Scientific Article

# Histological Evaluation of Enamel Matrix Derivative as a Pulpotomy Agent in Primary Teeth

Jumana Sabbarini, BDS, MSc[1] • Maha Mounir, BDS, MSc, PhD[2] • Jeffrey Dean, DDS, MSD[3]

**Abstract:** ***Purpose:*** *The purpose of this study was to evaluate histologically the effect of an enamel matrix derivative as a pulpotomy agent in primary canines.* ***Methods:*** *Ten carious primary canines among teeth deemed for serial extraction were selected for this study. Emdogain gel was used as the pulp dressing material on the amputated pulp stumps. Teeth were extracted postoperatively after: (1) 1 week; (2) 2 weeks; and (3) 6 months. The extracted teeth were examined histologically to assess the response of the pulp to Emdogain gel after the pulpotomy procedure.* ***Results:*** *Of the teeth extracted after 1 week, the amputated pulpal surface was lined by a thin, nearly continuous cellular layer. Generalized congestion was accompanied by an increase in angiogenesis. Of the teeth extracted after 2 weeks, most showed small islands of dentin-like tissue at different stages of mineralization. Of the teeth extracted after 6 months, several different histological pictures were viewed. Most of the teeth showed coalescing islands of dentin-like tissue trying to bridge the full width of the coronal pulp at the interface between the wounded and unharmed pulp tissue below the amputation site.* ***Conclusion:*** *Based on these experiments, Emdogain gel shows promising results as a valuable material for use in pulpotomy procedures, especially in the primary dentition. (Pediatr Dent 2007;29:475-9) Received December 8, 2006 / Revision Accepted March 14, 2007.*

KEYWORDS: ENAMEL MATRIX DERIVATIVE, PRIMARY CANINES, PULPOTOMY

The dental pulpotomy technique involves amputation of the coronal portion of the affected or infected dental pulp. Treatment of the remaining vital radicular pulp tissue surface should preserve the vitality and function of all or part of the pulp's remaining radicular portion.[1] Furthermore, it is an accepted procedure for treating both primary and permanent teeth with carious pulp exposures.[2] The pulpotomy technique has become the predominant pulp therapy for the primary dentition because of the: (1) complicated anatomy of the root canals in primary teeth; (2) proximity of the permanent tooth germ; and (3) difficulties in finding a root-canal filling material compatible with physiological root resorption.[3]

Pulpotomy therapy for the primary dentition has developed along 3 lines: (1) devitalization; (2) preservation; and (3) regeneration.[4] Regeneration is the induction of reparative dentin formation by the pulpotomy agent. Ideally, it should leave the radicular pulp vital, healthy, and completely enclosed within an odontoblast-lined dentin chamber. In this situation, the tissue would be isolated from noxious restorative materials, thereby diminishing the chances of internal resorption. Additionally, the odontoclasts of an uninflamed pulp could enter into the exfoliative process at the appropriate time and sustain it in a physiologic manner.[4]

The dental pulp is a highly vascular and innervated connective tissue, which is capable of healing by producing reparative dentin and/or dentin bridges in response to various stimuli and surgical exposure.[5] Innovative therapies attempt to apply biological modulators that have been: (1) identified during tooth and bone embryogenesis; and (2) cloned for experimental and clinical application. These modulators are intended to: (1) improve treatment modalities; and, ultimately (2) induce tissue regeneration. It is hoped that these biological modulators will be the promising materials that will successfully regenerate the exposed pulp tissue rather than devitalize it.[6]

Growth factors are biological modulators that are able to promote cell proliferation and differentiation. Naturally occurring osteogenic proteins, such as bone morphogenetic proteins (BMPs), are members of the "transforming growth factor" super family of bone-matrix polypeptides.[6] These

[1]*Dr. Sabbarini is specialist in pediatric dentistry , Ministry of Health, Irbid, Jordan;* [2]*Dr. Mounir is professor, Alexandria University Faculty of Dentistry, Alexandria, Egypt; and* [3]*Dr. Dean is professor and chairman, School of Dentistry, Indiana University, Indianapolis, Indiana.*
*Correspond with Dr. Dean at jadean1@iupui.edu*

bioactive molecules appear to modulate cartilage and bone deposition and/or resorption. The osteogenic properties of BMPs were initially demonstrated using demineralized bone matrix or reconstituted extracts of purified solubilized bone matrix.[7] This was followed by molecular cloning and expression of several recombinant human protein BMPs (osteogenic proteins-1 and osteogenic proteins-2).[8]

Recombinant human BMP-2, BMP-4, and OP-1 (BMP-7) initiate endochondral bone formation when: (1) simply implanted subcutaneously or intramuscularly; and (2) combined with insoluble collagenous bone matrix.[9] Other osteogenically active growth factors that have been identified are platelet-derived growth factor,[10] insulin-like growth factor, and fibroblast growth factor.[11]

