The biologic concept for the use of enamel matrix protein: True periodontal regeneration

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Guided tissue regeneration procedures have been used successfully to reestablish periodontal attachment. However, this new attachment reportedly differs from the original attachment in strength and continuity. Enamel matrix proteins secreted by Hertwig’s epithelial sheath play an important role in cementogenesis on roots and in the development of the periodontal attachment apparatus. Enamel matrix protein harvested from developing porcine teeth, or enamel matrix derivative (Emdogain), is reported to induce true periodontal regeneration (the attachment of new, acellular cementum to the underlying dentin surface). The results of experimental and clinical trials of Emdogain are reviewed, and the procedure for application of the material is described. (Quintessence Int 1998;29:621–630)

Key words: cementogenesis, Emdogain, enamel matrix protein, periodontal disease, true periodontal regeneration

Clinical relevance

An enamel matrix derivative has been used successfully to reestablish the biologic processes of developing tooth roots and their supporting tissues.

Periodontal disease is an infectious disease affecting tooth-supporting tissues. Clinically it results in color changes of the gingiva, periodontal pocket formation, bleeding, clinical attachment loss, increased tooth mobility, and loss of alveolar bone on the radiographs. The extrinsic etiology of periodontal disease is bacterial plaque in the periodontal pockets.

The main aim of conventional periodontal therapy is to halt and possibly reverse the attachment loss resulting from the disease. To this end, initial therapy is focused on removing bacterial plaque from teeth and periodontal pockets and preventing supragingival plaque accumulation. Subgingival plaque can be removed by nonsurgical forms of therapy, such as scaling and root planing, or by surgical means. The efficacy of nonsurgical methods is well documented.1,2

Several studies have confirmed the efficacy of mechanical subgingival plaque control in periodontal therapy, irrespective of the approach used.3,4 Adequate supragingival plaque control by patients is prerequisite for the success of the periodontal treatment.5,6

However, subgingival bacteria in deep pockets with complicated anatomy, in infrabony pockets, and in areas of furcal involvement are sometimes difficult to remove with nonsurgical therapy. In these pockets, open access with surgical therapy may be indicated to clean the root surfaces.

Mechanical removal of subgingival calculus and plaque may not be enough to control subgingival infection in specific cases. Thus, antibiotics are sometimes employed to enhance healing by suppressing specific bacteria that cannot be removed by mechanical therapy alone.7

Guided tissue regeneration or guided tissue repair?

Even when inflammation has been eliminated and healthy periodontal tissue has been established after pathogenic microorganisms are removed from periodontal pockets, the anatomy of healed defects can be a problem, particularly in areas where esthetics or maintenance is critical and maintenance is difficult, such as in the anterior dentition with gingival recession, infrabony defects, and furcations.

In the early 1980s, a series of experimental studies was conducted on a procedure to regenerate the lost attachment apparatus. A membrane was placed under the flap to prevent epithelial downgrowth and to make a space for periodontal re-formation.8 The procedure,
two cell layers of ectodermal origin are called Hertwig's epithelial root sheath. As in dentinogenesis in the epithelial root sheath, mesenchymal cells around the dental follicle, and cementoblasts are induced on the root dentin surface, resulting in the formation of root cementum. This root cementum, which is produced in the initial stage, contains no cells and is called acellular cementum. Principal fibers are embedded in this acellular cementum, and the cementum functions to maintain attachment to the tooth (Fig 1).

On the other hand, if Hertwig's epithelial sheath is not divided and the enamel proteins are not exposed to mesenchymal cells, an enamel pearl may be produced on the root dentin surface.

Following the production of root cementum, a series of cell inductions occur in the intrinsic dental follicle, and the periodontal ligament and alveolar bone proper are formed.

### Development of enamel matrix derivative

Thus, enamel matrix proteins secreted by Hertwig's epithelial sheath play an important role not only in cementogenesis on roots but also in the development of the periodontal attachment apparatus. Research was focused on enamel matrix proteins in a series of studies. These proteins were applied for the regeneration of tooth-supporting tissues that were lost to periodontal disease.

The enamel matrix protein harvested from developing porcine teeth is called enamel matrix derivative (EMD). Stable, freeze-dried EMD was purified (Emdogain, Biora) for use in periodontal tissue regeneration (Fig 2).

