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Efficacy of the enamel matrix derivative to induce cementogenesis in vital and endodontically treated teeth with osseous dehiscence defects

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Abstract – This experiment assessed the efficacy of the enamel matrix derivative (EMD) to regenerate cementum in vital and endodontically treated teeth with osseous dehiscence defects. Five adult female beagle dogs were used. Thirty maxillary teeth (bilateral maxillary canines and second and fourth premolars) were randomly divided into two experimental groups (groups A and B, containing 12 teeth each) and one control group (group C). Endodontic treatment was only performed on teeth in group A compared with teeth in groups B and C. Buccal osseous dehiscence defects were surgically created in teeth from all groups. Teeth in the experimental group were treated with the EMD, whereas the controls were not. After 5 months, the animals were sacrificed and block sections of the teeth in experimental and control groups were processed for histological analysis. Newly regenerated cementum was observed in all teeth in groups A and B. No cementum regeneration was observed in group C. There was a significant difference in cementum generation between the experimental and control groups ($P < 0.001$). EMD therapy induces cementogenesis in vital and endodontically treated teeth with osseous dehiscence defects.

In the past, attempts to regenerate periodontal tissues have mainly focused on either filling the defect space maintenance material or barrier to exclude the epithelium cells and allow the host cells to proliferate and generate new tissues or combination (1). Growth factors have also been used to encourage periodontal tissue regeneration, and histological studies have provided adequate evidence in this regard (2–4). Bone grafts have also been used as adjuncts to guided tissue regeneration (GTR), and studies have reported that bone grafts when used in combination with the enamel matrix derivative (EMD) yielded in enhancement of the osteoinductive activity of the bone grafts (3, 4). A recent study reported that EMD alone has the ability to promote new cementum formation (2); however, the significance of the protein in regenerating new bone remains debatable (2, 5).

It is known that proteins in the EMD play biological roles in the formation of dentine, acellular cementum and alveolar bone during tooth development (6). In a recent experimental study, the healing outcomes in dehiscence-type defects treated by the EMD were evaluated histologically and histometrically (2). The results showed that the created dehiscence defects in the test sites (sites treated with EMD) had formed functional connective tissue fibers inserted into regenerated cellular cementum compared with the control sites (no EMD treatment), which demonstrated absence of cementum and presence of long junctional epithelium (2). This study concluded that EMD alone effectively promoted new cementum and functionally oriented connective tissue formation (2). Likewise, a recent Cochrane systematic review (7) also concluded that compared with

conventional flap surgical procedures, treatment with EMD significantly improves the clinical attachment levels and reduces probing pocket depths in teeth with intrabony defects. It is notable that in earlier studies (3–7) that investigated the efficacy of the EMD in regenerating periodontal tissues, only vital teeth have been involved or the significance of pulp vitality was not highlighted.

Animal and human studies have indicated that the presence of periapical lesions or endodontic treatment may reduce the successful outcomes of periodontal therapy (8–10); however, the results remain debatable. Narang and Wells (11) reported that cementogenesis and osteogenesis can take place in non-vital teeth. Similar results were reported by Perlmutter et al. (12) and Ehnevid et al. (13). To our knowledge from indexed literature, the efficacy of EMD to regenerate cementum in teeth with intrabony defects is yet to be determined. In this context, the aim of the present study was to investigate the efficacy of the EMD to regenerate cementum in vital and endodontically treated teeth with osseous dehiscence defects.

Materials and methods

The study was approved by the research ethics review committee of the Eng. A. B. Growth factors and Bone Regeneration Research Chair, College of Dentistry, King Saud University, Riyadh, Saudi Arabia.

Five adult female beagle dogs with a mean age and weight of 15 months and 13.4 kg, respectively, were used. All procedures were performed under general sedation with an intramuscular injection of Ketamine Acepromazine (Ketalar®, Pfizer, NJ, USA), 10 mg kg^{-1} body weight, and local anesthesia (Xylocaine®, Astra, Sweden) containing 5 mg ml^{-1} epinephrine.

