

AN UPDATE OF EVIDENCE-BASED APPLICATIONS OF ENAMEL MATRIX PROTEIN DERIVATIVE IN REGENERATIVE PERIODONTAL THERAPY

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INTRODUCTION

Results from basic research identify different cementum types for attaching the tooth and for the reparative procedures in the entire periodontium. Acellular cementum is the most important tissue for the insertion of the collagen fibres¹ and plays thereby the largest role in attaching the tooth to the alveolar socket. Studies of SLAVKIN & BOYDE² and SLAVKIN³ show that proteins, which are delivered during the tooth development by the Hertwig's root sheath, play a crucial role in the emergence of the acellular root cementum. These proteins are enamel matrix proteins and 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, Tuffelin, and other serum proteins.¹ It is proven 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 of the root development in rats, apes and pigs, it has been immunohistologically proven that the concentration of the Amelogenin rises dramatically during tooth development.¹ In addition a close connection between acellular cementum and amelogenin exists.¹ These results are also confirmed with the investigation of human

teeth, whereby some histological sections show 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 one experiment the lateral incisors of two apes were extracted. Immediately after the extraction a standardized cavity in the root surface was created mesial and distal. The test cavities were then filled with enamel matrix derivative, while the control cavities remained untreated. All teeth were reimplanted into their original alveole. Histological investigation eight weeks after reimplantation observed the 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 Yet, a recent study on porcine teeth, analyzing the association between Hertwig's epithelial root sheath cells, enamel matrix proteins (EMPs) and cementogenesis, questions the causal link between EMPs and the formation of acellular extrinsic fiber cementum, as formulated by Hammarstrom.^{1,64} Based on these discoveries the enamel matrix derivative (EMD) from the tooth pouches of not erupted teeth from young pigs were isolated, purified and lyophilized. Since SMP are extreme hydrophobic, they were brought by means of a propylene glycol alginate (PGA) carrier into soluble form and used in the regenerative periodontal therapy.¹ However, in order to be classified as "regeneration-promoting" a technology or a material must fulfill the following criteria⁶:

1. In vitro studies, which confirm the effect mechanism.
2. Controlled histological animal studies, which exhibit a new formation of root cementum, periodontal ligament and alveolar bone.

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3. Human biopsies, which prove a new formation of root cementum, periodontal ligament and alveolar bone on a plaque- infected root surface.

4. Controlled clinical studies, which prove a gain of clinical attachment and radiological new bone formation. In the following overview, the existing evidence regarding the use of EMD is provided.

IN VITRO STUDIES

Several in vitro investigations were carried out to study the damage mechanism of the EMD on the desmodontal gingival and bone cells.⁷⁻¹⁷ Thus in a series of lab tests the migration, attaching, proliferation, biosynthesis activity and formation were examined in mineralized nodules. Immunoassays were formulated in order to determine the possible presence of existing polypeptide factors.^{8,9}

The results showed that:

a) under in vitro conditions EMD promotes the proliferation of periodontal ligament fibroblasts but not that of epithelium cells,

b) the total protein synthesis of the periodontal ligament fibroblasts increases,

c) the formation of mineralized nodules by periodontal ligament fibroblasts is promoted. Further, no specific polypeptid 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 significantly increased, if EMD were added in cell cultures.^{8-11,13} Furthermore periodontal ligament fibroblasts treated with EMD showed increased intracellular cAMP concentration and autocrine releasing of TGF-ss1, IL-6 and PDGF OFF in comparison to the control group (without auxiliary graft of EMD).¹³ Although the epithelium cells showed an increased release of cAMP and PDGF with the auxiliary graft of EMD, their proliferation and growth was inhibited.^{11, 13} It was concluded that EMD simultaneously promotes the growth of mesenchymal cells, inhibiting the epithelial cells. It was also concluded that EMD promotes the release of autocrine growth factors from the desmodontal fibroblasts.¹³ Furthermore desmodontal fibroblasts showed a strongly increased activity of the alkaline phosphatase with graft of EMD.¹⁷ Further investigations show that EMD significantly increased the mRNA synthesis of the matrix proteins Versican, Byglycan and Decorin and further lead 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 the gingival fibroblasts. Further experimental

