

Root maturation and dentin–pulp response to enamel matrix derivative in pulpotomized permanent teeth

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Abstract

The success of pulpotomy of young permanent teeth depends on the proper selection of dressing materials. This study aimed to evaluate the histological and histomorphometric response of dentin–pulp complex to the enamel matrix derivative (Emdogain[®] gel) compared to that of calcium hydroxide when used as a pulp dressing in immature young permanent dogs' teeth. Dentin-like tissues bridging the full width of the coronal pulp at the interface between the injured and healthy pulp tissues were seen after 1 month in both groups. With time, the dentin bridge increased in thickness for calcium hydroxide but disintegrated and fully disappeared for Emdogain-treated group. Progressive inflammation and total pulp degeneration were only evident with Emdogain-treated group. The root apices of Emdogain-treated teeth became matured and closed by cementum that attached to new alveolar bone by a well-oriented periodontal ligament. In young permanent dentition, Emdogain could be a good candidate for periodontium but not dentino–pulpal complex regeneration.

Keywords

Pulpotomy, enamel matrix derivative, calcium hydroxide, dentin bridge and histomorphometric analysis

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Introduction

Dental caries is a global health problem; it affects approximately 5 billion people all over the world.¹ The incidence of dental caries is higher in younger age groups;² 60%–90% of schoolchildren suffer from dental caries.¹ In highly progressed cases, infection and death of pulp are common sequences of dental caries. Loss of pulpal vitality before root maturation weakens the root and makes it prone to fracture.³ The principal goal of pediatric operative dentistry is to prevent the progression of dental disease and to restore damaged teeth to their healthy function. Removal of only the diseased while maintaining the healthy tissues of young permanent teeth (i.e. vital pulp therapy) remains the optimum treatment for root maturation (apexogenesis) to occur.^{4,5} The success of the vital pulp therapy depends largely on the proper selection of dressing/capping materials that are harmless to the remaining healthy tissues and capable of establishing a tight bacterial seal to promote apical closure.⁶

A variety of materials have been advocated for capping the pulp tissues. Many of them seem to empirically perform

better in either primary or permanent dentitions. Others appeared to be promising but did not succeed when used

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clinically. Calcium hydroxide paste, $\text{Ca}(\text{OH})_2$, represents one of the most commonly used materials for such purpose;^{7,8} they have been proved to stimulate the formation of a continuous layer of dentin-like tissues, called dentin bridge (DB), covering the remaining healthy pulp tissues. This calcific bridge helps to maintain the vitality of and prevents the ingress of bacteria into the remaining pulp tissues. There is, however, some concern about the cytotoxic effect of calcium hydroxide on the pulp tissue due to its high alkalinity.⁹ Furthermore, the success of calcium hydroxide as direct pulp capping material depends on its ability to provide a hermetic seal over the exposed pulp.¹⁰

With the introduction of adhesive dentistry, dental adhesives have been proposed as pulp capping material that can provide a hermetic seal which protected the exposed pulp from bacterial invasion; the formation of DB, however, has not been reported.^{11,12} Biological modulators identified during teeth development, for example, recombinant human bone morphogenetic protein^{13–15} or enamel matrix derivative (EMD; Emdogain® gel)¹⁶ or combination of both,¹⁷ have been proposed as pulp capping materials that would enhance dental tissues' regeneration.

Enamel matrix, as an important regulator of enamel mineralization, is made up of noncollagenous proteins mainly amelogenins (90%) and 10% nonamelogenins (enamelin and ameloblastin).¹⁸ Amelogenin enhances dentin formation by inducing differentiation of odontoblasts;^{19,20} its deposition normally precedes cementum formation during root development.²¹ The purified extracts of enamel matrix proteins, EMD or Emdogain gel, therefore play an important role in periodontal tissue regeneration^{22–26} and cementogenesis.^{21,27,28} It also induced hard tissue (dentin-like) formation when used as capping materials for exposed dental pulp in adult pigs.^{20,29,30}

The hypothesis of this study was that direct capping of young permanent pulp with Emdogain gel will facilitate reparative dentin formation and maintain the vitality of pulp tissues. The aim of this study was therefore to assess the effect of Emdogain gel as a pulp capping material when used in exposed young permanent teeth in a dog model. The calcium hydroxide paste was used as a control. The use of Emdogain gel has been assessed histologically and histomorphometrically. The parameters assessed include reparative dentin formation, apical closure, type of tissues responsible for apical closure, pulpal inflammation, pulpal degeneration (reference index), thickness of periodontal ligament (PDL), root resorption, and apical width.

Experimental study

Operative procedures

An experimental clinical trial with a split mouth design has been carried out on 12 healthy male mongrel dogs (5–6 months old) with an average weight of 6 kg. An appropriate local ethical committee approval has been obtained,

and the surgery was performed in accordance with national regulations and licensing procedures. All operations were carried out under general anesthesia (intravenously 3% sodium thiopental in a dose of 30 mg/kg)¹³ and full aseptic surgical conditions. All animals were kept under antibiotic coverage 2 days prior and after the operative procedures.

Preoperative histological examination and radiographic films were taken to show the normal architecture of the pulp tissue and to ensure wide apical foramina in the premolar region of all dogs, respectively. This was also done to address the big variability in the size of the pulp area and dentin-to-pulp ratio normally observed in mongrel dogs. For each dog, five teeth (maxillary second and third premolars but mandibular second, third, and fourth premolars)¹³ on each side were pulpotomized (i.e. the coronal pulp tissue was removed) using a sterile fissure bur after getting coronal access. The pulp chamber was then irrigated with sterile saline, and the bleeding was stopped using sterile cotton pellets and saline solution. The pulp was then capped with EMD (0.3 mL Emdogain and Emdogain PrefGel™; Biora AB, Mamo, Sweden) on one side, while injectable calcium hydroxide paste ($\text{Ca}(\text{OH})_2$, 1.2 mL UltraCal® XS; Ultradent Products, Inc., South Jordan, UT, USA) was used as a control on the contralateral side. A layer of light cure glass ionomer cement (GC Fuji II; GC Corporation, Tokyo, Japan) was then applied to all teeth, followed by an amalgam restoration. After 1, 2, and 3 months, the animals were euthanized by anesthetic overdose. Specimens were fixed in 10% neutral buffered formalin, after which they were prepared for histological examination.

