

Enamel matrix derivative Emdogain[®] as an adjuvant for a laterally-positioned flap in the treatment of gingival recession: an electron microscopic appraisal

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Enamel matrix derivative (EMD), such as Emdogain[®], has been suggested for the improvement of wound healing in periodontal surgical therapy. The present qualitative study seeks to illustrate the ultrastructural changes associated with a human gingival wound at 10 days after the application of EMD as an adjunct to a laterally-positioned flap in a patient with gingival recession. An otherwise healthy patient, who had been suffering from bilateral gingival recession defects on teeth #23 and #26, was studied. One defect was treated with a laterally-positioned flap, while the other was treated with a combination of EMD and a laterally-positioned flap. Ten days after the operation gingival biopsy specimens were obtained from the dentogingival region and examined using a transmission electron microscope. A considerable difference was found in both the cellular and extracellular phases of EMD and non-EMD sites. The fibroblasts of EMD site were more rounded with plump cytoplasm and euchromatic nuclei. A well-developed rough endoplasmic reticulum and numerous mitochondria could be detected. In contrast, the fibroblasts of non-EMD site were of flattened spindle-like morphology. While the signs of apoptosis could rarely be detected at EMD site, apoptotic bodies and ultra-structural evidence of apoptosis (crescent-like heterochromatic nuclei and dilated nuclear envelopes) were consistent features at non-EMD site. The extracellular matrix at EMD site mainly consisted of well-organised collagen fibres, while non-EMD site contained sparse and incompletely-formed collagen fibres. Coccoid bacteria were noted within the extracellular matrix and neutrophils at non-EMD site. It seems that EMD may enhance certain features of gingival wound healing, which may be attributable to its anti-apoptotic, anti-bacterial or anti-inflammatory properties.

Key words: Emdogain[®], gingiva, laterally-positioned flap, ultrastructure, wound

INTRODUCTION

Gingival wound healing is a highly orchestrated phenomenon, demanding harmonised interactions between numerous cellular and extracellular elements [6]. Enamel matrix derivative (EMD), such as Emdogain[®], has been found to favour cutaneous wound healing [11]. Likewise, the favourable effects of EMD on gingival wound healing have been suggested in the *in vitro* model [3, 4, 8]. However, the *in vivo* ultrastructural evaluation of a gingival epithelial wound after the application of EMD remains to be investigated. The present study describes the ultrastructural changes associated with gingival wound healing 10 days after the application of EMD as an adjunct for a laterally-positioned flap in a patient with a gingival recession defect.

MATERIAL AND METHODS

This study was a part of larger clinical and histopathological study consisting of two parts. The first part deals with the clinical efficacy of EMD in patients with gingival recession and with its light microscopic correlations. The second part, the qualitative study presented here was aimed at evaluating the potential EMD-induced ultrastructural changes of the gingiva in such patients.

Case selection

An otherwise healthy 34-year-old male patient with a bilateral gingival recession (Miller class III) on teeth to be extracted (#23 and #26) was studied. The patient was a non-smoker. The gingival recession defect was comparable on both sides.

The study was a part of a clinical project approved by the institutional research and medical ethics committee. The patient's informed consent was first obtained.

Surgical procedures

The surgical procedures were performed at the same session, each lasting about one hour. The root surfaces were conditioned using 24% EDTA gel for 2 minutes and thoroughly rinsed with sterile saline. One defect (EMD site) was treated with a laterally-positioned flap and EMD (Straumann[®]), while the contralateral one (non-EMD site) was treated with a laterally-positioned flap only. EMD was applied on the root of tooth #23. Aluminium foil was placed on the donor site and the surgical site was covered using a surgical pack. No post-operative complication was noted that might compromise the outcome of the procedure. On day 10 after surgery gingival

biopsy specimens (4 × 4 × 3 mm) were obtained from the dentogingival region immediately above the alveolar crest.

Tissue preparation

The tissue samples were fixed in 2% glutaraldehyde, 0.1 M phosphate buffer and then treated in 1% OsO₄ (Osmium tetroxide). The specimens were then dehydrated through graded concentrations of ethanol and embedded in resin. One-micron semi-thin sections were stained with toluidine blue. Ultra-thin sections from selected blocks were subsequently stained with uranyl acetate and lead citrate and examined using an LEO 906 transmission electron microscope. The examiner was unaware of the treatment applied to these specimens.