investigations could show that the application of EMD can regulate the expression of the genes associated with cementoblasts and can crucially affect the mineralization process.¹⁶ Kawase et al.¹⁸ examined the effect of EMD on the proliferation of oral epithelium cells (SCC25). After 3 days 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 EMD limited significantly the expression of Zytokeratin-18 (CK18). The authors concluded that EMD does not possess a cytostatic but rather, a cytotoxic effect on epithelial cells.¹⁸ In a further 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 therefore concluded that EMD possesses no osseoinductive, but rather osteopromotive characteristics in certain concentrations.⁷ Schwarz et al.¹⁵ showed that EMD stimulates the early stages of the osseoblast maturation by increased proliferation, with matured cell lines however, the main effect is reached by influencing cell differentiation. Other studies focused on the influence of the EMD on the viability, proliferation and attachment of periodontal ligament fibroblasts (PDLF) to diseased root surfaces.⁶¹ The results indicated that viability was negatively affected for higher concentrations of EMD (100mg/ml), while lower concentrations displayed viability effects similar to control. Proliferation appeared to be ameliorated following exposure to EMD, and the SEM analysis suggested that cellular attachment to diseased dentin was enhanced following the application of EMD. The study supported the concept that EMD may act as a suitable matrix for PDLF.⁶¹ As there are experimental evidences that EMD topically applied in instrumented periodontal pockets could enhance the early healing of periodontal soft tissue wounds⁶⁵, a study tried to clarify whether EMD can cause an angiogenic effect and, thus, possibly to enhance the wound healing. The study proved that EMD exhibits a certain angiogenic effect *in vitro*, as in a murine model. Yet, the clinical finding of faster initial healing after the application of EMD is not the result of the angiogenic effect of EMD alone.⁶⁶ Recently certain antibacterial effects and disturbances of bacteria adherence were proven by EMD.¹⁹⁻²² In this way from 24 patients with chronic periodontitis after 4 days of plaque accumulation a plaque sample was taken and divided into 5 equal parts.²¹ Each part was mixed with 5 μ l of the following solutions: 1) NaCl, 2) EMD dissolved in water, 3) EMD in PGA vehicle solved, 4) PGA vehicle, 5) Chlorhexidine digluconate (CHX). Subsequently, the vitality of the plaqueflora under the vital fluorescent microscope was evaluated. The results showed that

EMD in PGA vehicle solved and the PGA vehicle had a very strong antibacterial effect. It was therefore accepted that the antibacterial effect of EMD is achieved mainly by 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 after application of EMD no living colonies of these germs could be observed. Furthermore EMD demonstrated no negative effect on gram positive bacteria.²² The inhibiting effect of EMD on periodontal pathogenic germs has also been confirmed by other groups of researchers.²⁰

In summary, the data of in vitro studies show that EMD affects important periodontal 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 segment was histologically regenerated. The results showed 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 absorption occurred, while in the control defects the root absorption was a phenomenon found very frequently. It is important to mention that during the entire study period in the animal group, no oral hygiene measures occurred. In two further studies with apes, 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 showed 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 a new 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, created surgically as an experiment, was treated with EMD. After a healing period of 4 months, the tooth as well as the surrounding soft and hard tissue was extracted and prepared histologically. The histological investigation showed that a new layer of acellular root cementum covered 73% of the original defect depth. New alveolar bone regressed on 65% of the initial bone height. In another study Yukna and Mellonig²⁷ treated 10 intrabony periodontal defects in 8 patients with EMD.