Histological examination

A total of 15 teeth from each group were examined histologically, 5 for each time point. Gross resection of the premolar region with a portion of the surrounding periapical tissue and bone has been carried out. The specimens were decalcified in 5% trichloroacetic acid, dehydrated, infiltrated with paraffin, sectioned at 5 μm thickness, mounted, deparaffinized, and stained with hematoxylin and eosin for histological examination³¹ but with trichrome and periodic acid–schiff stains for histochemical examination.³²

Apical root closure and dentin–pulp response

To assess the dentin–pulp response and apical closure, the following parameters were assessed: apical root closure, type of tissues responsible for apical closure, inflammatory infiltrate, pulp degeneration (reference index), thickness/orientation of PDL, and root resorption. A histological descriptive analysis was performed. These parameters were classified by calibrated examiners using a 0- to 3-point scale; the histology of normal pulp tissue was used as a baseline (Table 1).

Table 1. The scores for apical closure, type of tissues responsible for apical closure, pulpal inflammation, pulpal degeneration (reference index), thickness of periodontal ligament, and root resorption.

Apical closure: ³³
0: complete closure
1: incomplete closure
2: absence of closure (open apex)
Tissues responsible for apical closure: ³³
0: closure with dentin
1: closure with cementum
2: closure with other tissues
Pulpal inflammation ^{34–36}
0: absent
1: mild (little scattering of inflammatory cells with no structural damage)
2: moderate (focal accumulation of inflammatory cells with some disruption of the tissue structure)
3: severe (extensive inflammatory cells infiltration and alteration in tissue formation)
Pulpal degeneration (reference index):
0: absent
1: slight
2: moderate
3: severe (total degeneration)
Thickness of periodontal ligament: ³⁴
0: normal
1: slight
2: moderate
3: severe
Root resorption: ³⁴
1: absent
2: present

Histomorphometric analysis

The thickness of DB and the apical width were measured from five different areas from each specimen using image J software (ImageJ 1.41; The MathWorks, Inc., Natick, Massachusetts, USA). Two teeth from each group at each time points were used for this analysis.

Statistical analysis

Descriptive statistics were displayed as frequencies and percents. The normal distribution of data was assessed using Mann–Whitney U test. The statistical differences between groups and time points were calculated using chi-square or Fisher’s exact test using SPSS 13.0 (UK Ltd, UK). The mean difference was considered to be significant at 0.05 and 95% confidence interval.

Results

Generally, all animals were in a good condition after the operation and remained healthy for the study period. The jelly-like consistency of Emdogain gel made the next

addition of light-cured glass ionomer and condensation of amalgam filling materials challenging.

Histological examination of the coronal portion of root canals

Histological examination of the normal pulp tissues of dog’s teeth showed the presence of numerous dilated blood vessels, undifferentiated pulp cells, defensive cells (lymphocytes, neutrophils, and macrophages), and fibroblasts across the core of the pulp. A layer of pseudostratified odontoblasts was also seen along the periphery of the pulp cavity (data not shown).

After 1 month, a calcified tissue barrier formed of a thick layer of dentin (DB) was observed covering the pulp. A remnant of Emdogain gel was also evident. A layer of odontoblast cells and predentin covered the pulpal dentin. Infiltration of inflammatory cells and accordingly a slight degeneration of pulp tissue were also evident (Figure 1(a)). In one case, a pulp stone has been identified. After 2 months of implantation, numerous odontoclasts with marked resorption pits, indicated by the presence of Howship’s lacunae, were seen associated with DB. The inflammatory reaction became severe, and scattered calcified masses as well as area of hyaline degeneration were seen in most specimens (Figure 1(b)). A complete pulp degeneration with resorbed DB occurred at 3 months, and the odontoblastic layer has been completely disappeared (Figure 1(c)).

Regarding the Ca(OH)_2 group, the DB observed after 1 month was thinner than that for Emdogain gel-treated group. The underlying pulp tissues showed signs of inflammatory infiltration, but numerous dilated blood capillaries, which are characteristic to normal pulp, could be distinguished (Figure 1(d)).

The thickness of DB was then increased with time while the normal pulp tissue with a well-defined odontoblastic layer and dilated blood vessels appeared. Some signs of mild inflammation were seen in a few cases (Figure 1(e) and (f)).

Apical root closure and dentin–pulp response

Histological examination of the apical portion of root canals. The root apex remained open after 1 month of Emdogain treatment (Figure 2(a)); the invasion of numerous blood capillaries, severe inflammatory reaction, and consequently partial pulp degeneration were noticed at 2 months (Figure 2(b)). Complete pulp degeneration with complete closure of the root apex, however, was observed at 3 months. The apex was closed by both cellular and acellular cementum (i.e. hypercementosis). This new cementum extended approximately to the apical half of the root. The normally oriented PDL fibers were embedded in the newly formed cementum and bone (Figure 2(c)).

