Periodontal regeneration with enamel matrix derivative in one human experimental defect

A case report


Abstract. The purpose of the present study was to histologically assess the effect of enamel matrix derivative on periodontal regeneration in one human experimental defect. Experimental surgery was performed in a healthy male volunteer to create a buccal dehiscence defect in a mandibular incisor. Following bone removal and conditioning of the exposed root surface, enamel matrix derivative was applied onto the root surface. The flaps were then replaced and sutured. Clinical healing was uneventful. After 4 months, the experimental tooth together with the surrounding soft and hard tissues were removed surgically for histological evaluation. The microscopic examination revealed formation of a new acellular extrinsic fibre cementum, which was firmly attached to the underlying dentin. A new periodontal ligament with inserting and functionally-oriented collagen fibres and an associated alveolar bone was also present. The new cementum covered 73% of the original defect. Regain of bone was 65% of the presurgical bone height. It was concluded that adjunctive use of enamel matrix derivative could provide a regenerative technology with a potential for true periodontal regeneration.

Key words: enamel proteins; periodontal regeneration; dehiscence defect; histology; human

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Periodontal healing with new functional tooth-supporting tissues requires the expression of specific cell phenotypes at the root surface. A mixture of cells from the periodontal ligament, endosteal/periosteal connective tissues, gingival connective tissues and epithelial tissues compete in this process. Consequently, the resulting healing varies widely from a completely regenerated and functional periodontal attachment, such as that seen following surface resorption incident to orthodontic tooth movement, to non-functional capsular arrangements, or downgrowth of a long junctional epithelium as seen on root surfaces following mechanical debridement, i.e. scaling and root planing.

Recent data on application of enamel matrix derivative (Hammarström 1997, Gestrelius et al. 1997), has been found to promote the reformation of acellular (extrinsic fibre) cementum that was well attached to the underlying dentin and alveolar bone in a dehiscence model and in a replantation model in monkeys (Hammarström 1997, Hammarström et al. 1997). The mechanism of action is not known in detail, but is suggested to mimic the role of enamel proteins in cementogenesis during nascent root development. It appears that the temporary deposition of enamel matrix proteins onto a root surface is an essential step preceding the (re)formation of acellular cementum and that the formation/regain of periodontal ligament and alveolar bone is dependent on formation of acellular cementum (Hammarström 1997). Thus, adjunctive use of enamel matrix proteins in conjunction with regenerative periodontal surgery could possibly provide a natural extracellular matrix for colonization of previously diseased root surfaces with cells expressing a cementoblast phenotype.

However, the preclinical experimental data from studies with Enamel Matrix Derivative (EMD) in monkeys give only limited information on the ability of EMD to promote periodontal regeneration in humans. Thus, the purpose of the present study was to assess histologically the effect of EMD on periodontal regeneration in one human experimental defect.
Material and Methods

One healthy male (age 49 years), with crowding in the lower front segment, wanted to have tooth 31 extracted followed by orthodontic treatment to align the lower incisors. The subject volunteered to have experimental periodontal surgery performed on tooth 31 before it was removed. The patient was given a full and adequate verbal and written information about the nature, purpose and possible risk of the experimental procedure. The patient was notified that he was free to withdraw from the study at any time. A signed informed consent was obtained before starting the study. Prior to experimental surgery there were healthy periodontal conditions around the experimental tooth, although the gingival margin had receded slightly (Fig. 1).

Test device

Enamel matrix derivative (EMD) with propylene glycol alginate (PGA; BIORA AB, Malmo, Sweden) as vehicle (Gestrelius et al. 1997) was used. The vial containing 10 mg sterile lyophilized EMD was reconstituted with 1.0 ml of a sterile aqueous solution of PGA prior to experimental surgery. Five minutes was allowed for the enamel proteins to dissolve. The test device was then withdrawn with a 3 ml syringe to prepare for application in the experimental area.