The histological analysis 6 months after the treatment showed in 3 biopsies a 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 against it healed by a long junctional epithelium and showed no signs of a periodontal regeneration. In a comparative clinical and histological investigation the healing of intrabony periodontal defects was compared with EMD or Guided Tissue Regeneration (GTR) with a bioabsorbable barrier.²⁸ Six months after therapy, clinical attachment level (CAL) showed a mean gain of 3.2 ± 1.2 mm in the EMD group and 3.6 ± 1.7 mm in the GTR group. The histological analysis showed that in both groups the healing was mainly characterized by regeneration of periodontal structures.²⁸ The mean value of new cementum and desmodont 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 in the GTR group 2.1 ± 1.0 mm. A reparative healing by a long junctional epithelium occurred only in one biopsy from the EMD group. The results of the study supplied the proof that the treatment with EMD promotes the regeneration of periodontal structures in humans and can lead to similar clinical and histological results like the GTR therapy. These results were confirmed in subsequent reports by other authors, not only in intrabony but also in recession defects.²⁹⁻³² The latest human immunohistological studies show that EMD remains up to 4 weeks post-operative on the root surface; and in addition the wound healing and/or remodelling process can be continued after the EMD therapy, up to a period of 6 months.^{33,34} However, no periodontal regeneration is observed, if EMD is applied in a non-surgical way into the periodontal defects.³⁵

CONTROLLED CLINICAL STUDIES

Side effects, such as incompatibility or allergic reactions were not reported in any published studies, even after repeated treatment with EMD. Data from controlled clinical studies prove that the treatment of intrabony defects with EMD results in a significant reduction of the probing depths and gain of clinical attachment. A preliminary randomized, placebo controlled multicenter-study examined the effectiveness of EMD in the split-mouth procedure in 33 patients.³⁶ 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 (flap debridement surgery). The radiologic determined bone gain amounted to 2.6 mm, in the test group, with a 66% replenishment of the bone defects. However the control teeth did not show a bone gain. In another controlled study Froum et al.³⁷, compared the treatment of deep intrabony defects by open flap surgery with and without EMD in 23 patients with 2 intrabony defects, at least each. 53 defects were treated with open flap surgery and EMD and 31 were treated only with open flap surgery. After a healing phase of 12 months the defects were again opened and the defect filling was measured. The results showed that the treatment with open flap surgery and EMD resulted in a 3x larger defect fill than the treatment with flap surgery alone (74% defect fill after flap surgery and EMD opposite 23% defect fill after flap surgery alone)³⁷ In a further prospective, controlled clinical study with a total of 40 split-mouth procedures, the patients received surgical therapy with EMD and with a non-absorbable and/or with 2 absorbable barriers, compared to the conventional flap surgery.³⁸ All 4 regenerative procedures were equally effective regarding the probing depth (PD) reduction and profit of CAL and, significantly better than the control treatment (open flap surgery). 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.³⁹ Altogether 83 test and 83 control defects were treated. After 1 year the results showed significantly higher CAL gain in the test group than in the control group.³⁹ Comparative studies reported similar results after treatment of intrabony defects with EMD or GTR, whereby the kind of the GTR barrier (not absorbable or absorbable) did not play a role.^{38,40-42} In a prospective, controlled, clinical study the treatment of intrabony defects was compared with EMD, GTR, combination of EMD + GTR and open flap surgery.⁴¹ The results showed that all 3 regenerative procedures resulted in a significantly higher improvement of the clinical

parameters in comparison to the conventional flap surgery, while the combination of EMD + GTR led to no additional improvement of the clinical parameters. 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 leads to statistically significant clinical improvements over flap surgery.^{36,38,39,41-44} The clinical results are comparable to those of GTR therapy. A multicenter clinical trial conducted on 98 patients with an interproximal intrabony defect, comparing the outcomes of GTR with non-resorbable membranes and EMD, showed a strong correlation in both groups of patients between CAL gains and full mouth bleeding scores, and between CAL gains and defect morphology and depth.⁶³ Further data from the newest studies show that the clinical results after treatment of intrabony defects with EMD are observed for a longer period (4 and/or 5 years).⁴⁵⁻⁴⁷ Periodontal intrabony defects were treated with EMD also by mean of microsurgical access flaps. In a controlled clinical study, Wachtel et al.⁶⁷ concluded that, in terms of PD reduction and CAL gain, the combination of microsurgical access flap with EMD application appeared to be superior to the microsurgical flap alone.

