



## Enamel matrix derivative proteins

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### Introduction

Enamel matrix derivative proteins (EMD) are secreted by the Hertwig's epithelial root sheath (HERS) during tooth development and may be critical in the formation of cementum (1). These proteins share similar amino acid sequences between bovine, porcine and human species (2). Because of these inter-species similarities, EMD for dental use is sequestered from developing teeth of fetal pigs and is marketed by the Straumann Company as Emdogain gel®. This gel is composed mainly of amelogenin, which makes up 90% of the enamel matrix derivative proteins and it is thought to be the key protein associated with cementogenesis during tooth formation. The remaining 10% of EMD is proline-rich non-amelogenins including among them: tuftelin, tuft protein, ameloblastin (3) and amelin (4).

### Biological properties of Emdogain®

Emdogain® appears to have significant roles in regeneration by the stimulation of the periodontal ligament (PDL), cementum, bone and vascular components. Specifically:

#### Role in periodontal ligament formation

*In vitro* studies have shown that Emdogain® enhances proliferation of PDL cells (5). Other investigations revealed that cultured PDL cells exposed to Emdogain® demonstrate increased attachment rate and metabolism. PDL cells exposed to Emdogain® release several growth factors such as transforming growth factor (TGF-β1), interleukin (IL-6) and platelet derived growth factor AB (PDGF-AB) all of which function to recruit and differentiate mesenchymal cells for regeneration. Conversely, Emdogain® inhibits epithelial cell growth. This inhibition may preferentially promote the proliferation of mesenchymal cells instead of epithelium by the PDL release of autocrine growth factors in a process mimicking natural root development (6).

#### Role in cementogenesis

Secretion of EMD by the inner layer of the epithelial root sheath is required prior to cementum deposition (1). This regenerative process, modified through the application of Emdogain®, results in cementum formation in both primates and humans (7,8).

#### Role in osteogenesis

*In vitro* studies demonstrated an overall stimulatory effect of Emdogain® on osteoblastic cells (9). Similar outcomes were noted *in vivo* in which the addition of Emdogain® to demineralized freeze-dried bone allograft material (DFDBA) resulted in enhanced bone formation (10).

#### Role in angiogenesis

The role of vascular ingrowth (angiogenesis) into healing periodontal sites is vital to the success of guided tissue regeneration procedures (11). *In vitro* wound studies investigating the effect of Emdogain® have shown increased angiogenesis and improved healing properties after its application (12).

#### Role as an antimicrobial

A secondary property of Emdogain® is the antimicrobial effect displayed in the *in vitro* studies showing inhibition of periodontal pathogens such as *Actinobacillus actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg) and *Prevotella intermedia* (Pi) (13). Further investigation revealed this inhibition is due to the alginate carrier and not the proteins in Emdogain® (14). More research is required in the *in vivo* model to substantiate this proposal.

#### Role as a membrane

Periodontal membranes prevent the epithelial downgrowth into intrabony defects and allow the repopulation of the diseased root surface with undifferentiated cells from the surrounding bone and PDL. Because of its epithelium inhibitory properties, Emdogain® may function as a periodontal membrane with varying degrees of clinical success (15).

### Clinical applications of Emdogain®

#### Periodontal defects

Emdogain® was approved by the FDA for the topical application to diseased root surfaces to treat intrabony and furcation type of defects (16). The use of Emdogain® in combination with flap debridement resulted in 2.4 mm of greater osseous fill compared to flap debridement alone (17). Emdogain® can also be mixed with a graft material (autogenous or allograft) in the treatment of intrabony defects (18) but there are no clinical studies that demonstrate the clinical significance of this combined therapy.

#### Periodontal plastic procedures

Recession type defects treated with Emdogain® plus connective tissue grafts resulted in the histologic evidence of 1.87 mm of new bone and PDL over the previously diseased root surface (19,20). In a recent study, Emdogain® plus a coronally positioned flap (CPF) compared to a connective tissue graft demonstrated similar clinical outcomes of 95.1% and 93.8% root coverage respectively. Even though the results clinically similar, the Emdogain® plus coronally positioned flaps (CPF) eliminated the need for a donor site that is required for the connective tissue grafts (21).

#### Treatment of avulsed teeth

Emdogain® may play a role in reducing external root resorption following avulsion and subsequent reimplantation. In a prospective study, trauma induced ankylosed teeth treated with application of Emdogain® prior to reimplantation showed reduced rate of external root resorption compared to teeth that did not receive its application (22).

### How supplied? How to use it?

Emdogain® is supplied in a pre-filled, pre-mixed syringe that is available in two sizes - 0.7 ml for multiple defects and 0.3 ml for single defect sites. Each kit contains three syringes of Emdogain® in addition to EDTA (24% ethylenediaminetetraacetic acid) which functions as a root conditioner. Each syringe is intended for single use only. The kits must be maintained below 37°C during transport and must be refrigerated until use. Emdogain® has a shelf life of 24 months (17); assuming proper refrigerated storage.

After removal of all granulation tissue and calculus, the root surface is conditioned with EDTA gel for 2 minutes by gently burnishing with a cotton pellet. After removing the root conditioner with irrigation, Emdogain® is applied to the root surfaces with a syringe. It is critical that no saliva or blood contaminate the root surface prior to Emdogain® application. The gingival flaps are sutured following placement of appropriate graft material or membrane if indicated.

### Safety

Use of Emdogain® in periodontal therapy in humans had no negative impact on periodontal wound healing (23). Serum samples of patients treated with Emdogain® in periodontal defects demonstrated low immunogenic potential even after repeated applications of Emdogain®. These results were confirmed in allergy prone patients indicating that its use in humans is safe (24). While possible transfer of viruses or other

infectious agents such as prions among humans and animals is a valid concern, no disease transmission has been reported from the use of Emdogain®.

