the time to complete epithelialization by 3 days, compared to the vehicle treatment. In cultured fibroblasts, vascular endothelial growth factor levels in conditioned media were increased more than 5-fold with EMD treatment over control, as measured by specific enzyme-linked immunosorbent assay. EMD also increased the release of matrix metalloproteinase-2 from fibroblasts and endothelial cells more than 3-fold. It was concluded that EMD significantly accelerated wound closure in rabbits, possibly by increasing the levels of growth factors and proteinases important for granulation tissue formation and granulation. It was shown that EMD may express some angiogenic effects which may play an important role in early wound healing [50]. Recent results pointed to the anti-inflammatory properties of EMD which attenuated the release of TNF- α and IL-8 in whole blood from healthy donors, challenged by lipopolysaccharide or peptidoglycan [51]. Furthermore, it was shown that EMD inhibits the attachment of a typical breast cancer cell line (MCF-7) to a bone matrix, thus suggesting that EMD might be useful as an antiadhesive agent for breast cancer cells to bone in vivo [52].

In conclusion, the data from in vitro studies strongly indicate that EMD affects important wound healing

mechanisms. However, to date, it appears that the underlying molecules and mechanisms are still not completely understood.

Controlled Histological Studies in Animals

In an experimental study in rats the effects and distribution of EMD in the periodontal tissues of maxillary rat molars transplanted to a subcutaneous position in the abdominal wall were studied [53]. Molars were transplanted with or without EMD, either immediately after extraction or after drying for 30 min. The rats were killed after 2 days or 1, 2 or 4 weeks and the teeth were examined by light microscopy and immunohistochemistry with anti-amelogenin antibodies. The teeth that were transplanted immediately after extraction showed formation of alveolar bone separated from the dental roots by a periodontal space, regardless of the use of EMD. New alveolar bone was formed in 5 out of 8 teeth after 2 and 4 weeks in the teeth transplanted with EMD after drying for 30 min. None of the teeth transplanted without EMD showed alveolar bone formation. Additionally 1 tooth transplanted with EMD showed root resorption after drying, while resorption was noted in all teeth transplanted without EMD. EMD was detected as early as 2 days on all the teeth transplanted with EMD and was still demonstrable after 4 weeks. In experimental dogs, it was shown that the application of EMD in intrabony defects may significantly stimulate the proliferation of PDL cells [54]. However, this effect was limited to the first 4 weeks following surgery, thus indicating that the main effect of EMD is limited to the early stage of periodontal wound healing. In another study defects were filled with either vehicle (control) or EMD (test) in rat periodontal window wounds, with no microbial biofilm or epithelial downgrowth [55]. The animals were sacrificed 7, 14 and 21 days after wounding. Specimens of periodontium were immunostained for osteopontin, bone sialoprotein and osteocalcin as markers of osteogenic differentiation and for α -smooth muscle actin, a myofibroblastic marker. The results indicated that EMD did not appear to affect the expression of differentiation markers or bone matrix protein synthesis in the repopulation response of wounded rat molar periodontium. It was suggested that the effect of EMD on wound healing in the periodontium may be independent of differentiation in the cell populations examined in the type of model used [55].

In a controlled histological study, recession defects were created and treated with EMD [56]. Standardized