Preoperative management

A thorough scaling and root planning was performed on all teeth followed by topical application of 0.12% chlorhexidine solution (3M, St. Paul, MN, USA) until the surgical phase.

Study groups

Thirty teeth (bilateral maxillary canines, second premolars, and forth premolars) were randomly divided into three groups (groups A, B, and C). Groups A and B served as the experimental groups, whereas the controls were included in group C. Twenty-four teeth were included in groups A and B (12 teeth per group), whereas six teeth were included in group C.

Only teeth in group A underwent endodontic treatment compared with teeth in groups B and C. Root canals of teeth in group A were initially instrumented with K-type hand- (JS Dental, Ridgefield, CT, USA) and rotary files (Profile, Dentsply, Addlestone, UK) and irrigated with 5.25% NaOCl. Following this, the root canals were obturated with gutta-percha and sealer (Pulp Canal Sealer EWT; SybronEndo, Orange, CA, USA) using the vertically condensed technique. The access cavities were then sealed with amalgam. Accuracy of root canal obturation was confirmed with periapical radiographs (Fig. 1a).

Surgical phase

After 8 weeks, buccal mucoperiosteal flaps (extending from the maxillary lateral incisors to the first maxillary molars) were reflected using intracrevicular incisions in all groups. The buccal alveolar bone (including the associated periodontal ligament and cementum) was removed using a number 4 high-speed round carbide bur (SS White, Lakewood, NJ, USA). The marginal half of the interradicular alveolar bone was also removed to avoid a possible accelerated healing of the adjacent bone. The distance between the cemento-enamel junction and the apical end of the buccal bony defect was standardized and set at 8 mm for canines and 5 mm for premolars. A notch was also prepared on the buccal root surface, at the most apical level of the reduced bone, which served as a reference point for the histomorphometric evaluation (Fig. 1b).

Application of the enamel matrix derivative

After flap reflection, a 0.7-ml film of EMD was applied on all exposed root surfaces in groups A and B. Group C

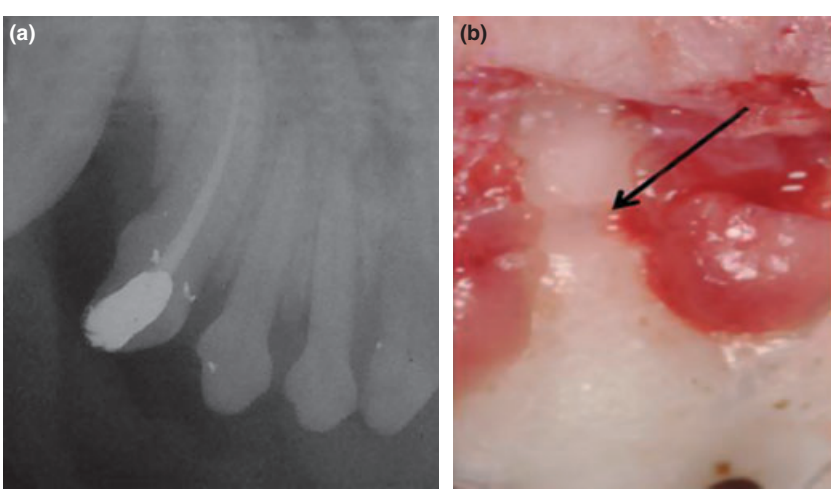

Fig. 1. (a) Postobturation periapical radiograph, (b) Clinical photograph following removal of bone and cementum. Note the notch (arrow) that was created to serve as histological landmark.

did not receive EMD treatment. The mucoperiosteal flaps were then repositioned to their original positions and sutured with 5–0 silk for 2 weeks. The wound areas were carefully swabbed with 0.12% chlorhexidine solution to minimize contamination and plaque accumulation.