RESULTS

The cellular phase

Fibroblasts, as the main cellular elements of the periodontal wound, showed obvious differences at the two sites. Non-EMD site contained flattened spindle-shaped fibroblasts with peripherally-located and crescent-like heterochromatin (Fig. 1). Furthermore, the nuclear membrane was dilated and appeared vesicular (Fig. 1). In contrast, the fibroblasts of EMD site were characterised by a more rounded morphology, plump cytoplasm, and euchromatic nuclei (Fig. 2). Numerous mitochondria and well-developed rough endoplasmic reticulum could be detected throughout the cytoplasm. Elongated fibroblast processes were in close contact with the fibres of the extracellular matrix. Some elongated thin cellular processes were also seen at EMD site in close contact with the fibres of the extracellular matrix.

While signs of apoptosis could rarely be detected at EMD site, apoptotic bodies and ultrastructural signs of apoptosis, such as crescent-like heterochromatic nuclei and dilated nuclear envelopes, were consistent ultrastructural findings in fibroblasts of non-EMD site. The apoptotic bodies were often engulfed in the neutrophils.

The extracellular phase

Matrix. Well-organised collagen bundles traversing the extracellular matrix and communicating with each other and the intracellular filaments were characteristic findings of EMD site (Fig. 2). In addition, junctions of intracellular filaments and the extracellular fibres were more common in the microscopic fields of EMD site (Fig. 2). However, at non-EMD

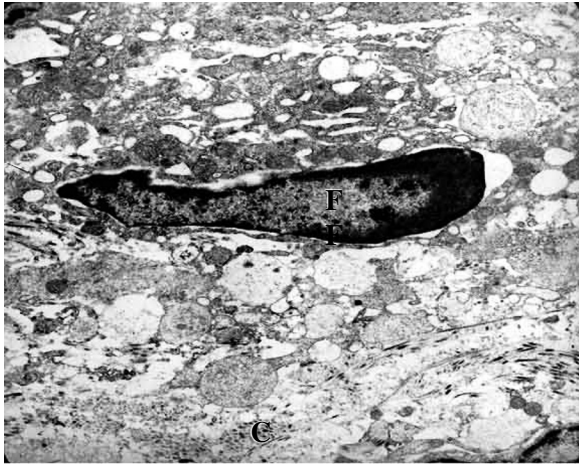


Figure 1. An electron micrograph of the gingiva from non-EMD site. Note the crescent-like heterochromatic nucleus of the fibroblast with dilated nuclear envelope (apoptotic features). The ground substance displays few collagen fibres and several vacuoles. Original magnification, $\times 7500$; F — fibroblast; C — collagen fibres.

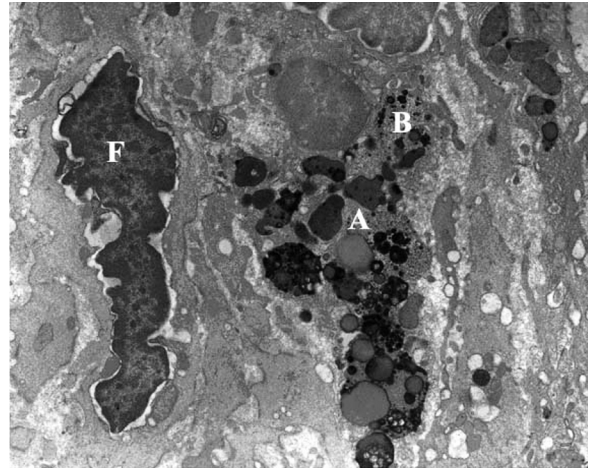


Figure 3. An electron micrograph of the gingiva from non-EMD site. Note a fibroblast with condensed nuclei and dilated nuclear membrane. A macrophage with phagocytic vacuoles is seen. Original magnification, $\times 7500$; F — non-activated, created fibroblast; A — apoptotic bodies; B — bacteria.

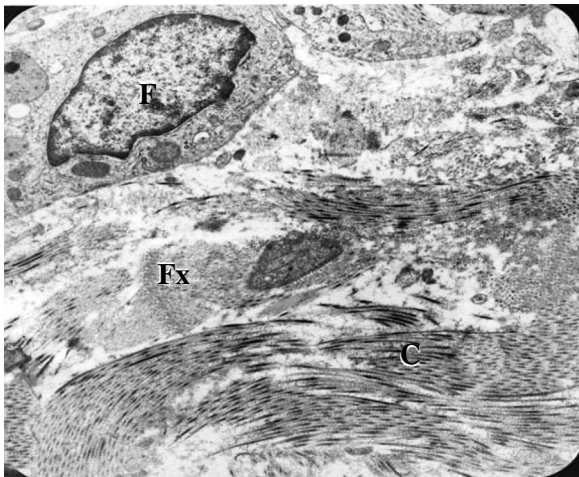


Figure 2. An electron micrograph of the gingiva from EMD site. A rounded fibroblast with euchromatic nuclei as well as plenty of collagen fibres is seen in the ground substance. Original magnification, $\times 7500$; F — rounded active fibroblast; C — collagen fibres; Fx — fibronexus, the connection between extracellular fibres and intracellular filaments.