defects were created, by surgically removing the entire buccal bone plate and the root cementum. The test defects were treated with EMD, while in the control defects a coronally repositioned flap was made. Eight weeks after surgery the animals were sacrificed and the appropriate jaw segments histologically evaluated. The results showed that in all test defects a new periodontium (acellular cementum with inserting collagen fibers and new alveolar bone) developed. In the control defects, the healing was characterized by a long junctional epithelium with very limited cementum and new bone formation. If in the control defects new cementum was formed, it was mostly cellular and only partly attached at the root surface. Interestingly, in this study, in the test defects no root resorption occurred, but in the control defects, root resorption was a very frequent phenomenon. It is important to note that during the entire study period no oral hygiene measures were carried out. In an experimental study in monkeys acute fenestration-type defects were surgically created and subsequently treated with (a) guided tissue regeneration (GTR), (b) EMD or (c) coronally repositioned flaps (control) [57]. All 3 treatment approaches enhanced the formation of new connective tissue attachment and new bone, but there was no major difference between the treatment groups. The results also indicated that acute fenestration-type defects do not seem to be the suitable test model for determining the potential of any type of regenerative approach [57]. In 2 subsequent studies in monkeys, recession-type and intrabony defects were surgically created and exposed to dental plaque infection [58, 59]. Following initial periodontal therapy consisting of oral hygiene measures and topical application of chlorhexidine, the defects were treated with 1 of the following therapies: (a) GTR, (b) EMD, (c) EMD + GTR or (d) open flap debridement surgery (control). Histological investigation showed healing in the control defects, characterized by a long junctional epithelium and a limited periodontal regeneration. Treatment with GTR, EMD or EMD + GTR resulted in the formation of cementum with inserting collagen fibers as well as of alveolar bone [58, 59]. Comparable results were also reported in rat, dog and monkey defects, with either spontaneous intrabony and experimentally created intrabony, recession or dehiscence-type defects [60–64]. A histomorphometrical study in dogs evaluated the effectiveness of EMD to induce regeneration of periodontal tissues in class II furcation lesions with or without GTR [65]. Experimental class II furcations were made in the premolars of 4 dogs. The furcation defects were filled with gutta percha to induce an inflammatory response and to prevent spontaneous

repair. Twenty-one days later, the defects were treated with (a) GTR, (b) EMD or (c) open flap debridement surgery (control). Histological analysis at 8 weeks following therapy showed healing characterized by formation of a long junctional epithelium and limited bone formation in the control group. Treatment with EMD led to significant regeneration of the furcation lesions but association with membranes was detrimental. Another study, using monkeys, histologically evaluated the healing of mandibular class III furcation defects following treatment with (a) GTR, (b) EMD, (c) EMD + GTR or (d) open flap debridement surgery (control) [66]. The results showed that treatment with GTR or EMD + GTR resulted in formation of new cementum with inserting collagen fibers, and new bone was filling the defects in the situation where the membrane was not exposed. The sites treated only with EMD exhibited new attachment and new bone formation to a varying extent, while the control sites presented only limited new attachment and bone formation.

In conclusion, animal data indicate that EMD is present on the treated root surfaces for a period of at least 4 weeks and predictably it promotes formation of cementum, PDL and bone in fenestration, recession, intrabony and mandibular class II furcation defects.

Human Histological Studies

Results of the first human histological biopsy were published by Heijl [67]. A recession defect on a lower incisor was surgically created and treated with EMD. After a healing period of 4 months, histological evaluation of the tooth as well as the surrounding soft- and hard-tissue extracts showed that a new layer of acellular root cementum covered 73% of the original defect depth. New alveolar bone had regenerated on 65% of the initial bone height. In another study Yukna and Mellonig [68] treated 10 intrabony periodontal defects in 8 patients with EMD. Histological analysis 6 months later showed complete periodontal regeneration (i.e. new formation of root cementum, PDL and alveolar bone) in 3 biopsies and 3 further biopsies indicated healing characterized by a new connective tissue attachment (i.e. new cementum with inserting collagen fibers). The remaining 4 biopsies showed a long junctional epithelium without any signs of periodontal regeneration. In a comparative clinical and histological investigation, the healing of intrabony periodontal defects was evaluated following treatment with EMD or GTR with a bioabsorbable barrier [69]. Six months after therapy, clinical attachment level (CAL) showed a mean gain of