Experimental procedures

Surgery

The area around the experimental tooth was anesthetized (Xylocain® adrenalin, Astra AB) and disinfected. Following a buccal intracrevicular incision with vertical releasing incisions extending out into the alveolar mucosa on the buccal aspect of the surgical area, a full-thickness (muco-periosteal) buccal flap was raised. The buccal bone plate, i.e. the bone between the mesial and distal line angles and almost reaching the apex of the root, was removed with a large round bur and bone chisels (Fig. 2). There were no attempts to minimize the mechanical injury to the root surface. A bevel was prepared in the root surface at the level of the surgically established bone crest as well as a notch at the marginal level of bone prior to removal. These landmarks later served as reference points for histometric measurements to be made in histological sections (Fig. 3). Following bone removal the surgical area was rinsed with sterile saline and the exposed root surface was quickly “etched” with 37% ortho-phosphoric acid (DeTrey, Dentsply) for 15 seconds to remove the “smear layer” (Polson & Proye 1982, Polson et al. 1984, Blomlof & Lindskog 1995, Blomlof et al. 1996), thereby allowing the enamel matrix proteins to precipitate onto the root surface devoid of organic debris (Gestrelius et al. 1997). Following acid application, the root surface was thoroughly rinsed with saline. EMD in PGA vehicle was then immediately applied to the exposed root surface, starting at the most apical bone level and covering the entire root surface. The flaps were replaced and sutured appropriately with Goretex™ sutures.

After 4 months, the experimental tooth, together with the surrounding soft and hard periodontal tissues, was removed surgically for histological
EMD in a periodontal defect

Fig. 4. Lower front area with tooth 31 four months post-surgery. Note recession of gingival margin on tooth 31 (cf. fig. 1).

Fig. 5. Microphotograph of section of the experimental root surface (polarized light) showing the formation of a thin, extrinsic/intrinsic cementum with an associated periodontal ligament and alveolar bone.

Evaluation. Subsequent to post-surgical healing, the investigator provided orthodontic treatment which was fully satisfactory to the patient.

Histological preparation
The specimen containing the experimental tooth with surrounding alveolar bone and soft periodontal tissues was fixed in cold 10% buffered formalin for 48 hours, demineralized in 5% formic acid, embedded in paraffin and sectioned parallel to the long axis of the tooth in a bucco-lingual direction. The microtome was set at 5 μm. The sections were cut step-serially at levels 20 μm apart and stained with hematoxylin and eosin.

Evaluation
The sections were examined by transmitted and polarized light microscopy. The distances, expressed in percent of the experimental defect (as delineated by the landmarks prepared in the root surface), covered by new cementum and new bone were measured.

Results
Clinical observations
Clinical healing was uneventful throughout the observation period and no subjective adverse experiences were recorded. The patient suffered no discomfort.

Macroscopic examination
The gross appearance revealed a healthy gingiva around the experimental tooth with no apparent gingival inflammation. The gingival margin demonstrated a further recession compared to the presenting presurgical condition (Fig. 4). No objective adverse effects were recorded.

Microscopic examination
Microscopic examination (Fig. 5) showed the formation of an acellular extrinsic fibre cementum, which was firmly attached to the underlying instrumented dentin surface. The new cementum was thin and contained collagen fibres, which had a direction that was at right angles to the long axis of the root and also extended into an associated periodontal ligament. An alveolar bone attached to the ligament was also present. The morphometric measurements showed that there was an apical recession of the soft gingival tissues leaving an exposed root surface amounting to 15% of the original defect as measured from the coronally placed notch to the apical bevel. The new cementum layer covered 73% of the original defect and the junctional epithelium had proliferated slightly in an apical direction to cover 12% of the defect. Alveolar bone gain was 65% of the presurgical bone height.

Discussion
The results demonstrated periodontal healing characterized by the formation of acellular cementum with an associated periodontal ligament and alveolar bone in one human experimental defect...
following adjunctive use of EMD. The study utilized a setting identical to that of experiments performed in monkeys and almost identical to the setting proposed for clinical use of EMD in regenerative periodontal surgery (Heijl et al. 1997, Zetterstrom et al. 1997).

The usual approach as a first step to establish efficacy for regenerative procedures, is to perform experiments in suitable animal models. However, a major disadvantage in animal experimentation is the interpretation relative to predictability in humans. In this respect it needs to be emphasized that controlled histological trials in humans can hardly, for ethical reasons, be performed due to the need to obtain large tissue blocks containing the tooth including its surrounding tissues, the need to have matching defects etc. Thus, specimens retrieved from experimental defects in humans will strengthen the interpretation of predictability for clinical outcome in human trials.

So far, there has been no single paradigm for research as to how to obtain or enhance the potential for true periodontal regeneration. It is only recently that there has been some concordance between theory and clinical practice by the advent of, for example, the guided tissue regeneration technology (Melcher 1976, Stahl et al. 1990, Stahl & Froum 1991, Gottlow et al. 1992) and research into the use of naturally occurring biological substances (Bowers et al. 1989, Lynch et al. 1989). The results obtained in the present study indicate that adjunctive use of enamel matrix derivative could provide an alternative technology with a potential for periodontal regeneration in clinical practice.

References


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