Effect of enamel matrix derivative protein on the healing of standardized epithelial wounds: a histomorphometric analysis in vivo

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ABSTRACT

The enamel matrix derivative (EMD) is a preparation of the enamel matrix proteins secreted by the Hertwig’s epithelial root sheath. It has been shown that EMD promotes periodontal wound healing; however, the significance of the protein in repairing skin wounds is insufficiently addressed. The aim of this in vivo histomorphometric investigation was to analyse the effect of EMD protein on the healing of standardised epithelial wounds. Dorsal skin of 22-week-old female guinea pigs (n = 33) was scarified and divided into test- (topical application of EMD) and control-sites (sutured and allowed to heal). Animals were euthanised at specific time intervals and the specimens were then evaluated histomorphometrically. The mean widths of the external wound gaps (WGs) in the test- and control-sites at the 5th, 20th and 35th day of healing were 5·89, 3·6 and 1·01 mm and 6·41, 5·02 and 3·43 mm, respectively. Histomorphometric analysis showed a statistically significant difference in the WGs between the test- and control-sites. A significant increase in the formation of organised connective tissue matrix, collagen fibres and early muscle formation was observed in the test-sites as compared with the control-sites. Within the limits of this study, it is concluded that topical application of the EMD on standardised epithelial allows early wound closure and promotes healing as compared to when the defects are merely sutured.

Key words: amelogenin • enamel matrix derivative • skin • wound healing

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INTRODUCTION

Wound healing is a complex process involving various events including cellular attachment to various extracellular matrices, cell migration and proliferation. These events are regulated by an intricate array of cytokines and growth factors (1,2). Formation of fibrous granulation tissue is crucial during wound healing, providing the necessary matrix for final wound closure through epithelialization (1,2). A mechanism that has been proposed to regulate the healing of skin and muscle tissues following injury is the expression of inducible nitric oxide synthase (NOS) in these tissues (2). NOS is produced by a variety of cells including skeletal and smooth muscle cells, fibroblasts and epithelial cells (2). The impact of NOS on wound repair may be attributed to its functional influences on angiogenesis, inflammation, cell proliferation, matrix deposition and remodelling (1,2).

The enamel matrix derivative (EMD) is a preparation of the enamel matrix proteins secreted by the Hertwig’s epithelial root sheath. Amelogenin, a 20 kDa hydrophobic protein, is the chief ingredient in the EMD. Studies have shown that amelogenin promotes healing and regeneration of the supporting structures of teeth including cementum (3,4). Similar results were reported by Berlucchi et al. (5). Although amelogenin has been reported to play a significant role in the healing process (6), the exact underlying mechanism remain unclear. Results by Khedmat et al. (7) showed that EMD significantly enhanced mitosis in, and proliferation of, human periodontal ligament fibroblasts. In a recent study, Almqvist et al. (8) reported that amelogenin protein promotes DNA synthesis and angiogenesis. It may therefore be hypothesised that treatment with the EMD protein may also promote healing of dermal epithelial wounds. Although previous studies (3,9–11) investigated the effect of EMD protein on oral tissues have yielded promising results, to our knowledge from indexed literature, the effect of EMD protein on the healing of standardised dermal epithelial wounds is yet to be investigated. It is hypothesised that topical application of EMD protein enhances healing of standardised dermal epithelial wounds as compared with dermal wounds epithelial that are merely sutured.

The aim of this in vivo histomorphometric experiment was to assess the effect of EMD protein on the healing of standardised epithelial wounds.

METHODS

Ethical considerations

The study was approved by the ethical committee at the Engineer Abdullah Bagshahn Research Chair for Growth Factors and Bone Regeneration, College of Dentistry, King Saud University Riyadh, Saudi Arabia. The animal study was performed in compliance with Research ethical guidelines.

Study animals

Thirty-three female guinea pigs (with a mean age and weight of 22-weeks and 4 kg, respectively) were used in this experiment.

Surgical protocol

The surgical procedures were performed under general anaesthesia using pentobarbital (Narcoren, Merial, Hallbergmoos, Germany) at 6 mg/kg body weight, which was given by intraperitoneal injection. Local anaesthesia was administered using lidocaine hydrochloride 2% with epinephrine 1:100000 (Xylocaine, AstraZeneca International, Molndal, Sweden).