3.2 ± 1.2 mm in the EMD group and of 3.6 ± 1.7 mm in the GTR group. Histologically, both groups showed healing, mainly characterized by periodontal regeneration [69]. The mean values of new cementum and PDL fell to 2.6 ± 1.0 and 2.1 ± 1.0 mm in the EMD and GTR group, respectively. The mean value of new alveolar bone was 0.9 ± 1.0 mm in the EMD group and 2.1 ± 1.0 mm in the GTR group. Reparative healing by a long junctional epithelium occurred only in 1 biopsy from the EMD group. These results provide evidence that treatment with EMD promotes periodontal regeneration in humans and may lead to clinical and histological results comparable with GTR therapy. These findings are confirmed in subsequent reports by others, not only in intrabony but also in recession-type defects [70–75]. Subsequent immunohistological studies have shown that following surgery, EMD remains on the root surface for up to 4 weeks and the wound healing and/or remodelling process can be followed for up to 6 months after treatment with EMD therapy [76–78]. A very recent human histological study attempted to characterize the tissues developing on the root surface at 2–6 weeks following treatment of intrabony defects with EMD [79, 80]. The results showed that the newly formed tissues on the root surfaces were thick, collagenous, devoid of extrinsic fibers and had an irregular surface contour. The presence of electron-dense, organic material in the collagenous matrix indicated at least partial mineralization. Embedded cells were numerous and the cells on the matrix surface were very large in size. It was concluded that following treatment with EMD, a bone-like tissue resembling cellular intrinsic fiber cementum may develop on the root surfaces, instead of acellular extrinsic fiber cementum. Furthermore, EMD may induce de novo formation of a mineralized connective tissue on scaled root surfaces and also stimulate matrix deposition on old native cementum. External root resorption was observed in 2 cases at 6 and 24 months following treatment with EMD, while no periodontal regeneration was observed when EMD was applied in a nonsurgical way into intrabony periodontal defects [81, 82]. Based on the available evidence from human histological studies, it may be concluded that the application of EMD in conjunction with periodontal surgery may promote formation of new cementum, PDL and bone in intrabony and recession defects. Moreover, when applied during periodontal surgery EMD can be detected on the root surfaces for a period of at least 4 weeks. Based on current knowledge, there are no histological data from human material evaluating the regenerative potential of EMD in furcation defects.

Controlled Clinical Studies Evaluating the Effect of EMD on Early Wound Healing

Several studies have attempted to evaluate the effect of EMD treatment on early wound healing [83–85]. In a double-masked, split-mouth, placebo-controlled, randomized study 28 patients with moderately advanced chronic periodontitis were scaled and root planed, and the soft-tissue wall of the pocket was curetted to remove pocket epithelium and adjacent granulation tissue [83]. All experimental sites were carefully irrigated with saline. When the bleeding from the pocket had ceased, a 24% EDTA gel was applied in the sites and retained for 2 min. The sites were then thoroughly irrigated with saline to remove EDTA remnants. Subsequently, left and right quadrants were randomized to subgingival application of EMD (test) or vehicle (control). All sites were re-examined clinically after 1, 2 and 3 weeks. In addition, a visual analogue scale was used to score the degree of post-treatment discomfort. The results indicated that EMD topically applied in instrumented sulci enhanced early healing of periodontal soft-tissue wounds. Furthermore, at 1 week, the proportion of patients reporting a visual analogue scale score ≤ 20 was considerably higher for the EMD-treated quadrants than for controls. In another study, clinical evaluation and patient perception of post-operative events on the effect of EMD on the healing of soft-tissue wounds following periodontal surgery was done [85]. Patients scheduled for periodontal flap surgery were treated with either modified Widman flap and application of EMD (test) or with modified Widman flap alone (control). Clinical measurements were taken at 4 different points – at surgery and 1, 4 and 8 weeks after surgery. Of all parameters evaluated, none showed a significant difference between the control and EMD groups, except for gingival swelling at the 1-week assessment, where the EMD group exhibited a higher swelling score. It was concluded that the early wound healing of periodontal flap surgeries in the sites treated with EMD is not different from control sites.

Based on the available data, at the current time it appears that no definitive conclusions can be drawn regarding the extent to which additional application of EMD may further enhance early wound healing following conventional periodontal therapy.

Controlled Clinical Studies in Intrabony Defects

Nonsurgical Periodontal Therapy

Two randomized, placebo-controlled clinical studies evaluated the effect of EMD as adjunct to nonsurgical periodontal therapy in intrabony defects [86, 87]. Both these studies failed to show any significant benefit of using EMD during nonsurgical periodontal therapy.