Euthanasia

Five months later, the animals were euthanized and segments of the jaws containing the teeth associated with the buccal dehiscence defects were removed *en bloc* together with their adjacent teeth and alveolar bone. The specimens were then prepared for histological evaluation and histomorphometric analyses.

Histomorphometric evaluation of specimens

The specimens were decalcified in an equal mix of formic acid and sodium citrate at 38°C for a period of 3 weeks. This solution was refreshed every 48 h. The specimens were then washed with sterile water and placed in an automated tissue processor where they were dehydrated in ascending grades of ethyl alcohol, infiltrated in xylene, and embedded in paraffin. Buccolingual sections of 7-μm thickness parallel to the long axis of the tooth were obtained using a microtome with a diamond blade and stained with Retic and Masson trichrome.

Measurements of histological sections

Measurements (in pixels) for each section were carried out with a computerized microscope linked to a video camera (Buehler, NJ, USA). A new Michigan periodontal probe (Hu-Friedy, Chicago, IL, USA) was placed over the histological sample, and a picture was taken measuring only 1 mm of the probe. One pixel was calibrated as 0.0023 mm. The descriptive analysis of the histomorphometric parameters was performed by a single investigator who assessed the presence of periodontal connective tissues and cementum and the type of wound healing following EMD treatment.

A comparison was made in the defect compartments in the vital and endodontically treated teeth. The following comparisons were made:

1 Apico-coronal cementum formation: measurements were made from the base of the notch to the most coronal extension of the newly formed cementum.
2 Bone formation: measurements were made from the base of the notch to the most coronal part of the newly formed bone.

Statistical analysis

The statistical analysis was performed using a software program (SPSS, Chicago, IL, USA). Data were analysed using the Mann–Whiney *U*-test at a 95% level of confidence.

Results

A continuous layer of newly generated cementum was observed in all the specimens of groups A and B (Figs 2 and 3). The new cementum layer extended coronally from the notch reference point averaging 2.82 mm in group A and 2.77 mm in group B (Table. 1). The newly regenerated cementum layer appeared cellular in nature (Figs 2b,c and 3a). A cementocyte lacuna was evident in the EMD-treated sites (Fig. 2c). New connective tissue fibers were inserted into the newly formed cementum in perpendicular position (Figs 2b and 3b).

In group C, no evidence of new cementum layer could be observed. The periodontal attachment in this group was characteristic of a long junction epithelial attachment (Fig. 4a,b). There was no significant difference in the different defect size in the premolar or the canine sites (Tables 2 and 3). Absence of new bone formation was evident, and the connective tissue was parallel to the root surface.

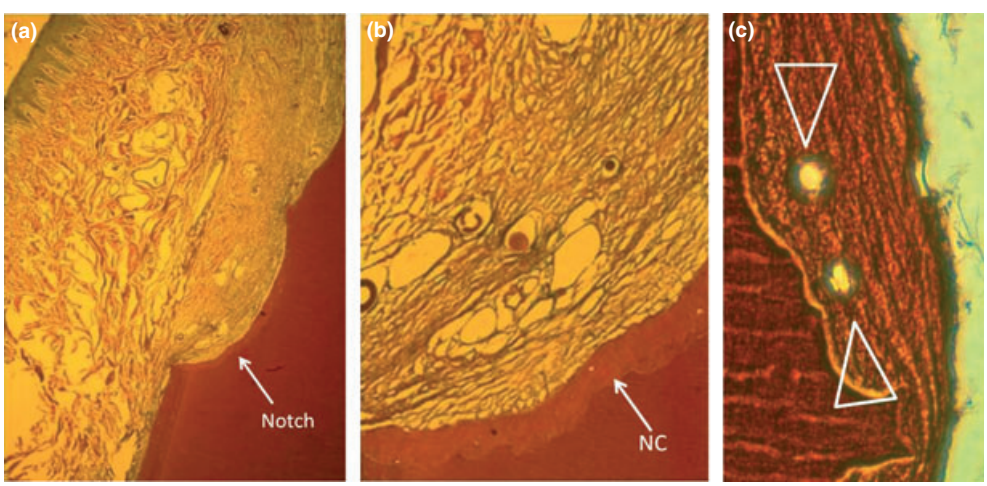

Fig. 2. Light microscopy: (a) A new cementum layer (NC) has formed coronally to the base of the notch (N) without evidence of bone regeneration. Original cementum layer (OC) can also be observed (original magnification × 20). (b) Higher magnification shows the newly formed cementum (original magnification × 40). (c) Higher magnification showing the presence of cementocytes lacunae (arrow) (original magnification × 60).