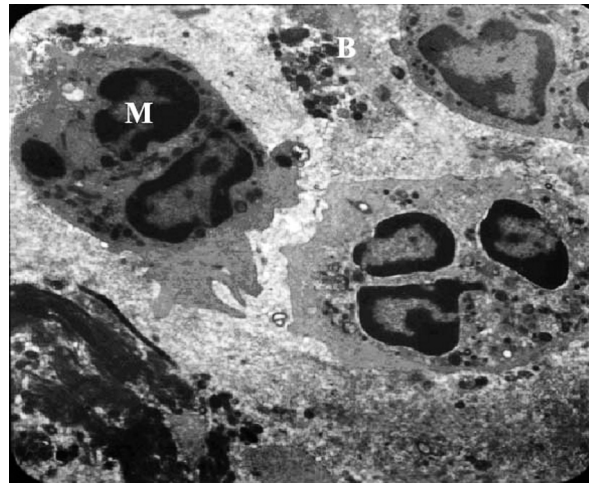


Figure 4. An electron micrograph of gingiva from non-EMD site. The ground substance has sparse darkly stained collagen fibres and several bacteria. Original magnification, $\times 7500$; M — macrophage; B — bacteria.

site the collagen fibres were sparse and not completely formed. Inter-collagenous fibre and cell-collagen fibre communications were also rarely observed (Fig. 1).

Microbial involvement. At non-EMD site numerous micro-organisms of round coccoid morphology were noted throughout the extracellular matrix and within the cytoplasm of neutrophils scavenging the

area (Figs. 3, 4). No such observation was made at EMD site.

DISCUSSION

The change in the morphology of gingival fibroblasts brought about by Emdogain[®] has been reported previously. We found that EMD induced transformation of gingival fibroblasts to more rounded

morphology with abundant mitochondria and rough endoplasmic reticulum and euchromatic nuclei. We concluded that EMD favours the transformation of fibroblasts to metabolically and translationally active cells. This notion is in agreement with the findings of Cattaneo et al. [3]. These authors postulated that this morphological transformation of periodontal fibroblasts following the application of EMD may favour the colonisation of the root surface and the formation of new periodontal attachments. The results of the present study also show that non-EMD site frequently figured in cellular apoptosis. He et al. [7] revealed that EMD inhibited tumour necrosis factor-induced osteoblast apoptosis. In contrast, Kawase et al. [9] found no such anti-apoptotic effects of EMD in human oral squamous cell carcinoma.

We also found that the extracellular matrix fibres, especially collagen, were more abundant and organised at EMD site. Hasse and Bartold [5] reported that EMD upregulated the synthesis of extracellular matrix elements. Likewise, in a study by Keila et al. [10], a two-fold increase in the number of gingival fibroblasts and collagen production was noted after the administration of EMD. In contrast, Palioto et al. [12] found no difference in the expression of collagen fibres through the effect of enamel matrix proteins.

Another unique finding of the present qualitative study was the inhibition of bacterial growth or bacterial decontamination at EMD site. The potential antibacterial properties of EMD have been suggested by other investigations [1, 13]. The enhanced viability of bacterial micro-organisms in the wound milieu can delay periodontal healing [14].

The effect of EMD on periodontal fibroblast proliferation is controversial. The *in vitro* study of Ashkenazi and Shaked [2] showed that EMD decreased the percentage of periodontal ligament fibroblasts with the capability of causing colonies with a confluence of 75–100% of confluence (covering the well area). However, Cattaneo et al. [3] found that EMD enhanced human periodontal fibroblast proliferation *in vitro*. Both these authors cited enhanced cellular differentiation as a potential explanation for their observations [2, 3]. In the present study gingival fibroblasts at EMD-treated sites showed features of active secretory cells with obviously decreased apoptosis. These findings may represent alternative explanations for the potential efficacy of EMD in gingival healing.

Finally, the present qualitative study indicates that EMD may enhance certain features of gingival wound

healing, which may be attributable to its anti-apoptotic, anti-bacterial or anti-inflammatory properties.

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