Dental pulp capping: effect of Emdogain Gel on experimentally exposed human pulps

H. Olsson[1], J. R. Davies[2], K. E. Holst[3], U. Schröder[4] & K. Petersson[1]

[1]Department of Endodontics, Faculty of Odontology, Malmö University, [2]Department of Oral Biology, Faculty of Odontology, Malmö University, [3]Biora AB, [4]Department of Pedodontics, Faculty of Odontology, Malmö University and Specialist Clinic for Pediatric Dentistry, Public Dental Health Service, Malmö, Sweden

Abstract

Olsson H, Davies JR, Holst KE, Schröder U, Petersson K. Dental pulp capping: effect of Emdogain Gel on experimentally exposed human pulps. *International Endodontic Journal*, **38,** 186–194, 2005.

Aim To investigate the effect of Emdogain Gel (Biora AB, Malmö, Sweden), consisting of a enamel matrix derivative (EMD) in a propylene glycol alginate (PGA) vehicle, on experimentally exposed human pulps and to register postoperative symptoms.

Methodology Nine pairs of contralateral premolars scheduled for extraction on orthodontic indications were included. Following a superficial pulp amputation performed with a small (016) diamond bur, either EMDgel or a mix of calcium hydroxide and sterile saline was placed at random in contact with the pulp wound. The subjects made records of symptoms and were also interviewed about pain/discomfort by a blinded examiner. After 12 weeks the teeth were extracted, prepared and subjected to light microscopic examination in which the inflammation and newly formed hard tissue in the pulp were analysed. Immunohistochemistry was performed using affinity-purified rabbit anti-EMD polyclonal antibodies.

Results Postoperative symptoms were less frequent in the EMDgel-treated than in the calcium hydroxide-treated teeth, especially during the first six weeks. In the EMDgel-treated teeth, new tissue partly filled the space initially occupied by the gel and hard tissue was formed alongside the exposed dentine surfaces and in patches in the adjacent pulp tissue. EMD was detected in the areas where new hard tissue had been formed. The wound area of the EMDgel-treated teeth exhibited inflammation in the majority of the teeth whereas less inflammation was seen in the calcium hydroxide-treated teeth where the hard tissue was formed as a bridge.

Conclusions In the EMDgel-treated teeth, postoperative symptoms were less frequent and the amount and pattern of hard tissue formation were markedly different than in the teeth treated with calcium hydroxide. However, the operative procedure and the formulation with EMD in a PGA vehicle do not seem to be effective for the formation of a hard tissue barrier.

Keywords: calcium hydroxide, dental pulp exposure, dentinogenesis, histology, immunohistochemistry, randomized controlled trial.

Received 4 May 2004; accepted 26 November 2004

Introduction

There have been many attempts to find a substance that will predictably induce a hard tissue barrier after pulp exposure. Calcium hydroxide has been shown to induce a hard tissue bridge (Nyborg 1955, Schröder 1972, Cvek 1978). However, the clinical outcome of pulp capping performed with calcium hydroxide has varied (Cvek 1978, Hørsted *et al.* 1985, Mejàre & Cvek 1993, Barthel *et al.* 2000). This suggests that the newly formed hard tissue may be of insufficient quality to be able to act as a functional barrier protecting the pulp against bacterial microleakage along the restoration margins.

Studies where growth or differentiation factors have been used as a pulp capping material on pulp exposures in animals have shown interesting results (Rutherford *et al.* 1993, Nakashima 1994, Decup *et al.* 2000).

Correspondence: Helena Olsson, Department of Endodontics, Faculty of Odontology, Malmö University, SE-205 06 Malmö, Sweden (Tel.: +46-40-665 85 60; fax: +46-40-665 85 71; e-mail: helena.olsson@od.mah.se).