Table 1. Shows the mean of the apico-coronal length of the newly formed cementum for all the groups

Group	*N*	Mean	Stand deviation	Stand error mean
A	15	2.8267	1.28819	0.33359
B	15	2.7791	1.29199	0.33489
C	15	0.0000	0.00000	0.00000

Minimal or no bone formation was found coronally to the notch in either group (Figs 2a, 3a and 4a). No replacement resorption was found in any of the experimental groups or the controls.

Discussion

It was previously believed that the EMD exclusively regulates the propagation, termination, and maturation of the enamel hydroxyapatite crystals; however, the presence of enamel proteins in initial cementum formation during normal tooth development hinted at a profound role of the protein in biomineralization (6, 14). Although several studies (13–18) have shown that the EMD can successfully be used for GTR, however, the exact mechanism behind the bioactivities of the enamel proteins remains unclear. Hammarström et al. (15) conducted a study to investigate the effect of locally applied EMD protein on the regeneration of periodontal tissues. The results showed that the application of homogenized EMD resulted in an almost complete regeneration of acellular cementum, firmly attached to the dentin and with collagenous fibers extending over to newly formed alveolar bone (15). Researchers believed that the bioactivities of the EMD are caused by the amelogenin family, a major component in EMD; however, the amelogenins and their derivatives separated by ammonium sulfate precipitation fractionation or gel filtration have demonstrated no cementum regeneration activity (16, 19).

Also, most of these studies involved only vital teeth (13–18). In their study, Cortellini and Tonetti (17) investigated the null hypothesis that there are no differences in GTR outcomes vital and endodontically treated teeth with intrabony defects. In this study (13), 208 patients (41 with non-vital teeth and 167 with vital teeth) were included. The results showed that teeth in the non-vital and the vital groups showed a significant gain in clinical attachment levels (17). The present study supports these results; however, it is notable that Cortellini and Tonetti study (17) used barrier membranes instead of the EMD to achieve clinical attachment levels in vital and endodontically treated teeth. It is also noteworthy that in the Cortellini and Tonetti study (17), the conclusions were derived solely from the clinical and radiological investigations, and histological confirmation was not obtained. The present results provided a histological evidence regarding the ability of the EMD to induce cementogenesis in endodontically treated as well as in vital teeth. Our results may be explained by an *in vivo* study (16), which demonstrated that the cementum regeneration-promoting factor in enamel proteins may be used for periodontal regeneration to induce cementogenesis. The results from this study (16) showed that cementogenesis was found in the aggregate comprising 13- to 17-kiloDaltons (kDa) sheath proteins. In these proteins, cementum regeneration activity was detected upon application of the 17-kDa sheath protein, but not by other lower molecular weight sheath proteins. In this context, it may be hypothesized that the role of pulp vitality in cementogenesis is rather secondary. Further studies are warranted to prove and/or explain this hypothesis.

In the present experiment, a characteristic outcome of EMD treatment was the formation of new functional connective tissue fibers and cementum without bone formation. This suggests that in clinical traumatic cases like avulsion associated with buccal or labial bone fracture, EMD treatment could promote the formation of new functional connective tissue fibers and cementum. The present study supports the results by Filippi et al. (20, 21), which reported that EMD treatment in traumatized teeth promotes the formation of new connective tissues and prevents ankylosis; however, controversy persists in this regard (22).