Surgical Periodontal Therapy

Side effects, for example incompatibility or allergic reactions even after repeated treatment with EMD, were not reported in any published studies [88–91]. A multicenter study evaluated the potential for sensitization to EMD in a subgroup of periodontal patients treated at least twice, with an interval of at least 2 months between treatments [91]. Intrabony defects in 376 patients from 11 university-based postgraduate periodontics programs and 5 private practices were treated with open flap debridement, root conditioning with either citric acid (pH = 1) or 24% EDTA, followed by defect irrigation with sterile saline and application of EMD. The second test defect was treated in a similar manner at least 8 weeks after the first surgery. No clinical adverse reactions to multiple applications of EMD were noted. The results demonstrated a lack of clinical adverse reactions following 2 separate applications of EMD. Any subjective/objective adverse reactions experienced by the patient were typical complications following routine periodontal surgery and were not directly related to the use of EMD. Data from controlled clinical studies have demonstrated that treatment of intrabony defects with EMD results in a significant reduction of the probing depths and gain of clinical attachment. Moreover, it was shown that EMD attenuated the release of TNF- α and IL-8 in whole blood from healthy donors challenged by lipopolysaccharide or peptidoglycan, but the release of IL-10 remained unchanged. EMD also produced a 4-fold increase in the cAMP levels of peripheral blood mononuclear cell lysates, which in turn suggested that EMD has anti-inflammatory properties [91]. A randomized, placebo-controlled multicenter study examined the effectiveness of EMD in the split-mouth procedure in 33 patients [92]. The results after 36 months showed a mean CAL gain of 2.2 mm in the test group and of 1.7 mm in the control group (open flap debridement). The radiologically determined bone gain amounted to 2.6 mm in the test group, with a 66% fill of the bone defects. However, the control teeth did not show any bone gain. In another controlled clinical study, Froum et al. [93] compared the treatment

of deep intrabony defects by open flap surgery with and without EMD therapy. Twenty-three patients each with ≥ 2 intrabony defects were treated. Thus, a total of 53 defects were treated with open flap surgery + EMD and 31 with open flap surgery alone. After a healing phase of 12 months the defects were opened again, to measure the defect fill. The results showed that the treatment with open flap surgery + EMD resulted in a 3 times larger defect fill than the treatment with flap surgery alone (74% defect fill after flap surgery + EMD vs. 23% defect fill after flap surgery alone) [93]. In a further prospective, controlled clinical study, a total of 40 patients were treated by surgical therapy with either EMD or GTR with a nonbioabsorbable or with 2 bioabsorbable barriers and compared to open flap surgery (control) [94]. All 4 regenerative procedures were equally effective regarding probing depth, reduction and CAL gain and were significantly better than the control treatment. A prospective, randomized, multicenter clinical study reported the treatment of intrabony defects with the papilla preservation technique with and without auxiliary application of EMD [95]. From a total of 166 defects, 83 were treated with EMD and the remaining 83 acted as control. After 1 year the results showed a significantly higher CAL gain in the test group than in the control group [95]. However, a recent randomized, double-masked, placebo-controlled clinical trial in 2005 failed to show significant differences in clinical and radiographic parameters following treatment of intrabony defects with open flap debridement and application of EMD or placebo [96]. Generally, most data from controlled clinical studies indicate that the additional application of EMD in the context of surgical therapy of deep intrabony periodontal defects may lead to significantly higher gains of clinical attachment and defect fill compared to open flap debridement [92–95, 97–102]. Surgical treatment with EMD was also demonstrated to significantly improve supracrestal soft-tissue density compared to open flap debridement alone [103, 104]. However, neither postoperative administration of amoxicillin and metronidazole nor selective cyclo-oxygenase-2 inhibitor appeared to enhance the clinical results [105, 106]. Furthermore, 2 studies suggested that the clinical outcomes of intrabony defects treated with EMD do not depend on the use of root conditioning of EDTA [107, 108]. Comparative studies reported similar results after treatment of intrabony defects with EMD or GTR, whereby the type of GTR barrier (nonbioabsorbable or bioabsorbable) did not play a role [94, 98–101, 109, 110]. The clinical results are comparable to those after GTR therapy. A recent prospective multicenter, random-

ized, controlled clinical trial compared the clinical outcomes of EMD and GTR with a bioabsorbable membrane [110]. Seventy-five patients with advanced chronic periodontitis were recruited in 7 centers from 3 countries. The surgical procedures included access for root instrumentation using the simplified papilla preservation flap and either the application of EMD or placement of a resorbable GTR membrane. The results of the trial failed to demonstrate superiority of 1 treatment over the other. It was interesting to note that all cases treated with GTR presented at least 1 surgical complication, mostly membrane exposure, while only 6% of EMD-treated sites displayed complications.

The data also indicate that the clinical outcomes after treatment of intrabony defects with EMD can be maintained over a longer time period (up to 5 years) [111–114].