Amelogenin and amelin are proteins that have been suggested to participate in the final differentiation of odontoblasts and subsequent dentine formation during dentinogenesis (Inai *et al.* 1991, Spahr *et al.* 2002, Papagerakis *et al.* 2003). An amelogenin-rich fraction of porcine enamel matrix derivative (EMD) that also contains amelin has been used in patients with severe periodontitis to induce cementogenesis as the EMD induces processes that seem to imitate normal odontogenesis (Hammarström 1997, Heijl *et al.* 1997, Gestrelius *et al.* 2000). Emdogain Gel (Biora AB, Malmö, Sweden) is a commercial product containing EMD used in the treatment of periodontal disease. Studies in miniature pig, where this product has been tested as a pulp capping material, demonstrated the potential of EMD to induce hard tissue after experimental pulp exposures (Nakamura *et al.* 2001, 2002). There are no published reports on pulp reactions after pulp capping with EMD in human teeth.

The aim of this study was to investigate pulp tissue reactions in humans after the placing of Emdogain Gel (EMD in a PGA vehicle) on experimentally created pulp exposures in premolars planned for extraction prior to orthodontic treatment, and to record and evaluate symptoms.

Materials and methods

The study was approved by the Committee on Investigations Involving Human Subjects at Lund University, Sweden.

The material consisted of nine contralateral pairs of premolars, seven mandibular and two maxillary, scheduled for extraction on orthodontic indications from eight healthy subjects, aged 12–16 years (Table 1). Only teeth with no signs of caries were included. Criteria for exclusion were subjects included in other clinical trials or subjects with systemic illnesses. Orthodontists and general dentists enrolled the subjects to the project that was undertaken at the Department of Endodontics, Faculty of Odontology, Malmö University. Written informed consent was obtained from the subjects and from the parents of each subject. Subjects were included from November 2001 and the 12-week follow-up procedure was completed by September 2002. As this study was considered a pilot study on human material, the sample size was set to nine pairs of premolars. Adverse events were recorded throughout the study period.

Table 1 Distribution of subjects according to age

Age (years)	No. of subjects
12	2
13	2
14	3
15	0
16	1

Operative procedure

The teeth were anaesthetized with Citanest and Octapressin (30 mg mL^{-1} + 0.03 IE mL^{-1}) (AstraZeneca, Södertälje, Sweden), isolated with a rubber dam and cleaned with 30% hydrogen peroxide and chlorhexidine with alcohol 5 mg mL^{-1}. All instruments used were sterile.

To facilitate the application of the pulp capping materials a cylindrical cavity was prepared on the occlusal surface with a high-speed bur until a small pulp exposure was created. The bur and pulp wound were continuously irrigated with sterile saline. A superficial pulp amputation was performed with a gentle surgical technique modified after the method described by Granath & Hagman (1971) in order to cause as little surgical trauma as possible. With a small (016) round diamond bur the pulp tissue was amputated approximately 2 mm apical from the newly cut cavity floor, creating a cavity in which the test or control material could be placed. The pulp wound was carefully irrigated with sterile saline until the bleeding stopped in order to avoid formation of a blood clot. A drop of the test material Emdogain Gel [EMD 30 mg mL^{-1} in propylene glycol alginate (PGA)] (Biora AB) was placed on the pulp wound. In the control cases a newly prepared paste of sterile calcium hydroxide and sterile saline was applied in close contact with the pulp tissue under gentle pressure. The pulp capping materials were covered with a disc of Teflon, the margins of which were fixed to the dentine walls by wax, and the cavity was sealed with zinc oxide eugenol cement covered with a glass–ionomer restoration (Ketac-fil; ESPE, Seefeld, Germany) as seen in Fig. 1. The same operator performed all procedures. The capping materials were placed in a 'semi-blind' fashion, i.e. when the tooth had been prepared to the point of application of the pulp capping material, the randomization envelope for the subject number was opened to reveal the treatment assignment for the site. Each subject received treatment with both test and control in a split mouth design. A computer program generated the randomization schedule and Biora provided envelopes containing randomization codes. Five pairs of teeth were treated in sequence EMDgel-calcium hydroxide and four

