

The Application of Enamel Matrix Protein Derivative (Emdogain[®]) in Regenerative Periodontal Therapy: Which Applications are Evidence-Based?

Anton Sculean
Adrian Kašaj
Britta Willershausen

Department of Conservative Dentistry and Periodontology
Johannes Gutenberg University, Mainz

Summary

The goal of regenerative periodontal therapy is the reconstitution of the lost periodontal structures (i.e. the new formation of root cementum, periodontal ligament and alveolar bone). Results from basic research have pointed to the important role of the enamel matrix protein derivative (EMD) in periodontal wound healing. Histological results of experiments in animals and of human case reports 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. The goal of the current overview is to present, based on the existing evidence, the clinical indications for regenerative therapy with EMD.

Keywords: *enamel matrix protein derivative, cementogenesis, surgical periodontal therapy, periodontal regeneration.*

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Address for correspondence:

Dr. Anton Sculean, M.S.
Department of Conservative Dentistry and Periodontology
Augustusplatz 2
D-55131 Mainz, Germany
phone: 0049/6131/17-3052
fax: 0049/6131/17-3406

Introduction

Results from basic research have indicated the role of the different types of cementum for attaching the tooth and for the reparative processes in the entire periodontium. Acellular cementum is the most important tissue for the insertion of collagen fibres (1) and plays thereby the largest role in attaching the tooth to the alveolar socket. Studies of SLAVKIN and BOYDE (2) and SLAVKIN (3) show that proteins, which are secreted during the tooth development by the Hertwig's root sheath, play a crucial role in the formation of acellular root cementum. These proteins, referred to as enamel matrix proteins, constitute the largest part of the enamel matrix

(1, 4). They consist of a whole family of proteins, from which 90 % are Amelogenin, and the remaining 10% consist of prolin-rich non-Amelogenins, Tuftelin, and other serum proteins (1). It has been shown that the chemical structure of Amelogenin remained more or less constant during evolution, even among the individual animal species, exhibiting only slight differences (5). 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). In addition a close connection between acellular cementum and amelogenin exists (1). These results have also been confirmed in investigations of human teeth, where-

by some histological sections showed a thin layer of highly-mineralized enamel between dentin and root cement. This observation permits the assumption that the attachment of enamel matrix must occur on the dentin surface before the emergence of acellular cementum (1). Based on these results several *in vivo* experiments in animal models were conducted (1). In an experiment the lateral incisors of two monkeys were extracted. Immediately after the extraction a standardized cavity in the root surface was created mesially and distally. The test cavities were then filled with an enamel matrix derivative, while the control cavities remained untreated. All teeth were reimplanted into their original alveoles. Histological evaluation eight weeks after reimplantation resulted in formation of acellular cementum in the defects in which enamel matrix derivative was applied; whereas in the untreated control defects only a reparative, cellular cementum developed (1). On the basis of these findings the enamel matrix derivative (EMD) from the tooth pouches of non-erupted teeth from young pigs were isolated, purified and lyophilized. Since EMD are extreme hydrophobic, they were brought by means of a propylene glycol alginate (PGA) carrier into soluble form before their use in regenerative periodontal therapy (1). A technique or a material must, however, fulfill the following criteria in order to be classified as "regeneration-promoting" (6):

1. *In vitro* studies, which confirm the action mechanism.
2. Controlled histological animal studies, which demonstrate formation of new root cementum, periodontal ligament and alveolar bone.
3. Human biopsies, which show formation of root cementum, periodontal ligament and alveolar bone on a plaque-infected root surface.
4. Controlled clinical studies, which prove 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 were carried out to study the mechanism of the EMD on the desmod-

ontal gingival and bone cells (7-17). Thus in a series of laboratory studies the migration, attaching, proliferation, biosynthesis activity and formation of mineralized nodules following the application of EMD were examined. To determine the possible presence of existing polypeptide factors immunoassays were performed (8, 9). The results have shown that: a) under *in vitro* conditions EMD promotes the proliferation of periodontal ligament fibroblasts, but not that of epithelial cells, b) the total protein synthesis of the periodontal ligament fibroblasts increases, and c) the formation of mineralized nodules by periodontal ligament fibroblasts is promoted.