Pre-operative management

Guinea pig dorsal hair were removed using a depilatory cream (Veet, Reckitt Benckiser, Slough, UK), and the skin was disinfected with povidone-iodine. Two incisions of standardised length and depth (20 mm long and 12 mm wide), extending through the underlying panniculus and adipose tissue to the muscular fascia, were made on each side of the dorsum using a surgical blade (#15). The two incisions were categorised as either ‘test-sites’ or ‘control-sites’ depending on the treatment offered. The test-sites received topical application of 0.2 ml EMD protein (Straumann, Basel, Switzerland), whereas the control-sites were closed with resorbable polyglactin 910 sutures (VICRYL, Johnson & Johnson, Livingston, UK).

Post-operative management

Postoperatively, intramuscular analgin 50% (0.5 gm IM/10 kg) analgesic was given immediately every 12 hours, for the first 3 days. The animals were euthanised at 5, 20 and 35 days by an intracardiac perfusion of ketamine–xylazine solution.

Key Points

- the enamel matrix derivative (EMD) is a preparation of the enamel matrix proteins secreted by the Hertwig’s epithelial root sheath
- amelogenin, a 20 kDa hydrophobic protein, is the chief ingredient in the EMD
- studies have shown that amelogenin promotes healing and regeneration of the supporting structures of teeth including cementum
- although amelogenin has been reported to play a significant role in the healing process, the exact underlying mechanism remain unclear
- the aim of this in vivo histomorphometric experiment was to assess the effect of EMD protein on the healing of standardised epithelial wounds
- thirty-three female guinea pigs (with a mean age and weight of 22-weeks and 4 kg, respectively) were used in this experiment
Key Points
- histomorphometric and histological results showed that at 5, 20 and 35 days, wounds in the test-sites showed more connective tissue matrix and muscle formation as compared with the control-sites.

Histomorphometric analysis
External skin specimens from the test- and control-sites were excised and fixed in neutral phosphate-buffered 4% formaldehyde solution. The formalin-fixed tissues were dehydrated, embedded in paraffin and sectioned at 7 μm thickness. The sections were deparaffinised in xylene, rehydrated in a descending alcohol series and stained with haematoxylin and eosin, or Masson’s trichrome stain. The parameters were assessed independently, and measurement means of 10 wounds in each site were calculated.

Histomorphometry was performed with an image analysis system (OmniMet 9.5, Buehler, Lake Bluff, IL) linked to a light microscope. Pixel calibration was performed using a digitised image of a stage micrometer for transmitted light (Ted Pella, Inc., Redding, CA). The descriptive analysis of each histomorphometric parameter in each animal was performed by a single investigator. Measurements were made from the middle outermost dermal surface to the outermost opposite dermal surface (Figure 1). The wound gap (WG) distance was calculated in pixels and then converted into millimetres (12). The descriptive analyses of each histomorphometric parameter in each animal were obtained after analysing three sections per site, 50 μm apart for each position. Descriptive analyses were then evaluated by one examiner, scoring two parameters: (i) WG and (ii) characteristics of newly formed granulation tissue.

Statistics
Descriptive and analytic statistical analyses were performed using the software program (SPSS Statistics 18.0, Chicago, IL). In the descriptive part, the mean wound width was calculated for each treatment site, and at each time interval mean ± standard deviation were calculated.

The wound width measurement data were analysed with respect to two factors: treatment and time, using two-way analysis of variance. This was performed with the consideration of having one dependent value (healing) and two independent values (treatment method and time). The analysis consisted of comparing the mean wound width among the test- and control-sites, in addition to comparing the mean wound widths in the different time intervals for each of the test- and control-sites. Level of significance at α = 0.05.

RESULTS
At 5 days, the mean widths of WG in the test- and control-sites were 5.89 ± 0.26 mm and 6.41 ± 0.18 mm, respectively (P < 0.001) (Figure 1A, B). At 20 days, the mean widths of WG in the test- and control-sites were 3.6 ± 0.31 mm and 5.02 ± 0.24 mm, respectively (P < 0.001) (Figure 1C, D). At 35 days, the mean widths of WG in the test- and control-sites were 1.01 ± 1.16 mm and 3.43 ± 0.49 mm, respectively (P < 0.001) (Figure 1E, F).