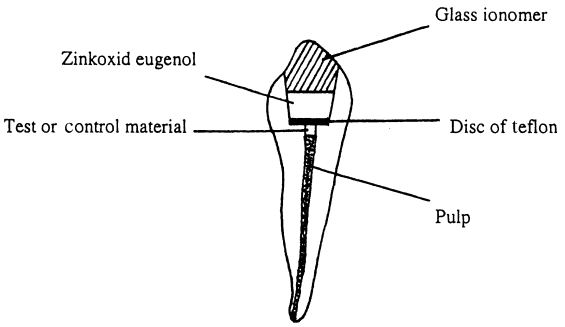

Figure 1 Schematic model of the operative procedure.

pairs in the sequence calcium hydroxide-EMDgel. Because of the characteristic nature of the capping materials they could not be blinded to the operator. Prior to starting the study, the surgical procedure was practised in one subject not included in the study. The two teeth from this subject were immediately extracted, prepared and subjected to light microscopic examination to ensure that the pulp amputation had been performed atraumatically according to the method described above (Granath & Hagman 1971).

Assessment of symptoms

Examination

One examiner, not involved in the operative procedure, examined the teeth clinically on two occasions. Immediately prior to the operative procedure and immediately prior to the extraction of the teeth, the teeth were tested for tenderness to percussion, tenderness to apical palpation and mobility.

Telephone interview

The blinded examiner made telephone interviews based on a structured questionnaire about postoperative pain and discomfort. The subjects were unaware of which capping material had been placed in which tooth. The subjects were asked about experiences of spontaneous pain, intake of analgesics and if they felt pain or discomfort when eating or drinking cold or warm food, as well as when chewing. The subjects were asked to evaluate any symptoms as mild, moderate or severe. The interviews were performed 1 day, 2 weeks, 6 weeks and 12 weeks after the treatment.

Diary

The subjects were given a diary and asked to record pain experience using a 100 mm Visual Analogue Scale (VAS) with anchor definitions: 0 = 'no pain' and 100 = 'unbearable pain' (Huskisson 1983). The subjects were required to make a daily recording on the scales for each of the first 10 days postoperatively, so that there would be 10 recordings of each tooth. As a part of the diary the subjects were also asked to record use of any analgesics during the first 10 days postoperatively.

Sampling and histological technique

Twelve weeks after the operative procedure the teeth were anaesthetized with Citanest and Octapressin (30 mg mL^{-1} + 0.03 IE mL^{-1}) and extracted with an elevator and forceps. The apices were sectioned under irrigation with sterile saline in order to facilitate fixation of the pulp tissue. The specimens were fixed in 4% neutral buffered formaldehyde for 7 days and thereafter demineralized in 12.5% EDTA and subsequently embedded in paraffin. After longitudinal serial sectioning (5 μm), every fifth section was stained with haematoxylin and eosin. The sections covering the pulp tissue were then examined in a light microscope equipped with a digital camera and a computer for histometry (Olympus Microimage; Media Cybernetics, Silver Spring, MD, USA). The dentist who had performed the operative procedure assessed the histological sections blindly.

Immunohistochemical staining

In order to investigate the presence and location of the EMD after 12 weeks in the EMD-treated teeth, immunohistochemistry was performed. Affinity-purified rabbit anti-EMD polyclonal antibodies were supplied by Biora AB. The antibodies have been described previously (Gestrelius *et al.* 1997b).