In the present study, the dehiscence defects represented a state of acute infection as the defects were surgically created. It may therefore be argued that a difference in the healing response to EMD therapy may be expected in chronic inflammatory conditions (depending on the nature of the infection).

In conclusion, within the limits of the present study, the EMD can induce cementogenesis in vital as well as

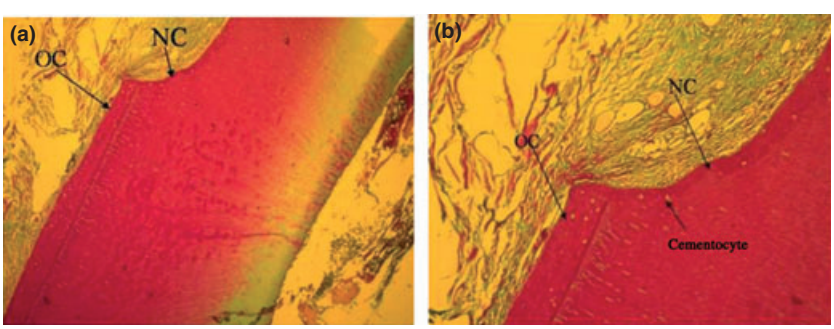

Fig. 3. Light microscopy (a) vital teeth stained with modified Masson's trichrome stain. A. New cementum (NC) has formed at the base of the notch (original magnification × 10), (b) Higher magnification showing cementocytes (chevron arrow) associated with the newly formed cementum and the original cementum (OC) (original magnification × 60).

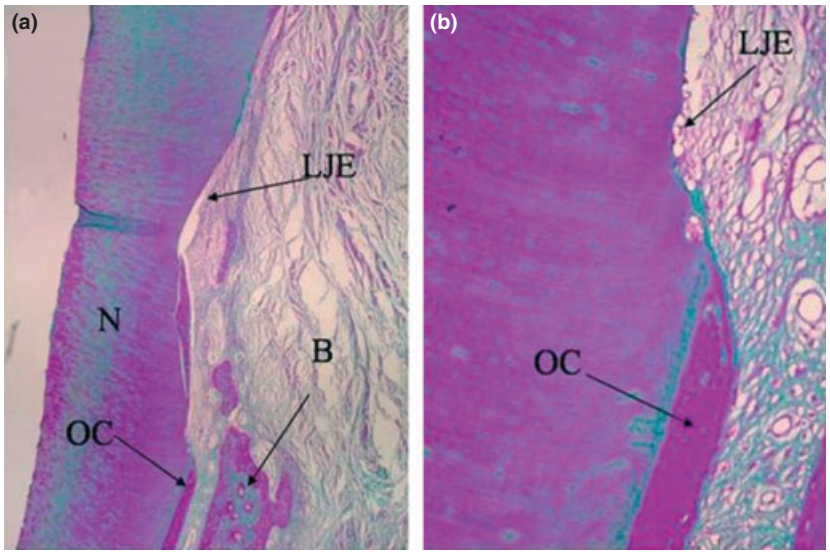

Fig. 4. Light microscopy (a) control group stained with modified Masson trichrome stain showing the original cementum (OC), landmark notch (N), and bone (B). (a) No newly formed cementum was observed. The epithelial down growth resulted in a long junctional epithelium (LJE) to the base of the notch (original magnification × 4). (b) Higher magnification shows the epithelial attachment without the formation of new cementum (original magnification × 20).

Table 2. Shows the mean of the apico-coronal length of the newly formed cementum for the experimental groups in the canine defect sites

Group	*N*	Mean	Stand deviation	Stand error mean
A	4	2.8267	1.28819	0.33359
B	4	2.7791	1.29199	0.33489

Table 3. Shows the mean of the apico-coronal length of the newly formed cementum for the experimental groups in the premolar defect sites

Group	*N*	Mean	Stand Deviation	Stand Error Mean
A	8	2.8267	1.28819	0.33359
B	8	2.7791	1.29199	0.33489

endodontically treated teeth with osseous dehiscence defects.

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