### Conclusion

Emdogain® is a material recently available for general use as a periodontal regenerative product that is based on the concept of bioengineering. Further investigations are needed to elucidate the specific functions of the proteins in Emdogain®. More clinical studies are needed comparing the efficacy of Emdogain® with other treatment modalities presently available.

### References

1. Hammarstrom L. Enamel matrix, cementum development and regeneration. *J Clin Periodontol.* 1997 Sep;24(9 Pt 2):658-68.
2. Fincham AG, Belcourt AB, Termine JD, Butler WT, Cothran WC. Amelogenins. Sequence homologies in enamel-matrix proteins from three mammalian species. *Biochem J.* 1983 Apr 1;211(1):149-54.
3. Krebsbach PH, Lee SK, Matsuki Y, Kozak CA, Yamada KM, Yamada Y. Full-length sequence, localization, and chromosomal mapping of ameloblastin. A novel tooth-specific gene. *J Biol Chem.* 1996 Feb 23;271(8):4431-5.
4. Cerny R, Slaby I, Hammarstrom L, Wurtz T. A novel gene expressed in rat ameloblasts codes for proteins with cell binding domains. *J Bone Miner Res.* 1996 Jul;11(7):883-91.
5. Gestrelus S, Andersson C, Lidstrom D, Hammarstrom L, Somerman M. In vitro studies on periodontal ligament cells and enamel matrix derivative. *J Clin Periodontol.* 1997 Sep;24(9 Pt 2):685-92.
6. Lyngstadaas SP, Lundberg E, Ekdahl H, Andersson C, Gestrelus S. Autocrine growth factors in human periodontal ligament cells cultured on enamel matrix derivative. *J Clin Periodontol.* 2001 Feb;28(2):181-8.
7. Lindskog S, Hammarstrom L. Formation of intermediate cementum. III: 3H-tryptophan and 3H-proline uptake into the epithelial root sheath of Hertwig in vitro. *J Craniofac Genet Dev Biol.* 1982;2(2):171-7.
8. Yoneda S, Itoh D, Kuroda S, Kondo H, Umezawa A, Ohya K, et al. The effects of enamel matrix derivative (EMD) on osteoblastic cells in culture and bone regeneration in a rat skull defect. *J Periodontol Res.* 2003 Jun; 38(3):333-42.
9. Boyan BD, Weesner TC, Lohmann CH, Andreacchio D, Carnes DL, Dean DD, et al. Porcine fetal enamel matrix derivative enhances bone formation induced by demineralized freeze dried bone allograft in vivo. *J Periodontol.* 2000 Aug;71(8):1278-86.
10. Rose LF, Rosenberg E. Bone grafts and growth and differentiation factors for regenerative therapy: a review. *Pract Proced Aesthet Dent.* 2001 Nov-Dec; 13(9):725-34; quiz 736, 721-2.
11. Yuan K, Chen CL, Lin MT. Enamel matrix derivative exhibits angiogenic effect in vitro and in a murine model. *J Clin Periodontol.* 2003 Aug; 30(8):732-8.
12. Rincon JC, Haase HR, Bartold PM. Effect of Emdogain on human periodontal fibroblasts in an in vitro wound-healing model. *J Periodontol Res.* 2003 Jun;38(3):290-5.
13. Spahr A, Lyngstadaas SP, Boeckh C, Andersson C, Podbielski A, Haller B. Effect of the enamel matrix derivative Emdogain on the growth of periodontal pathogens in vitro. *J Clin Periodontol.* 2002 Jan;29(1):62-72.

14. Newman SA, Coscia SA, Jotwani R, Iacono VJ, Cutler CW. Effects of enamel matrix derivative on *Porphyromonas gingivalis*. *J Periodontol.* 2003 Aug;74(8):1191-5.
15. Silvestri M, Sartori S, Rasperini G, Ricci G, Rota C, Cattaneo V. Comparison of infrabony defects treated with enamel matrix derivative versus guided tissue regeneration with a nonresorbable membrane. *J Clin Periodontol.* 2003 May;30(5):386-93.
16. <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMA/PMA.cfm?ID=10993>
17. Froum SJ, Weinberg MA, Rosenberg E, Tarnow D. 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 Jan;72(1):25-34.
18. Rosen PS, Reynolds MA. A retrospective case series comparing the use of demineralized freeze-dried bone allograft and freeze-dried bone allograft combined with enamel matrix derivative for the treatment of advanced osseous lesions. *J Periodontol.* 2002 Aug;73(8):942-9.
19. Rasperini G, Silvestri M, Schenk RK, Nevins ML. Clinical and histologic 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 Jun; 20(3):269-75.
20. 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.* 2003 Aug;74(8):1126-35.
21. 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.* 2003 Aug;74(8):1110-25.
22. Filippi A, Pohl Y, von Arx T. Treatment of replacement resorption with Emdogain--a prospective clinical study. *Dent Traumatol* 2002 Jun;18(3): 138-43.
23. Heard RH, Mellonig JT, Brunsvold MA, Lasho DJ, Meffert RM, Cochran DL. Clinical evaluation of wound healing following multiple exposures to enamel matrix protein derivative in the treatment of intrabony periodontal defects. *J Periodontol.* 2000 Nov;71(11):1715-21.
24. Zetterstrom O, Andersson C, Eriksson L, Fredriksson A, Friskopp J, Heden G, et al. Clinical safety of enamel matrix derivative (EMDOGAIN) in the treatment of periodontal defects. *J Clin Periodontol* 1997 Sep;24(9 Pt 2):697-704. 29.

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