One central section (5 μm) from each of the formalin-fixed, paraffin-embedded EMDgel-treated teeth was dewaxed and rehydrated. Endogenous peroxidase activity was quenched in all sections using 3% (v/v) hydrogen peroxide in water for 20 min, and the sections were washed with Tris-buffered saline (TBS; 0.15 mol L^{-1} NaCl, 0.05 mol L^{-1} Tris–HCl buffer, pH 7.6). Nonspecific binding was blocked using normal goat serum diluted 1 : 5 in TBS for 1 h and endogenous biotin was blocked by treatment with the Dako biotin blocking kit according to the manufacturer's instructions (Dako, Glostrup, Denmark). For immunohistochemical localization, sections were then incubated with rabbit anti-EMD (diluted 1 : 1000 in TBS)

overnight at 4 °C, followed by the StreptABComplex/HRP Duet kit (Dako). Antibody binding was visualized using DAB (diaminobenzidine) (0.6 mg mL^{-1}) in TBS containing 0.03% (v/v) hydrogen peroxide for 15 min and sections were counterstained with Mayer's haematoxylin. The dentist who had performed the operative procedures assessed the histological sections.

Analyses of the pulp and of new hard tissue

The degree of inflammation was classified in groups from 0 to 4 as None, Slight, Moderate, Severe or Abscess formation (Table 2), modified after Heyeraas *et al.* (2001).

In each experimental tooth, all sections throughout the pulp exposure were examined to identify the representative sections ($n = 5$) in which the pulp exposure was assessed. In two teeth it was difficult to find one area representing the pulpal status of the exposure site, thus two areas were chosen and two series of sections ($n = 10$) were analysed and noted in each of the two teeth.

In each section the pulpal status was judged in three different areas; the proliferated pulp tissue coronal of the exposure, directly below the exposure, and in the centre of the pulp.

The area (μm^2) of new hard tissue formed in the wound area was assessed in the representative central sections as described above. The bridging was judged as none, partial or complete as recommended by Federation Dentaire International CoDM, Instruments, Equipment and Therapeutics (1980). The thickness (μm) of the newly formed hard tissue was measured alongside the dentine wall. All data were entered into an SPSS database.

Table 2 Description of criteria for classification of pulp inflammation, modified after Heyeraas *et al.* (2001)

0	None: normal pulp structure
1	Slight: increased number of cells, predominately fibroblasts. A few inflammatory cells are involved. An increased number of capillaries are noted, and a few extravasated red blood cells may be found
2	Moderate: predominantly characterized by more cells in the area than in the slight reaction. Increased number of capillaries and vessels are found
3	Severe: Marked cellular infiltration, including local abscess formation. Numerous blood vessels are found in the tissue surrounding the intense cellular infiltration
4	Abscess formation or extended lesions not localized only to the tissue beneath the cavity floor

Results

All subjects who entered the study completed the treatment according to the protocol (Fig. 2). No adverse events related to the treatment were reported.

Assessment of symptoms

Examination

At baseline, prior to the treatment, no teeth had any symptoms, i.e. no tooth was tender to percussion or apical palpation and all teeth showed normal mobility. Twelve weeks after the treatment two of the nine EMDgel-treated teeth showed mild tenderness to percussion.

Telephone interview

The results from the telephone interviews are shown in Table 3. No severe symptoms were reported. Moderate symptoms were reported 2 weeks after the treatment in one of nine calcium hydroxide-treated teeth and after 6 weeks in two other calcium hydroxide-treated teeth. Mild symptoms were reported in three of the nine EMDgel-treated teeth and in eight of the nine calcium hydroxide-treated teeth at different observation times (Table 3). One subject reported intake of analgesics 6 weeks after a tooth had been treated with calcium hydroxide.

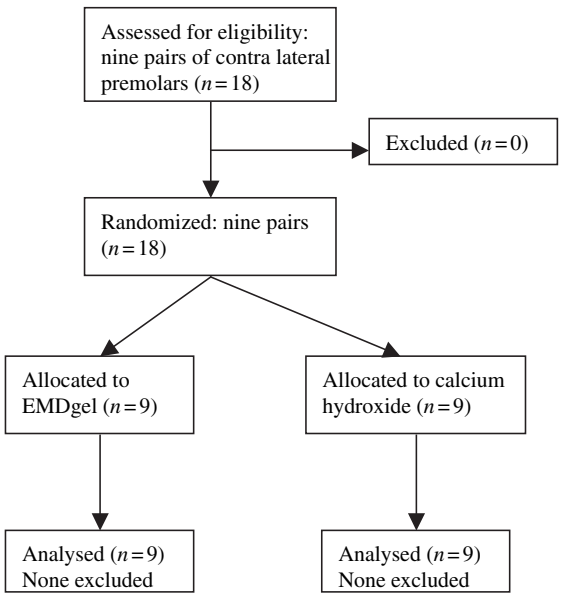

Figure 2 Participant flow.