Histomorphometric and histological results showed that at 5 days, wounds in the test-sites showed more connective tissue matrix and muscle formation (Figure 1A), as compared with the control-sites (Figure 1B). At 20 days, more abundant organised connective tissue matrix was formed in the test-sites (Figure 1C) as compared with the control-sites (Figure 1D). At 35 days, over 50% of the WG in the test-sites was almost completely closed (WG < 0.8 mm) (Figure 1E); whereas in the control-sites, WGs remained apparent (Figure 1F). The degree of connective tissue maturation in the test-sites was apparent in the wound side closure and on the well pattern formation of collagen compared with wounds in the control-sites (Figure 1E, F). Absence of muscle formation was also observed in the wounds in the control-sites as compared with those in the test-sites (Figure 1E, F).

DISCUSSION
Previous studies (3,9,10) have largely focused on the ability of EMD to repair and regenerate periodontal tissues; however, results from the present experimental study showed that the benefits of EMD are not only restricted to oral rehabilitation but can augment healing of external skin wounds as well. After a vigilant review of the pertinent indexed literature, we observed that not many studies have addressed the impact of EMD on skin wounds. In a study, Mirastschijski et al. (12) investigated the effects of EMD on skin wound healing with particular emphasis on the proliferation of dermal fibroblasts and microvascular endothelial cells. The results showed that the treatment increased the
Figure 1. Representative histomorphometric and histological (using light microscopy 10× magnification) of the tissue sections taken from the test- and control-sites at: (A) At 5 days, connective tissue matrix forming at the proximity of the wound edges in the test-sites; (B) At 5 days, a larger gap between both edges of the wound in the control-sites; (C) At 20 days, partial closure of the wound site and evidence of connective tissue matrix formation (arrows) in the test-sites; (D) At 20 days, minimal presence of the connective tissue matrix and the large distance between the wound edges in the control-sites; (E) At 35 days, complete closure of the wound gap (WG), matured connective matrix (arrows) and evidence of the muscle formation; (F) At 35 days, partial closure of the WG with no evidence of complete connective tissue formation or muscle formation.
in conclusion, treatment of granulation tissue and accelerated time to complete epithelialization by the third day of treatment. This study supports these results because a significant difference in skin wound closure was observed in the test sites (EMD-treatment) as compared with the control-sites (suture-treatment) within the first week of therapy.

Several studies (13–16) have reported that the vascular endothelial growth factor (VEGF) plays an evident role in wound repair. In a study (13) on diabetic mice, impaired wound healing capacity was successfully corrected by administering the VEGF gene to the wounds. Furthermore, studies (12,14,17) attributed defective collagen remodelling by chronic wound fibroblasts to decreased active matrix metalloproteinase-2 (MMP-2) expression – a key feature of defective wound healing. Experimental studies (12,15–18) have shown that treatment with EMD significantly augments the levels of MMP-2 and VEGF in cultured fibroblasts. These studies seem to be an explanation for our results where an enhanced skin wound healing was observed following EMD therapy.

Muscle development is a complex mechanism that involves an interaction between several proteins and their receptors (19). In the study by Pizza et al. (20), the role of nitric oxide in muscle inflammation was investigated. The results showed that nitric oxide or one of its intermediates promote muscle inflammation (20) Similar results were reported in the study by Paulsen et al. (2). An interesting finding in this study was that EMD-treatment did not only enhance skin wound closure but also promoted the formation of new muscle fibres. To our knowledge from indexed literature, this is the first study that showed that EMD promotes new muscle formation. Although an absolute explanation in this regard is yet to be established, it may be postulated that the EMD may significantly modulate the levels of NOS around the wound sites there by promoting healing and tissue regeneration.

EMD may also enhance mesenchymal cell recruitment to wound sites, thereby promoting the formation of granulation tissue and angiogenesis. It may explain the rapid formation of granulation tissue matrix and complete closure in cutaneous wound healing following EMD application (23). A limitation of this investigation is that medically healthy animals were used. Should EMD yield the same effects in medically compromised animals (such as those with streptozotocin-induced diabetes) requires further investigations.

CONCLUSION
Treatment of standardised epithelial wounds with the EMD protein promotes healing and facilitates early wound closure as compared with dermal wounds that are merely sutured.

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REFERENCES
Enamel matrix protein and skin wound healing