Combination Therapies in Intrabony Defects

Experimental and clinical studies have indicated that the extent of the regeneration is determined by the available space under the mucoperiosteal flap [115, 116]. A collapse of the mucoperiosteal flap may limit the area needed for the regeneration process and may thus affect the result of the therapy. In order to avoid these disadvantages, combination therapies between EMD and GTR and/or EMD and bone substitutes were tested. Histological observations, from both man and animals, have demonstrated periodontal regeneration after treatment of intrabony defects with some of these combination treatments. In a prospective, controlled, clinical study, the treatment of intrabony defects was evaluated following treatment with EMD, GTR, combination of EMD + GTR and open flap surgery [99]. It was shown that all 3 regenerative treatment procedures resulted in a significantly higher improvement of the clinical parameters compared to the conventional flap surgery; however, combination therapy of EMD + GTR led to no additional improvement. Comparable results were also reported by others [117, 118]. A prospective, controlled split-mouth study in 11 patients with a total of 12 pairs of intrabony defects evaluated the clinical response of EMD with or without a combination of a tetracycline-coated expanded polytetrafluoroethylene barrier membrane at 6 and 12 months following therapy [118]. At 12 months, the mean CAL gain was 1.28 ± 2.04 mm in the EMD group and 1.65 ± 1.29 mm in the EMD + GTR group. Except for more postoperative discomfort at the membrane-treated sites, the results failed to reveal any significant differences between the 2 groups.

Several studies have evaluated the effect of a combination of EMD and various types of bone graft/bone substitute in the treatment of intrabony defects. Human histological studies indicate that a combination of EMD and a natural bone mineral or bioactive glass may indeed result in formation of root cementum, PDL and mineralization around the graft particles [119, 120]. However, the application of a natural bone mineral alone also resulted in periodontal regeneration [119]. On the other hand, when the defects were filled with a bioactive glass alone, the healing was characterized by formation of a long junction epithelium and connective tissue encapsulation of the graft particles [120]. Controlled clinical studies comparing treatment of intrabony defects with EMD alone or a combination of EMD and different types of bone graft/bone substitute seem to indicate that the combination of EMD and demineralized freeze-dried allogenic bone or a natural bone mineral may enhance the clinical outcome [121–124]. However, a recent study comparing the combination of EMD and a bioactive glass to EMD alone failed to show any significant differences between the 2 groups [125]. Furthermore, clinical studies comparing treatment with a combination of EMD and a bone graft/bone substitute to bone graft/bone substitute alone did not demonstrate any advantage of the combination approach [126–128]. Thus, it may be speculated that the type of the bone graft/bone substitute and the volume and configuration of the defects are also important factors which might influence the clinical outcomes. Further well-designed controlled clinical studies are necessary to evaluate the advantage of a combination therapy in relation to the single therapies.

Controlled Clinical Studies in Recession Defects

Histological findings in animal and human studies have shown that treatment of buccal recession defects with a coronally positioned flap and EMD can result not only in a covering of the gingival recession but also in the formation of cementum, PDL and bone [56, 58, 62, 63, 67, 73–75]. In 2 controlled clinical studies, the treatment of buccal Miller class I and II gingival recessions with a coronally positioned flap and EMD or coronally positioned flap alone were examined using the split-mouth procedure [129, 130]. Clinical outcome did not show any difference between the therapies in terms of root coverage over a short time period of 1 year. Additional application of EMD, however, induced a statistically significantly greater formation of keratinized tissue, compared to that with

coronally positioned flap alone [130]. A follow-up evaluation of this study showed that over 2 years, complete root coverage could be maintained in 53% of the EMD group compared to the control (23%) [131]. However, in the second year after therapy as many as 47% of the control group had deteriorated compared to only 22% in the treatment group.