Subject no.	EMDgel				Calcium hydroxide			
	Day 1	2 weeks	6 weeks	12 weeks	Day 1	2 weeks	6 weeks	12 weeks
1		○				○	○■	
2		○	○	○	○	○	○	○
3					○	○		
4a					○	○		
4b								
5						○		
6						○		
7						○	○■	○
8		○				○■	○	

Table 3 Mild symptoms (○) and moderate (■) in EMDgel- and calcium hydroxide-treated teeth reported at telephone interviews. No severe symptoms were reported

Diary

One diary was missing and another was incomplete, both regarding treatment with calcium hydroxide. Pain was recorded on several occasions in one tooth treated with calcium hydroxide. Another subject recorded taking analgesics during days 1–4 after the calcium hydroxide treatment, although he had not recorded any pain on the VAS. There were no recordings of pain or intake of analgesics in the teeth treated with EMDgel.

Evaluation of soft tissue reactions in the pulp

In the nine EMDgel-treated teeth, pulp tissue had proliferated into the space initially occupied by the gel between the pulp wound and the Teflon disc. In the proliferated pulp tissue, areas of moderate to severe inflammation were found. One tooth had an abscess in this area (subject no. 2). Below the pulpal wound area, five EMDgel-treated teeth exhibited no or mild inflammation, two exhibited moderate inflammation and two showed abscesses or extended lesions not localized only to the proliferated pulp tissue (Table 4).

In the nine calcium hydroxide-treated teeth, no proliferation of pulp tissue was observed. Eight calcium hydroxide-treated teeth exhibited no or mild inflammation in the pulp. One tooth showed total pulp necrosis (subject no. 7) (Table 4).

Evaluation of hard tissue formation in the pulp

In all but one EMDgel-treated teeth, the hard tissue was formed alongside the exposed dentine surfaces and in patches in the proliferated pulp tissue (Figs 3 and 4). In the EMDgel-treated teeth the hard tissue was not formed as a bridge, thus there are no measurements of the thickness in these teeth. In all calcium hydroxide-treated teeth the hard tissue was formed as a bridge covering the underlying pulpal tissue (Fig. 5). The amount of newly formed hard tissue was considerably larger in the EMDgel-treated teeth than in the calcium hydroxide-treated teeth (Table 5). The average thickness of the hard tissue bridge measured close to the dentine wall was 148 μm in the calcium hydroxide-treated teeth.

Table 4 Pulp inflammation classified from 0 (none) to 4 (abscess formation). The mean number of classifications from the representative central sections ($n = 5$) from each experimental tooth is given. The inflammation was judged above the exposure in the proliferated pulp tissue, directly below the exposure and in the centre of the pulp

Subject no.	EMDgel			Calcium hydroxide		
	Above	Below	Centre	Above	Below	Centre
1	3	0	0		0.2[a]	0[a]
2	4	4	0.6		0	0
3	3	1	0		0.6	0
4a	2	1	0		0.6	0
4b	3	0	0		0	0
5	3.5[a]	3.2[a]	0[a]		1	0
6	3	1.6	0		0	0
7	2	0.2	0		[b]	[b]
8		4	0		0	0

[a]There were difficulties in finding one representative area of the pulp, therefore the pulp was assessed in two different areas ($n = 10$).
[b]This tooth showed a total necrosis throughout the entire pulp.