TREATMENT OF PERIODONTAL SITES WITH HORIZONTAL TYPE OF BONE LOSS

Yilmaz et al.⁶² tried to assess the clinical and radiographic outcome of the horizontal type of bone loss over 8 months following periodontal surgery with or without using EMD. Results showed that for pockets of 4-6 mm, EMD was significantly better than the open flap debridement in terms of PD reduction, relative attachment (RAL) gain and less recession. Yet, no significant difference in the degree of bone levels between the two procedures was found after 8 months.

EVALUATION OF EMD AS AN ADJUNCT TO NON-SURGICAL PERIODONTAL THERAPY

Data of clinical studies indicate that EMD topically applied in instrumented pockets enhance the early healing of periodontal soft tissue wounds.⁶⁵ However, results of a controlled split-mouth longitudinal study on 22 patients displaying symmetric sites with PD=5mm and radiographic angular bone defects >3mm revealed no statistical difference in PD reduction or CAL gain between the test and the control group

when using EMD as a routine adjunct to non-surgical periodontal therapy.⁶⁸

COMBINATION THERAPIES

Experimental and clinical studies show that the extent of the regeneration strongly depends on the free space which is under the mucoperiosteal flap.^{28,48} A collapse of the mucoperiosteal flap limits the area needed for the regeneration process and effects the result of the therapy. In order to avoid these disadvantages, combination therapies of EMD and GTR and/or EMD and bone substitutes were tested. Observations from animal-histological and human-histological studies prove a periodontal regeneration after treatment of intrabony defects with some of these combinations. The data from controlled clinical studies shows however no advantage of a combination therapy in relation to the single therapy with EMD.^{24,25,41,49-55} A particular type of combined therapy – Er: YAG laser in combination with EMD – for the treatment of intrabony periodontal defects was evaluated by Schwarz and Sculean in a controlled study on 22 intrabony defects.⁶⁹ Within the limits of the study, it could be concluded that the combination of laser and EMD does not seem to improve the clinical outcome of the therapy additionally compared to SRP+EDTA+EMD.

TREATMENT OF RECESSION DEFECTS

Histological results from animal and humans show that the 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 a new formation of cementum, desmodont and even bone.^{23,24,26,29,31} In 2 controlled clinical studies with the split-mouth procedure the treatment of buccal Miller class I and II gingival recessions with a coronally positioned flap and EMD or coronally positioned flap were examined.^{56,57} The results did not show differences between the therapies concerning root coverage. The auxiliary application of EMD led however to statistically significantly higher new formation of keratinised tissue than the coronally positioned flap technology alone.⁵⁶ In a recently published controlled, clinical, split-mouth procedure study with 17 patients the therapy of buccal Miller class II recessions with a coronally positioned flap and EMD (test group) with a coronally positioned flap and connective tissue graft (control) was compared.⁵⁸ The results showed that 1 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 cases in the test group and in 79% of cases in the control group. The additional histological evaluation of two biopsies showed that the treatment of recession defects with a coronally positioned flap and EMD resulted in a new formation of root cementum, periodontal ligament and alveolar bone, whereby the treatment with a coronally positioned flap and a connective graft was characterized by a long junctional epithelium and even signs of root absorption.²⁹

TREATMENT OF FURCATION DEFECTS

The histological results from studies with apes show that the treatment of class III furcation defects in the lower jaw with EMD results in no foreseeable periodontal regeneration.⁵⁹ At present there are no human-histological data regarding the healing from furcation defects and treatment with EMD. Also data from controlled clinical studies are lacking regarding the treatment of furcation defects by means of flap surgery with and without EMD. In a multi-center, randomized, controlled, split-mouth, clinical study the treatment of lower jaw class II furcation defects was compared with EMD and GTR.⁶⁰ The results showed that the treatment with EMD resulted in significantly higher CAL gain and bone fill than the GTR therapy.