Similar results were obtained in a randomized controlled clinical study on 58 contralateral sites, in 17 patients with ≥ 2 -mm Miller class I, II and III buccal recessions treated with coronally positioned flap and EMD (test) or the flap alone (control) [132]. At 6 months, there was a mean increase of keratinized tissue of 0.60 mm for the test sites and a mean decrease of 0.05 mm for the control sites. The test sites demonstrated better root coverage (92.9% after 6 months) compared to the control sites (66.8% after 6 months) [132]. These results were recently corroborated by others [133]. In a controlled, clinical, split-mouth study involving 17 patients, the therapy of buccal Miller class II recessions with a coronally positioned flap and EMD (test group) or flap alone was compared to connective tissue graft (control) [134]. A year after therapy, the mean value for root coverage was 95.1% in the test group and 93.8% in the control group. Total root coverage (100%) was reached in 89.5% of the cases in the test group and 79% in the control group. Furthermore, histological evaluation of 2 biopsies showed that treatment of recession defects with a coronally positioned flap and EMD resulted in the formation of root cementum, PDL and alveolar bone, while treatment with a coronally positioned flap and a connective graft was characterized by a long junctional epithelium and even signs of root resorption [75]. Comparable results were also reported in a multicenter, controlled clinical trial comparing the clinical efficacy of a coronally advanced flap procedure with the additional use of EMD (test) and subpedicle connective tissue graft (control) [135]. At 12 months the root coverage in the test group was 71.7% (± 16.14) compared to 87.0% ($\pm 12.22\%$) of the control. The available data suggest that the use of EMD may enhance the outcome of root coverage procedures, but the additional application of a connective tissue graft seems to further enhance the formation of keratinized tissue [134–136]. It is interesting to note that most controlled clinical studies evaluating the treatment of gingival recessions with coronally repositioned flaps and EMD therapy reported stable clinical results after a longer time period, up to 2 years, and an increase in the width of keratinized tissue, thus indicating that EMD may have an effect upon the proliferation and keratinization of gingival fibroblasts [131, 137, 138].

Controlled Clinical Studies in Furcation Defects

Data from controlled clinical studies evaluating the treatment of furcation defects using flap surgery with and without EMD are lacking. A multicenter, randomized, controlled, split-mouth, clinical study compared the treatment of mandibular class II furcation defects with EMD or GTR [139, 140]. A total of 44 patients with 90 comparable defects on contralateral molars were included. Defects were randomly assigned to treatment with EMD or GTR with a bioresorbable membrane. Treatment effects including gingival margin levels, probing depths, bleeding on probing, vertical attachment levels and vertical bone sounding from a stent at 5 buccal sites per tooth were evaluated at 8 and 14 months. The results indicated that both the treatment modalities led to significant clinical improvements. The median reduction of open horizontal furcation depth was 2.8 mm with the corresponding interquartile interval (1.5–3.5) at the test sites compared with 1.8 mm (1.0–2.8) at the control sites. The frequency of complete furcation closure was 8/45 (test) and 3/45 (control), partial closure 27/45 in both groups, and no change 9/45 and 11/45, respectively. Deterioration was observed in 1/45 and 4/45 sites, respectively. The frequency of no pain or no swelling at 1 week after surgery was 62 and 44%, respectively, at the test sites and 12 and 6% at the control sites. It was concluded that there was a significantly greater reduction in horizontal furcation depth and a comparatively lower incidence of postoperative pain/swelling following EMD compared to GTR therapy.

Conclusions

Based on the evidence presented, the following inferences can be drawn.

- Surgical periodontal treatment of deep intrabony defects with EMD promotes periodontal regeneration. The application of EMD in the context of nonsurgical periodontal therapy has failed to induce periodontal regeneration.
- Surgical periodontal therapy of deep intrabony defects with EMD may lead to significantly higher improvements in clinical parameters compared to open flap debridement alone. The effect of treatment with EMD is comparable to that with GTR and can be maintained over a 2-year period.
- Treatment of intrabony defects with a combination of EMD + GTR does not seem to additionally improve the outcomes compared to treatment with EMD alone or GTR alone.
- The combination of EMD and some types of bone graft/bone substitute may result in certain improvements in the soft- and hard-tissue parameters compared to treatment with EMD alone. However, further studies are needed in order to definitively clarify the possible advantage of a combination therapy of EMD and bone grafts/bone substitutes in relation to the single therapies.
- Treatment of recession-type defects with coronally repositioned flaps and EMD may promote formation of cementum, PDL and bone and may significantly increase the width of the keratinized tissue.
- Application of EMD seems to provide better long-term results than coronally repositioned flaps alone.
- Application of EMD may enhance periodontal regeneration in mandibular class II furcations. The clinical results are comparable to those obtained with GTR.

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