Enamel matrix derivative (EMD), obtained from embryonic enamel as amelogenin, has been demonstrated in vitro (using a wound healing model) to be capable of stimulating periodontal ligament cell proliferation sooner when compared to gingival fibroblasts and bone cells.[12] The ability of EMD to facilitate regenerative processes in mesenchymal tissues is well established. The EMD-induced processes actually mimic parts of normal odontogenesis. It is believed that the EMD proteins participate in the reciprocal ectodermal-mesenchymal signaling that control and pattern these processes.[13] Based on these observations, it has been suggested that amelogenin participates in the differentiation of odontoblasts and the subsequent predentin formation.[14]

Emdogain gel (Straumann, Switzerland) has been successfully employed for pulpotomies in noninfected teeth in animal studies.[15-17] Its effect was also investigated on experimentally exposed human permanent pulps, but seems ineffective for formation of hard tissue barriers.[18] The aim of this present study was to investigate the effect of Emdogain on deeply decayed primary canines with possible pulpal inflammation.

## Methods

This study was approved by the committee on investigations involving human subjects at Alexandria University, Alexandria, Egypt. The patients selected for this study were children served by the pediatric dental clinics, Department of Pediatric Dentistry, Faculty of Dentistry, Alexandria University, Egypt. Ten carious primary canines indicated for pulpotomy were selected among teeth deemed for serial extraction. Prior to treatment, an appropriate informed consent was obtained from the parents. After achieving profound local anesthesia: (1) the teeth were isolated with a rubber dam, and (2) all dental caries and any overhanging enamel were removed.

When pulpal exposure occurred, a sterile high-speed bur was used to remove the pulp chamber's roof. The coronal pulp tissue was amputated using a sterile sharp spoon excavator. Gross hemorrhage control was achieved using a moist cotton pellet for a few minutes. A "protective" cotton pellet was used to cover the amputated pulp stumps, and the teeth were acid etched (35% phosphoric acid gel) and primed. Then, the cotton pellet was removed and the amputated pulpal stumps were covered with Emdogain gel from a 0.3-ml syringe. Finally, light-cured glass ionomer cement (Vitremer 3M ESPE, Seefeld,Germany) was applied as the restorative material.

Three teeth were extracted after 1 week, 3 teeth were extracted after 2 weeks, and then 4 teeth were extracted 6 months postoperatively. They were histologically prepared and examined in the following manner to assess the pulp's response to the Emdogain gel pulpotomy procedure. After sealing the foraminae, the extracted teeth were fixed in neutral formalin and decalcified in 5% trichloroacetic-acid. Buccolingual sections were processed and prepared for examination by light microscopy, using either hematoxylin and eosin or trichrome stain. Each specimen was observed for: (1) dentin bridge formation; (2) odontoblastic layer integrity; (3) pulp inflammation; and (4) pulp calcification. The amount of new hard tissue formed subjacent to the amputation site was assessed in the central section (N=3) from each experimental tooth. The areas covered by newly formed hard tissue in these sections were measured using digital histo-metry. Results were measured as a mean thickness of dentin bridging.

## Results

Teeth extracted after **1** week demonstrated amputated pulpal surfaces lined by a thin, nearly continuous cellular layer. Generalized congestion, accompanied by an increase in angiogenesis, was evident in the deeper parts of the pulp tissue below the application sites. For the most part, the teeth extracted after 2 weeks showed small islands of dentin-like tissue at different stages of mineralization (Figure 1). Some of these islands tended to coalesce together subjacent to the amputation site at the junction between vital pulp tissue and the amputated superficial part.

The teeth extracted after 6 months showed several different histological pictures. For the most part, the teeth demonstrated coalescing islands of dentin-like tissue trying to bridge the full width of the coronal pulp at the interface between the wounded and unharmed pulp tissue below the amputation site (Figure 2). This was accompanied by deposition of reparative dentin along pulpal walls narrowing the pulp canal. Some areas showed amputated pulpal surfaces covered by a thick, infiltrating layer of cellular condensation (Figure 3), accompanied by massive deposition of reparative dentin lining the whole pulpal wall, both in a coronal and apical direction. One tooth showed small coalescing dentin islands covering the surface of the amputated pulp, but not completely bridging the site (Figure 4).

The thickness of the dentin bridges formed in EMD-treated teeth after 6 months was assessed by histomorphometry. The mean thickness of dentin-like tissues bridging was measured in 3 central sections for the teeth. The results for mean thickness of dentin (mm±SD) were: (1) 0.137±0.105; (2) 0.021±0.004; and (3) 0.276±0.905.