CONCLUSIONS

So far, all evidence confirms the following:

1. Periodontal surgery treatment of deep intrabony defects with EMD promotes periodontal regeneration. The application of EMD in the context of the non-surgical periodontal therapy results histologically in no periodontal regeneration.
2. The periodontal surgery therapy of deep intrabony defects with EMD leads to a significantly higher improvement of clinical parameters than the flap debridement surgery alone as opposed to GTR therapy.
3. There is no clear evidence of an advantage of a combination therapy of EMD and GTR or EMD and bone substitutes in relation to the single therapy.

REFERENCES

1. Hammarström L. Enamel matrix, cementum development and regeneration. *J Clin Periodontol* 1997;24:658-668.
2. Slavkin HC, Boyde A. Cementum: An epithelial secretory product? *J Dent Res* 1975;53:157(abstr. 409).
3. Slavkin HC. Towards a cellular and molecular understanding of periodontics: Cementogenesis revisited. *J Periodontol* 1976;47: 249-255.
4. Lindskog S, Hammarström L. Formation of intermediate cementum III: 3H-tryptophane and 3H proline uptake into the epithelial

- root sheath of Hertwig in vitro. *J Craniofac Genet Dev Biol* 1982;2:172-177.
5. Brookes SJ, Robinson C, Kirkham J, et al. Biochemistry and molecular biology of amelogenin proteins of developing dental enamel. *Arch Oral Biol* 1995;40:1-4.
 6. World Workshop in Periodontology. The American Academy of Periodontology. *Ann Periodontol* 1996;1:618-670.
 7. Boyan BD, Weesner TC, Lohmann CH, et al. Porcine fetal enamel matrix derivative enhances bone formation induced by demineralized freeze dried bone allograft in vivo. *J Periodontol* 2000;71:1278-1286.
 8. Gestreluis S, Andersson C, Johansson AC, et al. Formulation of enamel matrix derivative surface coating. Kinetics and cell colonization. *J Clin Periodontol* 1997;24:678-684.
 9. Gestreluis S, Andersson C, Lidström D, et al. In vitro studies on periodontal ligament cells and enamel matrix derivative. *J Clin Periodontol* 1997;24:685-692.
 10. Haase HR, Bartold PM. Enamel matrix derivative induces matrix synthesis by cultured human periodontal fibroblast cells. *J Periodontol* 2001;72:341-348.
 11. Hoang AM, Oates TW, Cochran DL. In vitro wound healing responses to enamel matrix derivative. *J Periodontol* 2000;71:1270-1277.
 12. Hoang AM, Klebe RJ, Steffensen B, et al. Amelogenin is a cell adhesion protein. *J Dent Res* 2002;81:497-500.
 13. Lyngstadaas SP, Lundberg E, Ekdahl H, et al. Autocrine growth factors in human periodontal ligament cells cultured on enamel matrix derivative. *J Clin Periodontol* 2001;28:181-188.
 14. Okuda K, Miyazaki A, Momose M, et al. Levels of tissue inhibitor of metalloproteinases-1 and matrix metalloproteinases-1 and -8 in gingival crevicular fluid following treatment with enamel matrix derivative (EMDOGAIN®). *J Periodont Res* 2001;36:309-316.
 15. Schwartz Z, Carnes DL Jr, Pulliam R, et al. Porcine fetal enamel matrix derivative stimulates proliferation but not differentiation of pre-osteoblastic 2T9 cells, inhibits proliferation and stimulates differentiation of osteoblast-like MG63 cells, and increases proliferation and differentiation of normal human osteoblast NHOst cells. *J Periodontol* 2000;71:1287-1296.
 16. Tokiyasu Y, Takata T, Saygin E, et al. Enamel factors regulate expression of genes associated with cementoblasts. *J Periodontol* 2000;71:1829-1839.
 17. Van der Pauw MT, Van den Bos T, Everts V, et al. Enamel matrix-derived protein stimulates attachment of periodontal ligament fibroblast and enhances alkaline phosphatase activity and transforming growth factor b1 release of periodontal ligament and gingival fibroblasts. *J Periodontol* 2000;71:31-43.
 18. Kawase T, Okuda K, Yoshie H, et al. Cytostatic action of enamel matrix derivative (EMDOGAIN®) on human oral squamous cell carcinoma-derived SCC25 epithelial cells. *J Periodont Res* 2000;35:291-300.
 19. Arweiler NB, Ausschill TM, Donos N, et al. Antibacterial effect of an enamel matrix protein derivative on in vivo dental biofilm vitality. *Clin Oral Invest* 2002;6:205-209.
 20. Newman SE, Coscia SA, Jorwani R, et al. Effects of enamel matrix derivative on *Porphyromonas gingivalis*. *J Periodontol* 2003;74:1191-1195.
 21. Sculean A, Ausschill TM, Donos N, et al. Effect of an enamel matrix derivative (Emdogain®) on ex vivo dental plaque vitality. *J Clin Periodontol* 2001;28:1074-1078.