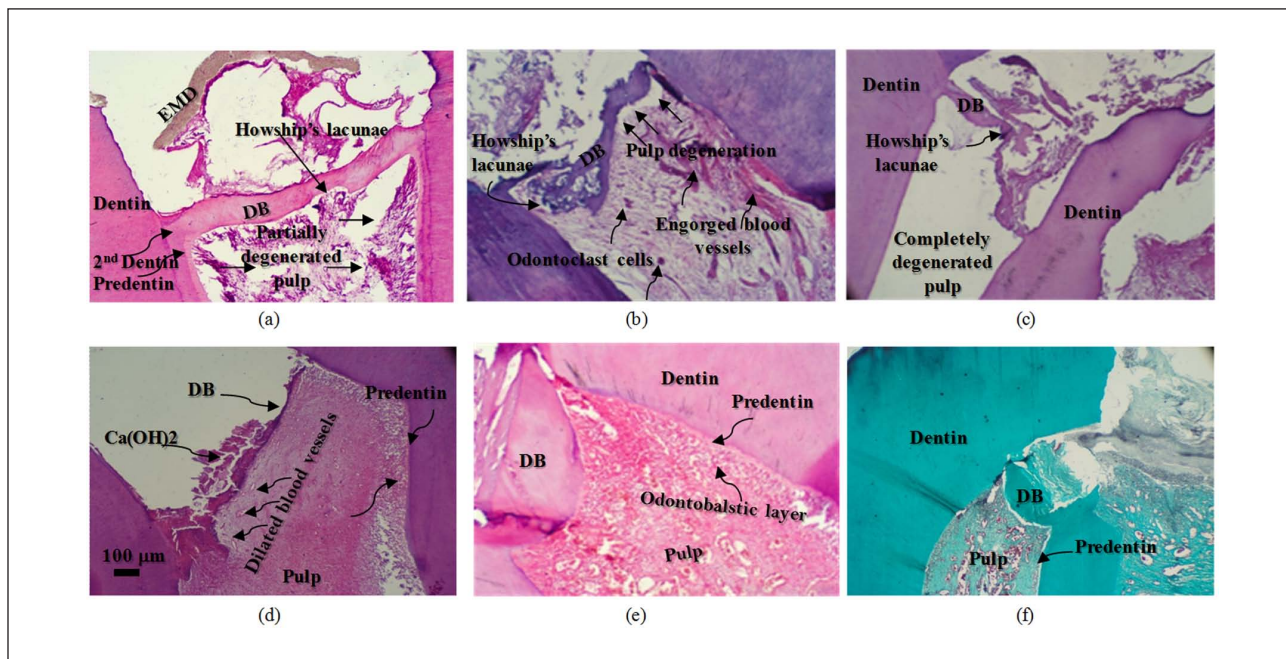


Figure 1. Light microscopy images of coronal sections: Emdogain gel–treated group—(a) after 1 month, the formation of thick dentin bridge (DB) with remnants of Emdogain gel (EMD) has been identified. Inflammatory infiltration of the coronal pulp tissue and the presence of ill-defined odontoblasts at the periphery of the root canal have been also observed. Partial degeneration of pulp tissues and a beginning of DB resorption, indicated by the presence of Howship's lacunae, have also been detected. (b) After 2 months, partial disintegration of DB, indicated by the presence of Howship's lacunae, and degeneration of odontoblastic layer have been observed. This finding was correlated with the presence of osteoclast-like cells (odontoclasts) underlying the disintegrated DB and hyperemic blood vessels as a sign of inflammation and subsequent degeneration. (c) After 3 months, complete disintegration of DB and degeneration of pulp tissues were seen. $\text{Ca}(\text{OH})_2$ -treated group—(d) after 1 month, a very thin DB has been seen associated with the presence of a mild inflammatory response. The presence of dilated blood vessels under the DB indicated an active dentinogenesis. The pulp tissues appeared normal and active. The activity of the pulp is indicated by its high vascularity and the absence of the cell-free zone. The migration of both preexisting odontoblasts and undifferentiated mesenchymal cells from the cell-rich zone to the defect area to form dentin could explain the absence of the cell-free zone. (e) After 2 months, the thickness of DB increased; the pulp tissues appeared normal and highly active. (f) After 3 months, the thickness of DB increased; the pulp tissues appeared normal.

EMD: enamel matrix derivative.

These images were hematoxylin and eosin–stained except (f) that was stained with Gomeri's trichrome.

Partial apex closure happened after 1 month for the $\text{Ca}(\text{OH})_2$ -treated group. This closure was achieved by a typical dentin and an island of irregular calcified tissues resembling osteodentin; both dentin and osteodentin were covered by a thin layer of cementum. The pulp tissue was normal with no signs of inflammatory reaction or resorption (Figure 2(d)). At 2 months, thick oriented PDL fibers, which were properly attached to the newly formed bone and cementum by Sharpey's fibers, have been observed (Figure 2(e)). Complete root closure with a typical normal dentin, however, was observed at 3 months. The newly formed dentin was covered by a thin layer of cementum with a normal alveolar bone and normally oriented PDL fibers (Figure 2(f)).

Incidence of root closure and type of tissues that close the apices. The percent of open apices has been decreased with time from 100% at the start of the experiment to 60% after 1 month of Emdogain use and finally to 5% after 2

and 3 months. The apex closure after 1 month of Emdogain treatment, however, was incomplete. The complete root closure has been seen after 2 months of treatment, and it was significantly increased from 65% at 2 months to 85% at 3 months. The rate of apical root closure was only statistically significant when compared with 1-month time point ($p < 0.001$) (Figure 3(a)). The cementum was the main tissue that closes the apex in 55% of the cases after 1 month, and this has been increased to 85% and 95% after 2 and 3 months, respectively. The remaining cases had other tissues responsible for the apex closure (Figure 3(b)).