### Experimental study of enamel matrix derivative

Hammarström et al. used Emdogain to attempt periodontal tissue regeneration in monkeys. After a mucoperiosteal flap extending from the maxillary canine to the maxillary first molar was elevated bilaterally, the buccal alveolar bone, periodontal ligament, and cementum were removed with a dental bur, creating an artificial dehiscence defect (Fig 3). On the test site, after
an acid-etching procedure on the root surfaces, EMD was applied to cover the root surfaces. The flap was then repositioned and sutured (Fig 4). On the control site, after acid etching, the flap was immediately repositioned and sutured without application of EMD.

The monkeys were killed after 8 weeks, and the buccolingual sections of the sites were analyzed under microscope. The test sites, where EMD was used, did not generally show gingival recession or formation of a long junctional epithelium. Almost complete (60% to 70%) regeneration of acellular cementum that was firmly attached to the root dentin was observed, and the regenerated collagen fibers were found to extend into the regenerated alveolar bone proper (Fig 5). The control sites displayed gingival recession, and the cementum, periodontal ligament, and alveolar bone were regenerated to a much smaller degree (10%) (Figs 6 and 7).

This animal study showed that, in artificial dehiscence defects, much of the periodontal attachment apparatus can be regenerated with the application of EMD.

**Histology from the application of enamel matrix derivative**

Heijl applied EMD on a human tooth to investigate its tissue-generating ability histologically. A mucoperiosteal buccal flap was raised around a mandibular left central incisor that was to be extracted for orthodontic purposes. The alveolar bone, periodontal ligament, and cementum were removed to create an experimental dehiscence defect similar to that created in the monkey model. Following acid etching of the root surface, EMD was applied to cover the denuded root surface. The flap was then repositioned and sutured.
Fig 5 The buccolingual tissue section of the test site shows periodontal tissue regeneration between the arrows.

Fig 6 The buccolingual tissue section of the control site does not show regeneration of the periodontium between the arrows. Apical migration of the epithelium has occurred.

Fig 7 Newly formed cementum and bone, expressed in percentage of total defect length since the time of surgery, in experimental sites (EMD) and control sites in monkeys.

Fig 8 Regenerated alveolar bone (B) and acellular cementum (arrow) are present around a human central incisor treated with EMD. (D) Dentin.

After 4 months, the tooth was extracted in block section, and its buccolingual sections were examined under microscope. The regenerated cementum was found to have covered 73% of the artificial defect, and 65% of the defect was filled with regenerated alveolar bone. The regenerated acellular cementum was firmly attached to the root surface, and the collagen fibers from the cementum were observed extending into the regenerated alveolar bone proper (Fig 8). These histologic images were similar to those observed in the test sites in the monkey study.

These results suggest that application of EMD may result in true periodontal regeneration.

Clinical study of enamel matrix derivative

Heijl et al. reported the results from a multicenter study of the clinical application of Emdogain. Thirty-three patients (7 men and 26 women; mean age, 48 years) were included in the study. Each patient had, in the same jaw, two or more infrabony defects with probing depths of 6
mm or more and exhibited a radiographic bone defect at least 4 mm deep and 2 mm wide. The defects had to be primarily one- or two-wall defects when examined at the time of surgery.

Modified Widman flap surgery was employed to treat the defects. After the mucoperiosteal flaps were raised, root surfaces were scaled and planed at the test and control sites. Following acid treatment of the root surfaces, Emdogain was applied to the denuded root surfaces of the test sites. The flap was then repositioned and sutured. At the control sites, a placebo was applied to the root surfaces after acid treatment. The patients were prescribed antibiotics for 3 weeks after surgery. Supragingival plaque control, including chlorhexidine rinsing, during weeks 3 to 6, was conducted throughout the experimental period. The patients were reexamined 8, 16, and 36 months after surgery.

The test sites where enamel matrix derivative was applied showed gradual radiographic bone level gain for 36 months, while significant changes in bone level were not observed in the control sites (Figs 9 and 10). The bone level gain observed on the radiographs was more predictable by far at Emdogain-treated sites than at control sites (Fig 11). The mean clinical attachment gain after 36 months was 2.2 mm at the test sites and 1.7 mm at the control sites; this difference was statistically significant (Fig 12).

**Technique for application of enamel matrix derivative**

**Preparation of Emdogain**

Emdogain consists of freeze-dried enamel matrix protein (enamel matrix derivative, packed in a vial with a green cap), of which the major protein is amelogenin, and its viscous carrier, propylene glycol alginate (packed in a vial with a silver cap). The vials are stored in a refrigerator (2°C to 8°C), and the Emdogain is prepared about 15 minutes before application of the material (Fig 13).