 22. Spahr A, Lyngstadaas SP, Boeckh C, et al. Effect of the enamel matrix derivative Emdogain® on the growth of periodontal pathogens in vitro. *J Clin Periodontol* 2001;29:62-72.
 23. Hammarström L, Heijl L, Gestreluis S. Periodontal regeneration in a buccal dehiscence model in monkeys after application of enamel matrix proteins. *J Clin Periodontol* 1997;24:669-677.
 24. Sculean A, Donos N, Reich E, Brex M, Karring T. Healing of recession-type defects following treatment with enamel matrix proteins or guided tissue regeneration. A pilot study in monkeys. *J Parodontol Implant Orale* 2000;19:19-31.
 25. Sculean A, Donos N, Brex M, et al. Treatment of intrabony defects with enamel matrix proteins and guided tissue regeneration. An experimental study in monkeys. *J Clin Periodontol* 2000;27:466-472.
 26. Heijl L. Periodontal regeneration with enamel matrix derivative in one human experimental defect. A case report. *J Clin Periodontol* 1997;24:693-696.
 27. Yukna RA, Mellonig J. Histologic evaluation of periodontal healing in humans following regenerative therapy with enamel matrix derivative. A 10-case series. *J Periodontol* 2000;71:752-759.
 28. Sculean A, Donos N, Windisch P, et al. Healing of human intrabony defects following treatment with enamel matrix proteins or guided tissue regeneration. *J Periodont Res* 1999;34:310-322.
 29. McGuire MK, Cochran DL. Evaluation of human recession defects treated with coronally advanced flaps and either enamel matrix derivative or connective tissue. Part 2: Histological evaluation. *J Periodontol* 2004;74:1126-1135.
 30. Mellonig JT. Enamel matrix derivative for periodontal reconstructive surgery: technique and clinical and histologic case report. *Int J Periodontics Restorative Dent* 1999;19:9-19.
 31. Rasperini G, Silvestri M, Schenk RK, et al. Clinical and histological evaluation of human gingival recession treated with a subepithelial connective tissue graft and enamel matrix derivative (Emdogain®): A case report. *Int J Periodontics Restorative Dent* 2000;20:269-275.
 32. Sculean A, Chiantella GC, Windisch P, et al. Clinical and histologic evaluation of treatment of intrabony defects with an enamel matrix protein derivative (Emdogain®). *Int J Periodont Rest Dent* 2000;20:375-381.
 33. Sculean A, Windisch P, Keglevich T, et al. Presence of an enamel matrix protein derivative on human teeth following periodontal surgery. *Clin Oral Invest* 2002;6:183-187.
 34. Sculean A, Junker R, Donos N, et al. Immunohistochemical evaluation of matrix molecules associated with wound healing following treatment with an enamel matrix protein derivative in humans. *Clin Oral Invest* 2003;7:167-174.
 35. Sculean A, Windisch P, Keglevich T, et al. Histologic evaluation of human intrabony defects following non-surgical periodontal therapy with and without application of an enamel matrix protein derivative. *J Periodontol* 2003;74:153-160.
 36. Sculean A, Donos N, Reich E, et al. Healing of recession-type defects following treatment with enamel matrix proteins or guided tissue regeneration. A pilot study in monkeys. *J Parodontol Implant Orale* 2000;19:19-31.
 37. Froum SJ, Weinberg MA, Rosenberg E, et al. A comparative study utilizing open flap debridement with and without enamel matrix derivative in the treatment of periodontal intrabony defects: a 12-month re-entry study. *J Periodontol* 2001;72:25-34.
 38. Pontoriero R, Wennström J, Lindhe J. The use of barrier membranes and enamel matrix proteins in the treatment of angular bone defects. A prospective controlled clinical study. *J Clin Periodontol* 1999;26:833-840.
 39. Tonetti MS, Lang NP, Cortellini P, et al. Enamel matrix proteins in the regenerative therapy of deep intrabony defects. A multicenter randomized controlled clinical trial. *J Clin Periodontol* 2002;29:317-325.
 40. Sculean A, Donos N, Blaes A, Lauerma M, et al. Comparison of enamel matrix proteins and bioabsorbable membranes in the treatment of intrabony periodontal defects. A split-mouth study. *J Periodontol* 1999;70:255-262.
 41. Sculean A, Windisch P, Chiantella GC, et al. Treatment of intrabony defects with enamel matrix proteins and guided tissue regeneration. A prospective controlled clinical study. *J Clin Periodontol* 2001;28:397-403.
 42. Zuchelli G, Bernardi F, Montebugnoli L, et al. Enamel matrix proteins and guided tissue regeneration with titanium-reinforced expanded polytetrafluoroethylene membranes in the treatment of intrabony defects: a comparative controlled clinical trial. *J Periodontol* 2002;73:3-12.