Regarding the $\text{Ca}(\text{OH})_2$ -treated group, 50% of the cases showed incompletely closed apices after 1 month; the remaining apices remained open. The percentage of apices remained opened has been reduced to 10% after 2 months, while all other specimens were divided equally between incomplete and complete closure. After 3 months, only 5% of apices remained open; 70% showed complete closure and 25% had incomplete closure (Figure 3(a)). There was

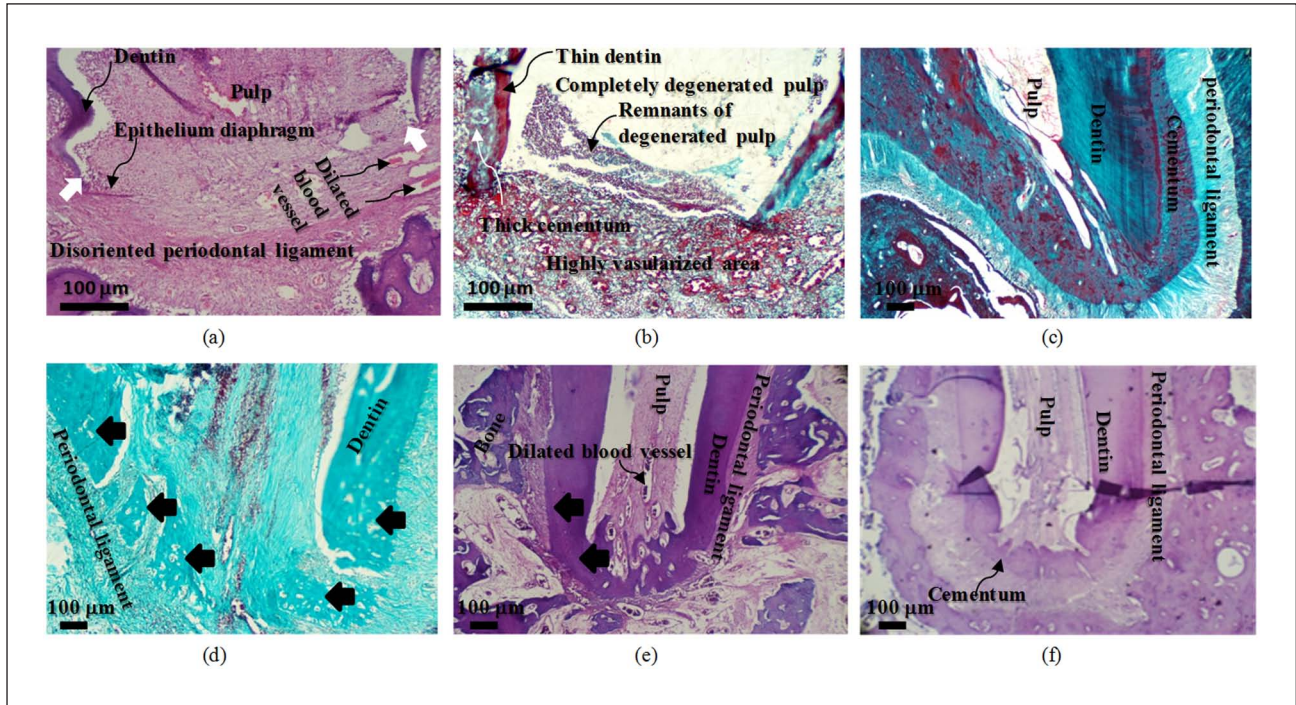


Figure 2. Light microscopy images of apical sections: Emdogain gel-treated group—(a) after 1 month, white arrows, delineating the border of the root apex, indicate that the root apex remained open. A mild inflammatory infiltration and thick fibrous tissues within the pulp were observed. Hertwig's epithelial root sheath, also known as epithelium diaphragm which is crucial for the root development, has been observed. The periodontal ligament appeared disoriented. (b) After 2 months, complete pulp degeneration was observed; the root apex was still opened and a new cementum formation was observed. (c) After 3 months, the root apex closure with cementum, which covered the apical half of the root length, was observed. The periodontal ligament appeared well oriented and attached to the new cementum. $\text{Ca}(\text{OH})_2$ -treated group—(d) after 1 month, partial root closure with osteodentin, marked with black arrows, was observed. The newly formed periodontal ligament was highly oriented. (e) After 2 months, complete root closure with dentin and osteodentin, marked with black arrows, was seen. (f) After 3 months, complete root closure with dentin covered by cementum was seen. A well-organized periodontal ligament, attaching the cementum to the surrounding alveolar bone, has been observed. The pulp tissue, periodontal ligament, and alveolar bone showed normal structure.

The images were hematoxylin and eosin-stained except (b), (c), and (d) that were stained with Gomeri's trichrome.

no statistically significant difference among different time points regarding the rate of closure of root apices across time ($p > 0.05$). Dentin was the main tissue that closes the apex in 50% of the cases after 1 month, and this has been increased to 80% and 90% after 2 and 3 months, respectively (Figure 3(b)).

Inflammatory infiltration and pulpal degeneration. After 1 month, 90% of Emdogain-treated cases showed inflammatory infiltrates ranging from mild (55%) to moderate (25%) and severe (10%). After 2 months, most of the Emdogain-treated specimens showed severe (40%), moderate (35%), or mild (10%) inflammation (Figure 4(a)). Total pulpal degeneration was observed in 15% of the cases. After 3 months, almost all the specimens (95%) of the Emdogain-treated group showed total pulp tissue degeneration (Figure 4(b)). The incidence rate of pulp tissue degeneration was statistically significant across time in the Emdogain-treated group ($p < 0.001$).

Regarding the $\text{Ca}(\text{OH})_2$ -treated group, all the cases showed inflammatory reactions ranging from mild (30%), moderate (40%), to severe (30%) after 1 month. A mild inflammatory reaction was seen in 90% of the cases after 2 months; the other 10% had no inflammatory reaction. After 3 months, however, the majority of the cases (90%) had no inflammatory reaction, and the remaining 10% only had a mild inflammation (Figure 4(a)). The incidence of the inflammatory reactions across time proved to be significant ($p < 0.001$). No pulpal degeneration has been observed except in 5% of the cases after 2 and 3 months (Figure 4(b)). The incidence rate of the pulp tissue degeneration was statistically insignificant across time ($p = 0.5$).