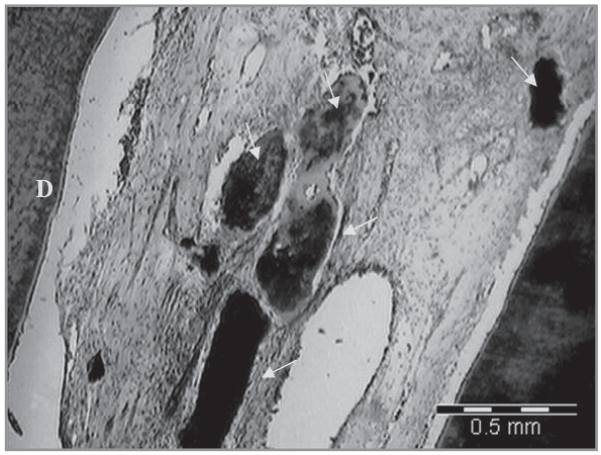


Figure 1. Micrograph showing primary canines 2 weeks after treatment with Emdogain. Trichrome micrograph at X80 magnification shows small islands of dentin-like tissue at different stages of mineralization (↓). Dentin (D).

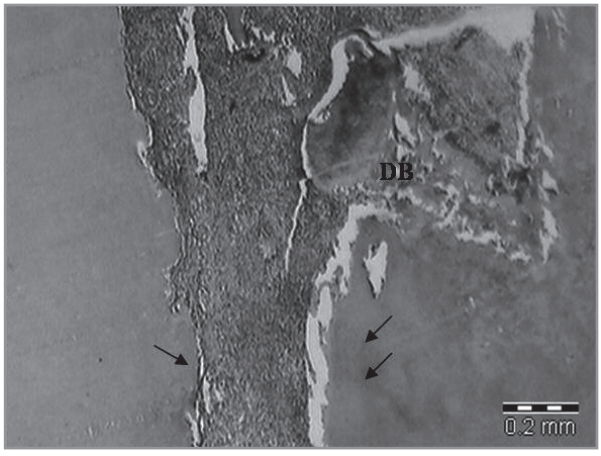


Figure 2. Micrograph showing primary canines 6 months after treatment with Emdogain. Trichrome micrograph of the amputated pulp at X110 magnification shows coalescing islands of dentin-like tissue in the process of bridging the full width of the coronal pulp at the interface between the wounded and unharmed pulp tissue below the amputation site, accompanied by deposition of reparative dentin along site pulpal walls narrowing the pulp canal (↓). Dentin bridge (DB).

## Discussion

The principal component of enamel matrix derivative (EMD) is amelogenin. It is one of the key factors in controlling mineral crystal growth. Amelogenin self-assembles to form nanospheres. Using atomic force microscopic phase images, nanospheres are seen as beaded rows surrounding the mineral crystallites at very early stages of mineral formation. These nanospheres provide the organized microstructures for crystal initiation, orientation, and growth.[19]

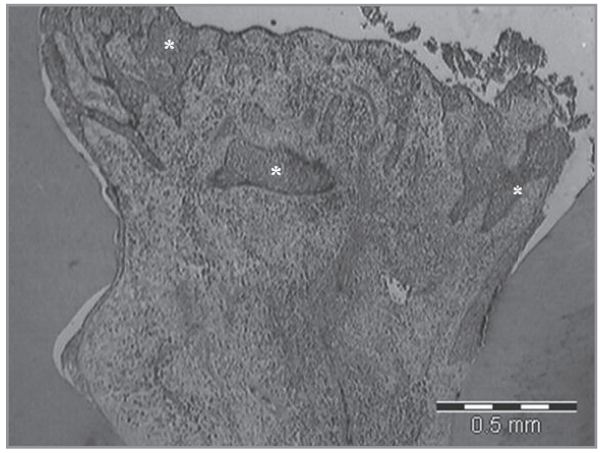


Figure 3. Micrograph showing primary canines 6 months after treatment with Emdogain. Trichrome micrograph of amputated pulp at X110 magnification shows the amputated pulpal surface covered by a thick infiltration layer of cellular condensation (*) accompanied by a massive deposition of reparative dentin.

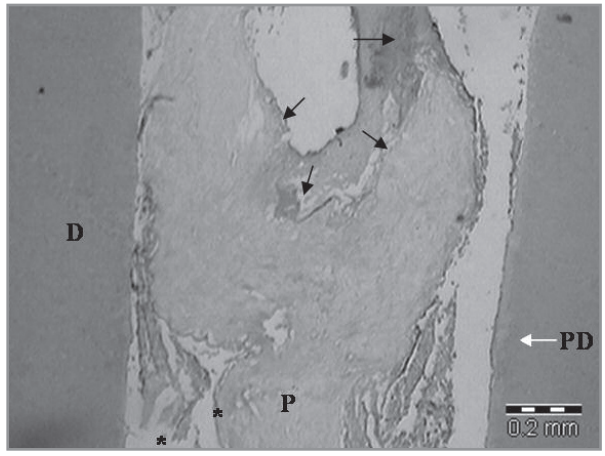


Figure 4. Micrograph showing primary canines 6 months after treatment with Emdogain. Trichrome micrograph of amputated pulp at X110 magnification shows small coalescing dentin islands covering the surface of the amputated pulp (↓). The pulp tissue is homogenous and shows signs of partial degeneration (*). Dentine walls shows resorption lacunae (↓) covered by predentin (PD). Dentin (D), and pulp (P).