Furthermore, no specific polypeptide factors such as IGF-1,2; PDGF, TNNF, TGF β , or IL-1 β could be identified. In further investigations it was shown that the attaching growth and metabolic rate of desmodontal fibroblasts increased significantly, when EMD was added in cell cultures (8-11, 13). Moreover, periodontal ligament fibroblasts treated with EMD displayed an increased intracellular cAMP concentration and autocrine releasing of TGF-1 β , IL-6 and PDGF in comparison to the control group (without addition of EMD) (13). Although the epithelial cells showed an increased release of cAMP and PDGF following the additional application of EMD, their proliferation and growth rate was inhibited (11, 13). It was concluded that EMD simultaneously promotes the growth of mesenchymal cells by inhibiting that of the epithelial cells. It was also concluded that EMD promotes the release of autocrine growth factors from desmodontal fibroblasts (13). Furthermore, desmodontal fibroblasts showed a significantly increased alkaline phosphatase activity following the application of EMD (17). Very recent investigations have demonstrated that EMD significantly increased the mRNA synthesis of the matrix proteins Versican, Byglycan and Decorin and led to an increased Hyaluronan synthesis in the gingival and desmodontal fibroblasts (10). However, it has to be emphasized that in all studies EMD had a much stronger effect on the desmodontal fibroblasts than on gingival fibroblasts. Further experimental investigations have shown that the application of EMD can regulate the expression of the genes associated with cementoblasts which in turn affects crucially the mineralization process (16). Kawase et al. (18) examined the effect of EMD on

the proliferation of oral epithelium cells (SCC25). After 3 days of treatment with EMD the cell division was prevented and at the same time the cell cycle was stopped in the G1 phase. Additionally, it was shown that the addition of EMD limited significantly the expression of Cytokeratin-18 (CK18). The authors concluded that EMD does not possess a cytostatic but rather, a cytotoxic effect on epithelial cells (18). In an *in vitro* study the combination of 4 mg EMD and active demineralized freeze-dried allogenic bone (DFDBA) showed an increased bone induction (7). It was concluded that EMD possesses no osseoinductive, but rather osteopromotive characteristics when applied in certain concentrations (7). Schwartz et al. (15) have shown that EMD stimulates the early stages of the osseoblast maturation by increasing cell proliferation. However, when applied on mature cell lines, the main effect was confined to the influence of cell differentiation. Recently, certain antibacterial effects and disturbances of bacterial adherence were found to be influenced by EMD (19-22). After 4 days of plaque accumulation a plaque sample was taken from 24 patients with chronic periodontitis and divided into 5 equal parts (21). Each part was mixed with 5 µl of the following solutions: 1) NaCl, 2) EMD solved in water, 3) EMD solved in PGA vehicle, 4) PGA vehicle, 5) Chlorhexidine digluconate (CHX). Subsequently, the vitality of the plaque flora was evaluated under the vital fluorescent microscope. The results have shown that EMD solved 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. In a further investigation it was shown that EMD inhibits the growth of the periodontal pathogenic bacteria *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*. 24 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 (22). The inhibiting effect of EMD on periodontal pathogenic bacteria was also confirmed by others (20).

In conclusion the data from *in vitro* studies indicate that EMD affects important wound healing mechanisms.

Controlled histological studies in animals

In a controlled histological study, recession defects were created and treated with EMD (23). 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 have shown that in all test defects a new periodontium, i.e. 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 more acellular and only partly attached at the root surface. An interesting aspect of this study is that in the test defects no root resorption occurred, while in the control defects the root resorption was a very frequently found phenomenon. It is important to mention that during the entire study period no oral hygiene measures were carried out. In two further studies in monkeys, intrabony defects were created surgically (24, 25). The defects were treated with one of the following therapies: a) Guided Tissue Regeneration (GTR), b) EMD, c) EMD + GTR or d) with conventional flap debridement surgery (control). The histological investigation has shown that the healing was characterized by a long junctional epithelium and a limited periodontal regeneration after flap debridement surgery. The treatment with GTR, EMD and EMD + GTR resulted in formation of cementum with inserting collagen fibres as well as of alveolar bone (24, 25).

Results from human histological studies

Results of the first human-histological biopsy were published by Heijl (26). A recession defect on a lower incisor, was surgically created and treated with EMD. After a healing period of 4 months, the tooth as well as the surrounding soft and hard tissue was extracted and histologically evaluated. The histological investigation 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 (27) treated 10 intrabony periodontal defects in 8 patients with EMD. The histological analysis 6 months after the treatment showed in 3 biopsies complete periodontal regeneration (i.e. new formation of root cementum, periodontal ligament and alveolar bone), while in 3 further biopsies, the healing was characterized by a new connective tissue attachment (i.e. new cementum with inserting collagen fibers). Four biopsies healed by a long junctional epithelium and 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 Guided Tissue Regeneration (GTR) with a bioabsorbable barrier (28). Six months after therapy, the 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. The histological analysis showed that in both groups the healing was mainly characterized by periodontal regeneration (28). The mean value of new cementum and periodontal ligament amounted to 2.6 ± 1.0 mm in the EMD group and to 2.1 ± 1.0 mm in the GTR group. The mean value of new alveolar bone was 0.9 ± 1.0 mm in the EMD group and of in the GTR group 2.1 ± 1.0 mm. Reparative healing by a long junctional epithelium occurred only in one biopsy from the EMD group. The results of the study have confirmed that treatment with EMD promotes periodontal regeneration in humans and may lead to comparable clinical and histological results than the GTR therapy. These results were confirmed in subsequent reports by other authors, not only in intrabony but also in recession-type defects (29-32). Very recent immunohistological studies in human have also shown that EMD remains up to 4 weeks following surgery on the root surface and, that the wound healing and/or remodelling process can be followed for a period of up to 6 months after treatment with EMD therapy (33, 34). However, no periodontal regeneration was observed, when EMD was applied in a non-surgical way into the periodontal defects (35).