Thickness of PDL and root resorption. After 1 month of the Emdogain treatment, the PDL thickness ranged from severe (20%), moderate (50%), to a slight increase (30%). After 2 months, 50% of the Emdogain-treated teeth restored their normal PDL thickness, while the remaining

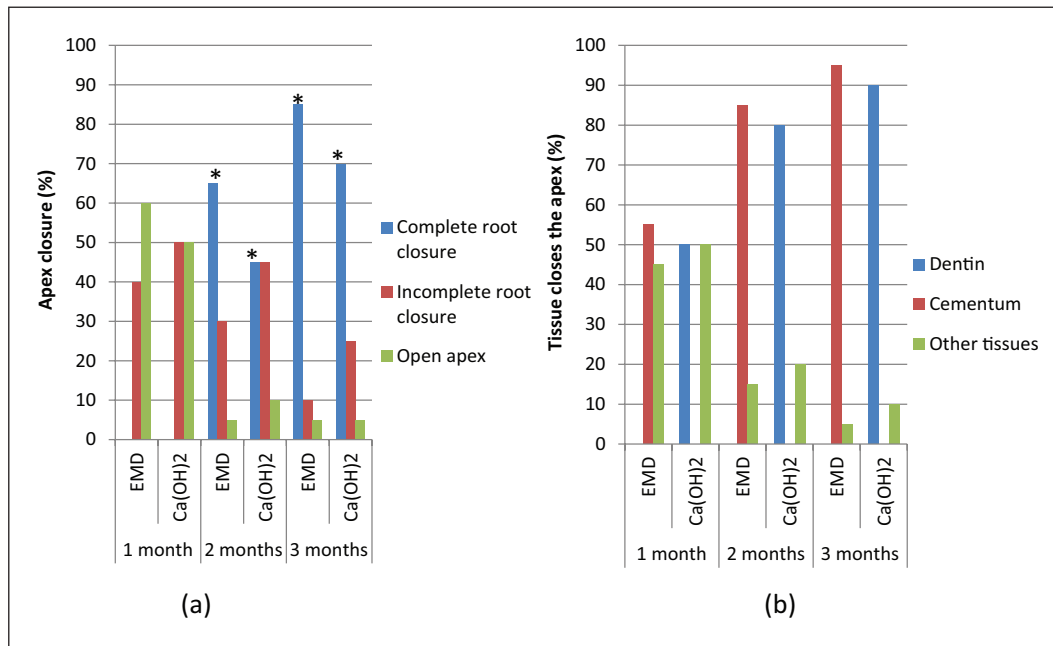


Figure 3. (a) Apex closure (%) and (b) type of tissues that close the apices (%) of teeth treated with Emdogain gel and Ca(OH)₂ up to 3 months after treatment.

EMD: enamel matrix derivative.

*Significant difference from 1-month time point.

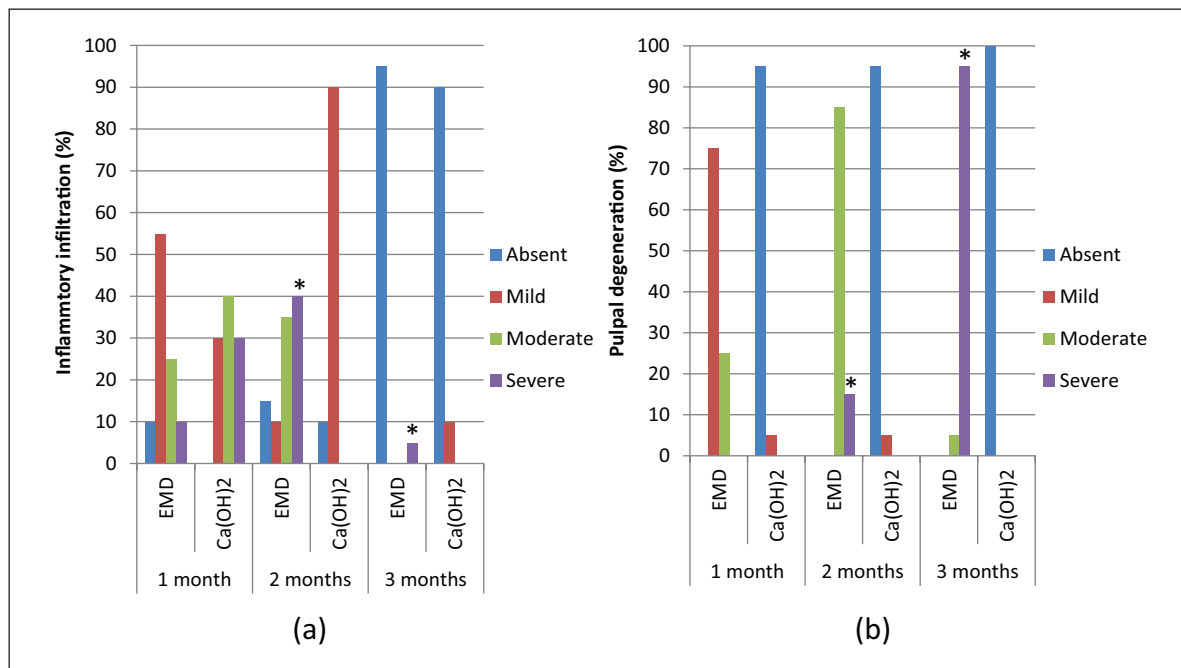


Figure 4. (a) Inflammatory infiltration (%) and (b) pulpal degeneration (%) of teeth treated with Emdogain gel and Ca(OH)₂ up to 3 months after treatment.

EMD: enamel matrix derivative.