A syringe with a long, large bore (diameter 1.2 mm) is used to draw the propylene glycol alginate solution from the vial (Fig 14). The solution is spread evenly...
over the freeze-dried EMD so it can begin to solubilize the EMD (Fig 15). Emdogain is drawn into a syringe with the same large bore, which is then replaced with a short, blunt needle before application. The mixture is generally enough to treat three teeth (Fig 16).

**Clinical application of Emdogain**

After initial therapy, which included scaling and root planning under local anesthesia, the mandibular right second molar still presented a Class II furcation involvement (Lindhe's classification) on the buccal aspect and bleeding on probing. A provisional fixed partial denture was placed between the second premolar and the second molar (Fig 17).

A radiograph at the site was taken with the Eggen method, and a gutta-percha point was inserted into the furcation area (Fig 18). The midbuccal probing depth measured 7 mm (Figs 19 and 20).

A local anesthesia that did not include a vasoconstrictor was administered (Fig 21). A minimal amount of anesthetic should be used in the papillae and marginal gingiva.

An intracrevicular incision was combined with a vertical releasing incision (Fig 22). A mucoperiosteal (full-thickness) flap was elevated. Inflammatory granulation tissue and pocket epithelium were removed to obtain complete access to the root surfaces (Figs 23 and 24).

Visible subgingival plaque and calculus were removed (Figs 25a and 25b). Scaling and root planing...
Figs 19 and 20. The probing depth measures 7 mm at the midbuccal aspect of the furcation defect.

Fig 21. An anesthetic without a vasoconstrictor is used for local anesthesia.

Fig 22. An intracrevicular incision with a vertical releasing incision is made.

Fig 23. A mucoperiosteal (full-thickness) flap is raised.

Fig 24. The alveolar bone is exposed.

Fig 25a. Hand instruments are used to scale the root surfaces.

Fig 25b. An ultrasonic scaler is then used to scale the root surfaces.
were completed (Figs 26a and 26b). The root surfaces were treated for a short period of time (about 15 seconds) to remove the smear layer and rinsed with saline (Figs 27 and 28). Care was taken to keep the area free of saliva and blood.

Prepared Emdogain gel was applied with a syringe to cover the entire denuded root surface (Figs 29 and 30). The flap was repositioned so that the soft tissue covered all the exposed root surfaces and was sutured (Figs 31 and 32). It is critical that the flap be sutured in such a way that the soft tissue is tightly attached to the root surfaces. Antibiotics were administered starting the day before surgery and lasting 3 weeks postsurgery.

The sutures were removed 2 weeks after surgery. An antimicrobial mouthrinse (chlorhexidine) was administered for 6 weeks after surgery. Mechanical tooth cleaning should not be performed at the surgical site for 3 weeks; after this period, the patient should clean the area with the roll method using a soft toothbrush. Interdental brushes should not be used for the first 6 weeks postoperatively. Postoperative plaque control is particularly important, and professional prophylaxis is provided if necessary (Figs 33 to 36).

The final restoration was delivered 8 months after surgery (Figs 37 and 38). The probing depth decreased from 7 mm at pretreatment to less than 3 mm at 8 months, and the furcation defect was clinically closed (Figs 39 and 40).

As in other types of periodontal surgery, thorough professional cleaning of subgingival plaque by the practitioner and adequate supragingival plaque control by the patient are required for the success of this technique. Also, this procedure is as technique sensitive as other tissue regeneration therapies.
Fig. 31 The flap is repositioned and sutured.

Fig. 32 Periodontal dressing is used.

Fig. 33 Healing after 1 week.

Fig. 34 Healing after 3 weeks.

Fig. 35 Healing after 3 months.

Fig. 36 Radiograph at 3 months.

Fig. 37 Healing after 8 months.

Fig. 38 Radiograph at 8 months.

Fig. 39 Furcation defect before surgery with probing depth of 7 mm.

Fig. 40 Same site at 8 months with probing depth of less than 3 mm.
Conclusion

Enamel matrix proteins play an important role in the formation of acellular cementum and set in motion a series of events that lead to periodontal attachment. Emdogain, an enamel matrix derivative, induces true periodontal regeneration by reestablishing the biologic process of developing cementum and other periodontal supporting tissues.

Acknowledgments

The author thanks Dr. Lars Heijl, Biora AB, for providing data and giving thoughtful advice.

References