43. Okuda K, Momose M, Miyazaki A, et al. Enamel matrix derivative in the treatment of human intrabony osseous defects. *J Periodontol* 2000;71:1821-1828.
44. Silvestri M, Ricci G, Rasperini G, et al. Comparison of treatments of intrabony defects with enamel matrix derivative, guided tissue regeneration with a nonresorbable membrane and Widman modified flap. A pilot study. *J Clin Periodontol* 2000;27:603-610.
45. Sculean A, Donos N, Miliuskaite A, et al. Treatment of intrabony defects with enamel matrix proteins or bioresorbable membranes. A four year follow up splith-mouth study. *J Periodontol* 2001;72:1695-1701.
46. Sculean A, Chiantella GC, Miliuskaite A, et al. Four-year results following treatment of intrabony periodontal defects with an enamel matrix protein derivative. A report of 46 cases. *Int J Periodont Rest Dent* 2003;23:345-351.
47. Sculean A, Donos N, Schwarz F, et al. Five year results following treatment of intrabony defects with enamel matrix proteins and guided tissue regeneration. *J Clin Periodontol* (in print).
48. Tonetti MS, Pini-Prato GP, Cortellini P. Factors affecting the healing response of intrabony defects following guided tissue regeneration and access flap surgery. *J Clin Periodontol* 1996;23:548-556.
49. Lekovic V, Camargo PM, Weinlaender M, et al. A comparison between enamel matrix proteins used alone or in combination with bovine porous bone mineral in the treatment of intrabony periodontal defects in humans. *J Periodontol* 2000;71:1695-1701.
50. Sculean A, Barbé G, Chiantella GC, et al. Clinical evaluation of an enamel matrix protein derivative combined with a bioactive glass for the treatment of intrabony periodontal defects in humans. *J Periodontol* 2002;73:401-408.
51. Sculean A, Chiantella GC, Windisch P, et al. Clinical evaluation of an enamel matrix protein derivative (Emdogain®) combined with a bovine derived xenograft (Bio-Oss®) for the treatment of intrabony periodontal defects in humans. *Int J Periodont Rest Dent* 2002;22:259-267.
52. Sculean A, Windisch P, Keglevich T, et al. Clinical and histologic evaluation of human intrabony defects treated with an enamel matrix protein derivative combined with a bovine-derived xenograft. *Int J Periodont Rest Dent* 2003;23:47-55.
53. Sculean A, Windisch P, Keglevich T, et al. Clinical and histological evaluation of an enamel matrix protein derivative combined with a bioactive glass for the treatment of intrabony periodontal defects in humans. *Int J Periodont Rest Dent* (in print).
54. Scheyer ET, Velasquez-Plata D, Brunsvold MA, et al. A clinical comparison of a bovine-derived xenograft used alone and in combination with enamel matrix derivative for the treatment of periodontal osseous defects in humans. *J Periodontol* 2002;73:423-432.
55. Velasquez-Plata D, Scheyer ET, Mellonig JT. Clinical comparison of an enamel matrix derivative used alone or in combination with a bovine-derived xenograft for the treatment of periodontal osseous defects in humans. *J Periodontol* 2002;73:433-440.