*Significant difference from 1-month time point.

cases ranged from a slight (30%) to a moderate increase (20%). After 3 months, 40% of the Emdogain-treated teeth restored their normal PDL thickness, while the remaining

cases ranged from a slight (20%), moderate (30%), to severe (10%) increase (Figure 5(a)). Comparing the changes in the PDL thickness across time in the Emdogain

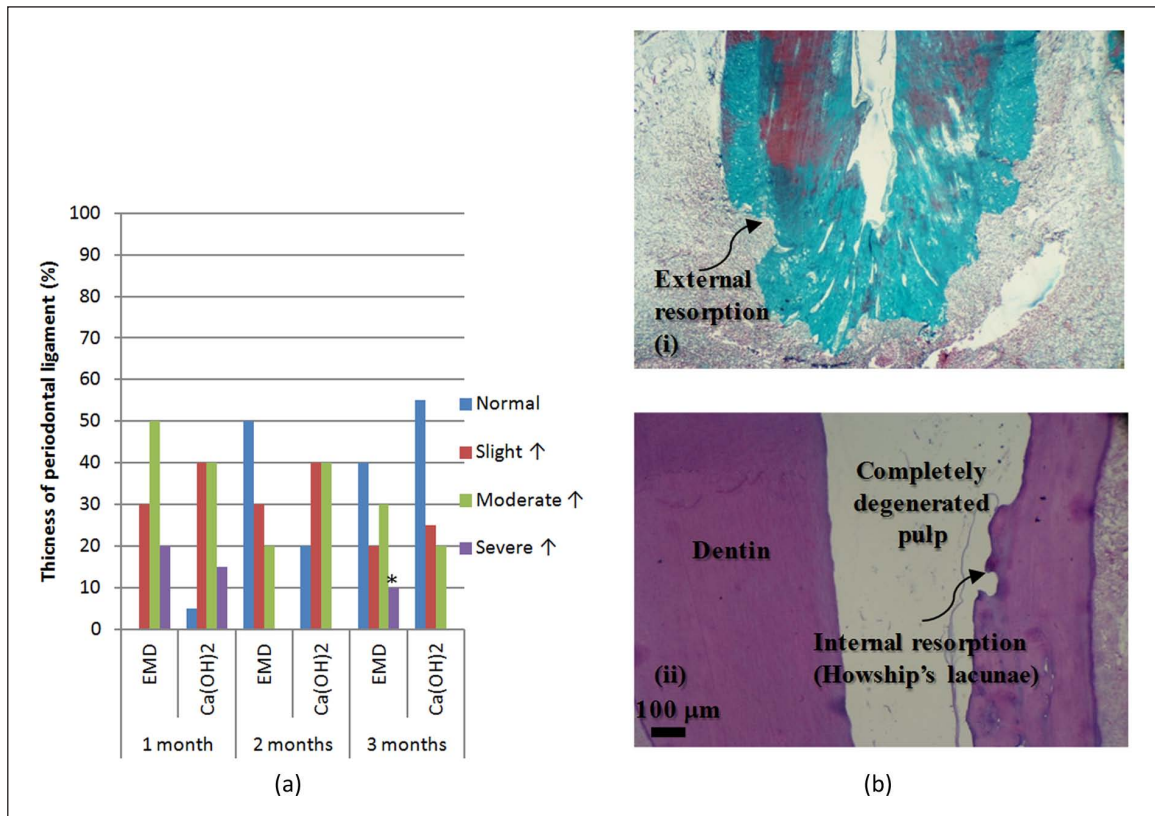


Figure 5. (a) Periodontal ligament thickness (%) of teeth treated with Emdogain gel and $\text{Ca}(\text{OH})_2$ up to 3 months after treatment. (b) Emdogain gel-treated group after 3 months showing the presence of both external (i, Gomeri's trichrome-stained section) and internal (ii, hematoxylin and eosin-stained section) resorptions as indicated by the presence of Howship's lacunae. EMD: enamel matrix derivative.

*Significant difference from 1-month time point.

group, the difference was only statistically significant between the first two time points ($p < 0.001$). Root resorption was observed in only 10% of the cases after 3 months. A total of 5% of the cases showed an external resorption, and the other 5% had an internal resorption (Figure 5(b)).

For $\text{Ca}(\text{OH})_2$ -treated group, 5% of the cases had normal PDL thickness after 1 month. A total of 40% had a slight to moderate increase in the PDL thickness; 15% showed severe increase in PDL thickness. After 2 months, 20% restored normal PDL thickness; 80% had a slight to moderate increase in PDL thickness. The percent of the cases with normal PDL thickness increased to 55% after 3 months; 25% had a slight increase and 20% had a moderate increase in PDL thickness (Figure 5(a)). Comparing the changes in the PDL thickness across time, the difference was statistically insignificant ($p > 0.05$). No root resorption was observed in any case at any time point.

Histomorphometric analysis

DB thickness (mm). Both the Emdogain- and $\text{Ca}(\text{OH})_2$ -treated groups had a comparable DB thickness after 1 month of treatment. Emdogain-treated group then showed a statistically significant reduction in DB thickness with

time until the bridge was completely disappeared by 3 months. The thickness of DB of those treated with $\text{Ca}(\text{OH})_2$ increased significantly with time (Figure 6(a)).

Apical width (mm). The apical width showed similar trends (i.e. a significant decrease with time) for both groups. At all time points, the $\text{Ca}(\text{OH})_2$ -treated group showed a significantly higher apical width than that of Emdogain-treated group (Figure 6(b)).