Controlled clinical studies

Side effects, such as for example incompatibility or allergic reactions even after repeated treatment with EMD, were not reported in any published studies (36-38). 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. A randomized, placebo controlled multicenter-study examined the effectiveness of EMD in the split-mouth procedure in 33 patients (39). 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. (40) compared the treatment of deep intrabony defects by open flap surgery with and without EMD. In 23 patients with at least 2 intrabony defects each a total of 53 defects were treated with open flap surgery + EMD and 31 were treated with open flap surgery alone. After a healing phase of 12 months the defects were again opened and the defect fill measured. The results showed that the treatment with open flap surgery + EMD resulted in a 3 x 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) (40). In a further prospective, controlled clinical study a total of 40 patients were treated by surgical therapy with either EMD or GTR with a non-bioabsorbable or with 2 bioabsorbable barriers and compared to the open flap surgery (control) (41). All 4 regenerative procedures were equally effective regarding probing depth (PD) reduction and CAL gain and were, significantly better than the control treatment. A prospective, randomized, multi-center clinical study reported the treatment of intrabony defects with the papilla preservation technique with and without auxiliary application of EMD (42). A total of 83 test and 83 control defects were treated. After one year the results showed significantly higher CAL gain in the test group than in the control group (42). Comparative studies reported comparable results after treatment of intrabony defects with EMD or GTR, whereby the type of the GTR barrier (not bioabsorbable or bioab-

sorbable) did not play a role (41, 43-45). 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 (44). The results have shown that all 3 regenerative procedures resulted in a significantly higher improvement of the clinical parameters compared to the conventional flap surgery; whereby the combination of EMD + GTR led to no additional improvement. The data from controlled clinical studies generally show that the additional application of EMD in the context of surgical therapy of deep intrabony periodontal defects may lead to statistically significantly better clinical results compared to open flap debridement (39, 41, 42, 44-47). The clinical results are comparable to those after GTR therapy. Very recent data have also shown that the clinical results after treatment of intrabony defects with EMD can be maintained over a longer time period (4 and/or 5 years) (48-50).

Combination therapies

Experimental and clinical studies have indicated that the extent of the regeneration is determined by the available space under the mucoperiosteal flap (28, 51). 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. Observations from animal-histological and human-histological studies have demonstrated periodontal regeneration after treatment of intrabony defects with some of these combinations. Data from controlled clinical studies are controversial and no clear advantage of a combination therapy in relation to the single therapies has been proven EMD (24, 25, 44, 52-58).

Treatment of recession defects

Histological results from animals and humans 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,

periodontal ligament and bone (23, 24, 26, 29, 31). In two 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 were examined using the split-mouth procedure (59, 60). The results did not show differences between the therapies concerning root coverage. The additional application of EMD led, however, to statistically significantly higher formation of keratinized tissue than the coronally positioned flap technique alone (59). In a recently published 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 with a coronally positioned flap and connective tissue graft (control) was compared (61). The results have shown that one year after therapy the mean value of root coverage was 95.1% in the test group and 93.8% in the control group. A 100% root coverage was reached in 89.5% of the cases in the test group and in 79% of the cases in the control group. The additional histological evaluation of two biopsies showed that treatment of recession defects with a coronally positioned flap and EMD resulted in formation of root cementum, periodontal ligament 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 (29).

Treatment of furcation defects

Histological results from studies in monkeys have indicated that the treatment of class III mandibular furcation defects with EMD results in no predictable periodontal regeneration (62). At present there are no human-histological data regarding the healing of furcation defects following treatment with EMD. Data from controlled clinical studies evaluating the treatment of furcation defects by means of flap surgery with and without EMD are also lacking. A multi-center, randomized, controlled, split-mouth, clinical study compared the treatment of mandibular class II furcation defects with EMD or GTR (63). The results have indicated that the treatment with EMD resulted in significantly higher CAL gain and bone fill than the GTR therapy

Conclusions

Based on the presented evidence the following conclusions can be drawn:

- a. Surgical periodontal treatment of deep intrabony defects with EMD promotes periodontal regeneration. The application of EMD in the context of non-surgical periodontal therapy has failed to result in periodontal regeneration.
- b. Surgical periodontal therapy of deep intrabony defects with EMD may lead to significantly higher improvements of the clinical parameters than open flap debridement alone. The results obtained following treatment with EMD are comparable to those following treatment with GTR.
- c. For the time being there is no clear evidence of an advantage of a combination of therapies, such as EMD and GTR or EMD and bone substitutes, in relation to single therapies.