56. Hägewald S, Spahr A, Rompola E, et al. Comparative study of Emdogain® and coronally advanced flap technique in the treatment of human gingival recessions. A prospective controlled clinical study. *J Clin Periodontol* 2002;29:35-41.
57. Modica F, Del Pizzo M, Rocuzzo M, et al. Coronally advanced flap for the treatment of buccal gingival recessions with and without enamel matrix derivative. A split-mouth study. *J Periodontol* 2000;71:1693-1698.
58. McGuire MK, Nunn M. Evaluation of human recession defects treated with coronally advanced flaps and either enamel matrix derivative or connective tissue. Part 1: Comparison of clinical parameters. *J Periodontol* 2004;74:1110-1125.
59. Donos N, Sculean A, Glavind L, et al. Treatment of mandibular degree III furcation involvements with a bioresorbable membrane and Emdogain in monkeys (Abstract 2337). *J Dent Res* 1998;77:294.
60. Hoffmann T, Boedeker RH, Jepsen S. Treatment of buccal Class II furcation defects (Abstract 284). *J Clin Periodontol* 2003;30:73 (Supplement 4).
61. Davenport DR, Mailhot JM, Wataha JC, et al. Effects of enamel matrix protein application on the viability, proliferation, and attachment of human periodontal ligament fibroblasts to diseased root surfaces, in vitro. *J Clin Periodontol* 2003;30:125-131.
62. Yilmaz S, Kuru B, Altuna-Kirac E. Enamel matrix proteins in the treatment of periodontal sites with horizontal type of bone loss. *J Clin Periodontol* 2003;30:197-206.
63. Silvestri M, Sartori S, Rasperini G, et al. Comparison of infrabony defects treated with enamel matrix derivative versus guided tissue regeneration with a nonresorbable membrane. A multicenter controlled clinical trial. *J Clin Periodontol* 2003;30:386-393.
64. Bosshardt DD, Nanci A. Hertwig's epithelial root sheath, enamel matrix proteins, and initiations of cementogenesis in porcine teeth. *J Clin Periodontol* 2004;31:184-192.
65. Wennstrom JL, Lindhe J. Some effects of enamel matrix proteins on wound healing in the dento-gingival region. *J Clin Periodontol* 2002;29:9-14.
66. Yuan K, Chen C-L, Lin MT. Enamel matrix derivative exhibits angiogenic effect in vitro and in a murine model. *J Clin Periodontol* 2003;30:732-738.
67. Wachtel H, Schenk G, Bohm S, et al. Microsurgical access flap and enamel matrix derivative for the treatment of periodontal intrabony defects: a controlled clinical study. *J Clin Periodontol* 2003;30:496-504.
68. Gutierrez MA, Mellonig JT, Cochran DL. Evaluation of enamel matrix derivative as an adjunct to non-surgical therapy. *J Clin Periodontol* 2003;30:739-745.
69. Schwarz F, Sculean A, Georg T, Becker J. Clinical evaluation of the Er:YAG laser in combination with an enamel matrix protein derivative for the treatment of intrabony periodontal defects: a pilot study. *J Clin Periodontol* 2003;30:975-981.