Discussion

The vital pulp therapy, aimed to maintain the vitality of the pulp and to allow continued development of the entire root (apexogenesis), remains one of the most controversial approaches for exposed pulp in permanent teeth. Emdogain gel has been found to be bio-inductive and hence widely used for vital pulp therapy in primary dentition with promising results.³⁷ In this study, Emdogain gel has been tried for vital pulp therapy in young permanent premolars in dogs and compared with calcium hydroxide paste as a control. Due to the close similarity to humans in the pulpal tissues and apical and periapical healing processes, the dog model was chosen.³⁸

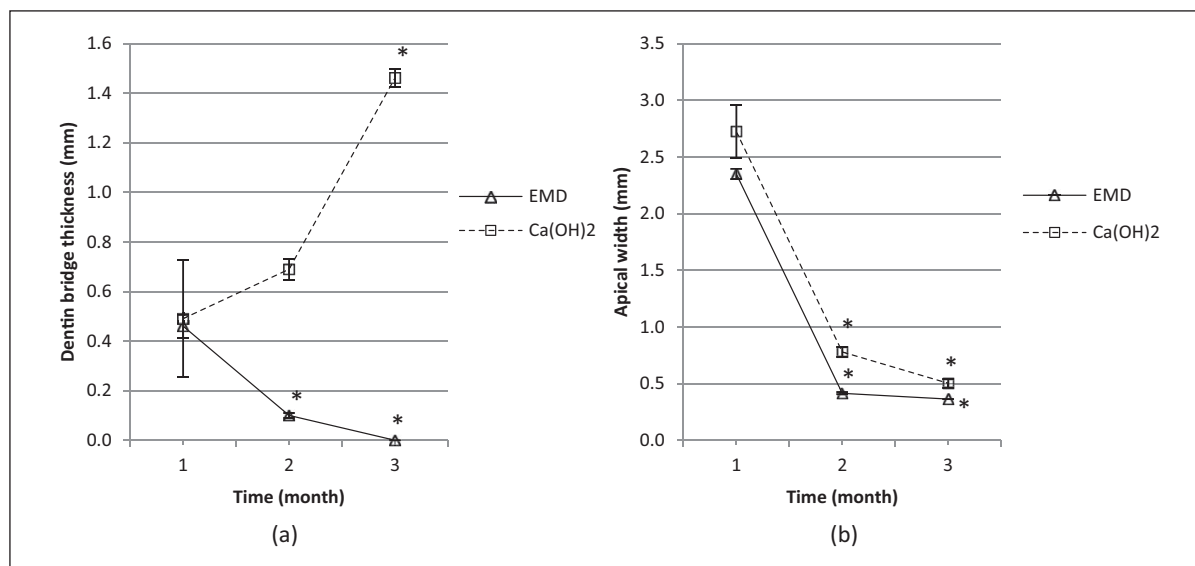


Figure 6. (a) Dentin bridge thickness (mm) and (b) apical width (mm) of teeth treated with Emdogain gel and Ca(OH)₂ up to 3 months after treatment.

EMD: enamel matrix derivative.

*Significant difference from 1-month time point.

Understanding the sequence of events involved in the healing process of the pulp and dentin, and hence, preserving the vitality of the tooth, is a key for the success of the vital pulp therapy, which is the least invasive treatment of pulp exposure. The surfaces of the human body have different physiological barrier functions to protect the body from the external noxious stimuli. In teeth, the tertiary dentin, also known as reactive or reparative dentin and formed by the preexisting odontoblasts or the newly differentiated odontoblast-like cells, respectively, plays a key role as a barrier against noxious agents that could damage the pulp tissues.^{39,40} The formation of DB over the pulp has been considered as classic wound healing with a subsequent neogenesis of normal pulp tissues and then repair of dental pulp.^{30,41} Unlike the primary or secondary dentin, the reparative dentin only formed as a response to various stimuli including dental procedure, caries, or attrition. When the cells forming the reparative dentin embedded in or lined the surface of tertiary dentin, the produced tissue is called osteodentin.⁴⁰ During development, several growth factors, for example, transforming growth factor-beta (TGF- β), are required for the inner enamel epithelium to stimulate the ectomesenchymal cells of dental papilla to differentiate into odontoblasts to form dentin.⁴² Like biological modulators during early dentinogenesis, Emdogain promoted the reparative process in the dental pulp^{19,20} by stimulating the differentiation of undifferentiated odontoblast-like cells to form a DB. Furthermore, Emdogain has a stimulatory effect on osteoblast cells. The soluble factors contained in it, for example, TGF- β 1 and small amelogenin peptides, could also be potential candidates responsible for its stimulatory effect on osteoblasts.⁴³

A slight inflammation of pulp tissues may cause demineralization of dentin lining the root and hence the release of TGF- β from demineralized matrix; the released TGF- β can accordingly activate the resident inactive odontoblasts to form DB.⁴⁴ On this basis, it can be assumed that the formation of DB with both Emdogain and Ca(OH)₂ could be secondary to the induced inflammation caused by both materials. The inflammatory reaction associated with Emdogain gel could be due to its acetic acid component. Furthermore, regardless of the ability of Emdogain gel to form a stable extracellular matrix that provides a protective action for the exposed pulp tissues,⁴⁵ its jelly-like consistency could allow direct contact between the glass ionomer base and the exposed pulpal tissues inducing further pulpal inflammation. A moderately infiltrated zone of chronic inflammatory cells under Emdogain gel has been previously observed.²⁰ The inflammatory reaction associated with Ca(OH)₂ paste, however, could be due to its high alkalinity.⁴⁶ The inflammation induced by the high alkalinity of Ca(OH)₂ is a reversible process;⁴⁷ this could allow the pulp tissue to recover and restore its normal structure and DB to increase in thickness. The increased severity and persistence of inflammation caused by Emdogain gel, however, could be responsible for the resorption of DB. The resorption was evident from the presence of Howship's lacunae and odontoclasts underlining the DB. Generally, a huge controversy remains around the effect of Emdogain gel as a pulp capping material. In pig permanent incisors, Emdogain gels showed more effective reparative dentin in bridging the pulpal wounds and higher capacity to induce rapid pulpal wound healing than Ca(OH)₂.⁴⁸ In dog permanent teeth, however, Emdogain gel did not show any sign

of reparative dentin formation at the exposure site but only along the root surface, and this was accompanied by severe inflammation and subsequent degeneration of the pulp tissue.¹⁹ In human, on the contrary, a randomized controlled trial, conducted on partially pulpotomized premolars, revealed that Ca(OH)₂ had less inflammation and more DB formation than Emdogain gel.⁴⁹ Furthermore, a systematic review study revealed that Emdogain gel was not effective in inducing the formation of a continuous DB; bacterial invasion and reduced ability of odontoblasts to repair the defect have been accordingly detected.³⁹