The ability of EMD (mainly amelogenins) to induce the regenerative processes in mesenchymal tissues is now well established. The EMD-induced processes actually mimic parts of normal odontogenesis, and it is believed that

EMD participates in reciprocal ectodermal-mesenchymal signaling.[20]

According to Nakamura et al,[15] when a pulp wound is exposed to EMD, substantial steps occur in a process resembling classic wound healing with subsequent neogenesis of normal pulp tissues and repair of dental pulp (ie, rapid fibrodentine matrix formation and subsequent reparative dentinogenesis). The present study is in full agreement with Nakamura et al. In the 2-week study period, the pulp matrix itself showed homogenous fibrous deposition together with reparative dentin islands. The formation of new dentin started from within the pulp at some distance from the amputated site. There was also a marked tendency for angiogenesis in the deeper parts of the pulps, indicating an increased level of cell growth and/or metabolism. After the initial phase of healing in these teeth, a fine web of odontoblast-like cells was also observed growing from the central parts of the pulp towards the pulp chamber walls, forming a dentin bridge. The EMD-induced hard tissue closely resembled osteodentin early in the process. Later, the hard tissue became more like secondary dentin. Finally, at the 6-month postoperative visit, most of the treated pulp tissues revealed morphological findings characteristic of extensive reparative dentin formation along the root canal walls and the apex itself, narrowing the pulp space and extending to the coronal pulp. This agrees with Hammarstron et al.[21]

The current study revealed that the incidence of inflammation did not significantly influence the outcome of hard tissue formation. In agreement with Nakamura et al,[15] none of the teeth treated with Emdogain showed signs of irreversible pulp damage. Instead, they resembled normal wound healing. This included the formation of a scab covering the amputation site, moderate inflammatory infiltrate beneath the injury, and a local increase in angiogenesis and cell proliferation. All these descriptions also coincide with previous animal studies by Nakamura et al[22] on miniature swine, and with Igarashi et al[23] on Wistar rats.

Ishizaki et al[16] reported different results in their study on the molar teeth of mongrel dogs, where congested capillaries in pulp tissue were observed in early stages. Later on, the pulp tissues revealed characteristic morphological findings of tertiary dentin along the root canal walls accompanied by atrophic pulpal degeneration. Dentin bridge formation, however, did not occur in their study.

Olsson et al[18] found hard tissue formation along side the exposed dentin surfaces in experimentally exposed human pulps, but the hard tissue was not formed as a bridge.

It is generally known that the ratio of surface area of tissue in contact with capping materials, relative to the remaining tissue volume, is considerably higher in pulpotomized teeth. Thus, if a material can elicit unwanted side effects, such as necrosis and/or internal root resorption, it is more likely to do so in narrow pulp canals than in the wider coronal pulp.[24] Few specimens showed internal root resorption in the present study, and none showed necrosis. A few degenerative changes accompanied by root resorption were only seen in **1** specimen in the 6-month period, while the rest of the pulp showed signs of total pulp regeneration.

The results reported in this study strongly supported the hypothesis that: (1) certain enamel matrix proteins, like amelogenins, participate in differentiation and maturation of odontoblastic cells; and (2) these enamel matrix molecules play important role(s) during early dentin formation.

When the pulp wound is exposed to EMD, a substantial amount of reparative dentin-like tissue is formed in a process resembling classic wound healing, with subsequent neogenesis of normal pulp tissue.[15] Thus, EMD components may:

1. act as a signal for induction of mesenchymal cell differentiation, maturation, and biomineralization; and
2. form a stable extracellular matrix that provides a beneficial and protective pulp environment.

Based on these experiments, EMD has several potential clinical applications and shows promising results as a valuable material for use in pulpotomy procedures, especially in primary dentition. More experimental data and further human research with larger sample sizes and longer follow-up periods is recommended, however, to determine if EMD can be developed as a material for predictable induction of dentin formation.

## Conclusions

Based on this study's results , the following conclusions can be made:

1. Emdogain is a bioinductive material that is compatible with vital human tissues. It offers a good healing potential and is capable of inducing dentin formation, leaving the remaining pulp tissue healthy and functioning.
2. Emdogain may act in a multitude of ways on mesenchymal cells that provide pulp protection.

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