Tooth root development is a complex, long-term process during which the root elongation in an apical direction and the formation of periodontium occur. A big controversy about the consistent detection of enamel proteins along forming roots exists. A transient expression of this protein, however, has been observed at early stages of root formation to induce the differentiation of odontoblasts and/or cementoblasts. On this basis, the EMD has been used clinically to stimulate the repair and regeneration of root.⁴⁰ However, whether these proteins act as “instructional messengers,” like growth factors, for odontoblasts and/or cementoblasts to start the processes of regeneration or merely alter the periodontal environment hence allowing efficient regeneration to proceed is still ambiguous.⁴⁰ Emdogain gel showed an enhanced apical closure tendency in comparison to Ca(OH)₂. The apex closure, however, was mainly by cementum in Emdogain-treated group. Osteodentin or dentin covered by a thin layer of cementum dominated the tissue responsible for root maturation in Ca(OH)₂-treated group. In addition to “instructional messengers-like action” of enamel proteins, the acetic acid component of Emdogain gel could be responsible for the stimulation of the surrounding cementoblasts to start cementogenesis to achieve apical closure with cementum. The latter action has been inferred from the formation of cementoma on top of an apical abscess as a means of healing process.⁵⁰ Similar finding was also observed in a non-vital pulp therapy conducted in rats where Emdogain gel encouraged the ingrowth of cementum-like tissues into the root canals.⁵¹ Normally, dentin is the most appropriate tissue to close the root apex; it is more resilient than any other tissues. Cementum, on the contrary, is fragile and therefore renders the root canal manipulation difficult.⁵² The absence of dentin in Emdogain gel-treated group could therefore be attributed to the complete degeneration of dental pulp tissue and the simultaneous absence of odontoblastic layer lining the pulp.⁵³

Calcium hydroxide demonstrated its success in stimulating Hertwig’s epithelial root sheath (HERS) to continue apical root formation in pulpotomized young permanent teeth.⁷ During the embryonic development, HERS is believed to be crucial for root development that normally starts after the completion of crown formation;⁵⁴ it induces the ectomesenchymal cells of dental papilla to

differentiate into odontoblasts to form dentin.⁵⁵ Then, HERS cells secrete extracellular matrix components (enamel-like proteins) to cover the dentin surface before the cells of dental follicle penetrate HERS network to contact dentin.⁵⁴ HERS cells are then capable of inducing the ectomesenchymal cells of dental follicle to differentiate into cementoblasts and promote the formation and maturation of both acellular and cellular cementum to overlay the formed dentin.^{56–58} Accordingly, from the results of this study, it can be inferred that the pulp and dental papilla were destroyed in teeth treated with Emdogain gel, and therefore, only cementum was formed. In case of Ca(OH)₂, however, pulp, dental papilla, and dental follicle remained, and accordingly, dentin overlaid by cementum was seen.^{59,60}

The PDL started to partially retain its normal thickness after 2 months but started to completely retain its normal thickness after 3 months of Emdogain gel treatment. As apical closure proceeds, with consequent deposition of newly formed bone, it is expected that the PDL resume normal appearance and distribution. Root resorption was observed with Emdogain gel-treated group after 3 months. This was also observed by St George et al.,⁶¹ who observed sporadic cases of external root resorption with the use of Emdogain gel while used for treating infra-bony pocket. The authors explained this external resorption as an unusual adverse event following the Emdogain gel treatment. This external resorption could be associated with the patent dentinal tubules which would encourage the Emdogain gel to act on the cells beyond the confines of the area of application. The internal resorption, however, may be caused by the inflammatory cellular infiltrate observed following Emdogain gel use; this could in turn stimulate the odontoclasts to start root resorption.

Conclusion

Under the circumstances of this study, the following can be concluded.

For regeneration of dentino–pulpal complex

- With Emdogain gel-treated teeth:
 - The formation of dentin-like tissues bridging the full width of the coronal pulp at the interface between the injured and healthy pulp tissues was seen after 1 month. With time, the DB became disintegrated and fully disappeared by 3 months. This could be due to the persistence and severity of pulpal inflammation induced by the Emdogain gel.
 - The pulp tissues showed an inflammatory infiltration with a slight degeneration after 1 month, and complete pulpal degeneration was seen after 2 months of the treatment.

- With calcium hydroxide-treated teeth:
 - The formation of dentin-like tissues bridging the full width of the coronal pulp at the interface between the injured and healthy pulp tissues was seen after 1 month of the treatment. Regardless of the slow formation of the DB, the thickness of this calcific barrier increased with time.
 - The pulp tissues showed an inflammatory infiltration with a slight degeneration after 1 month of the treatment. This inflammation was reversible, and the recovery of pulpal tissues was seen after 2 months of the treatment.

For root regeneration

- With Emdogain gel-treated teeth, the apexogenesis has been achieved by cementum that is attached to the newly formed alveolar bone by new attachment system of PDL. The newly formed PDL became well oriented only after 3 months of the treatment.
- With calcium hydroxide-treated teeth, the apexogenesis has been achieved by dentin overlaid by cementum. This cementum was then attached to the newly formed alveolar bone by a well-organized PDL.

Accordingly, Emdogain gel could be a good candidate for regeneration of the periodontium including cementum, PDL, and alveolar bone but not the dentino-pulpal complex in a young permanent dog's dentition. Calcium hydroxide, however, could be a good candidate for both dentino-pulpal and periodontium regeneration.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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