Evaluation of the immunohistochemical staining

EMD was detected in the wound area in all EMDgel-treated teeth with newly formed hard tissue. The staining was observed in close proximity with the newly formed hard tissue alongside the exposed dentine surfaces and centrally in the patches of hard tissue in the proliferated pulp tissue (Fig. 6).

Discussion

This study on human material was designed as a precursor to a clinical trial. Because of the pilot study

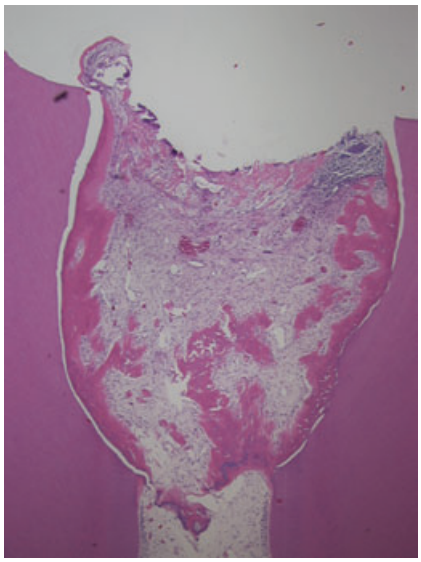

Figure 3 Micrograph of premolar 12 weeks after treatment with EMDgel (subject no. 1). The inflammation was classified as 'severe' in the proliferated pulp tissue and 'none' in the rest of the pulp tissue. Haematoxylin and eosin staining, original magnification ×88.

Figure 4 Micrograph of premolar 12 weeks after treatment with EMDgel (subject no. 4a). The inflammation was classified as 'moderate' in the proliferated pulp tissue, 'slight' directly below the exposure, and 'none' in the centre of the pulp. Haematoxylin and eosin staining, original magnification ×88.

design, the sample size consisting of eight subjects with nine pairs of premolars was limited. As the pulp tissue

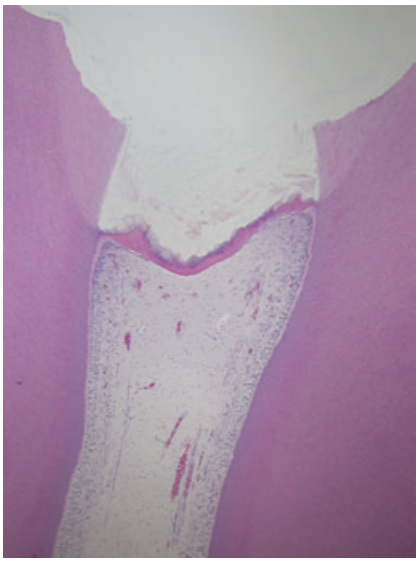

Figure 5 Micrograph of premolar 12 weeks after treatment with calcium hydroxide (subject no. 8). The inflammation was classified as 'none'. Haematoxylin and eosin staining, original magnification ×88.

Table 5 Area of newly formed hard tissue

	Area of newly formed hard tissue (μm^2)	
Subject no.	EMDgel	Calcium hydroxide
1	1 431 000	227 000[a]
2	13 000	188 000
3	677 000	326 000
4a	871 000	289 000
4b	467 000	288 000
5	146 000[a]	145 000
6	145 000	76 000
7	384 000	105 000[b]
8	114 000	126 000
Mean	472 000	197 000

[a]There were difficulties in finding one representative area of the pulp, therefore the pulp was assessed in two different areas ($n = 10$).
[b]This tooth showed total necrosis throughout the entire pulp.

reactions differed markedly in all EMDgel-treated teeth from the pulp tissue reactions in the control group, the observed reactions in the EMDgel-treated teeth may be considered representative in spite of the limited sample size.

The experimental pulp exposure was made as a superficial pulp amputation. This method was chosen to ensure that the odontoblastic layer was removed as the hypothesis was that EMDgel would promote differentiation of new odontoblasts with subsequent hard

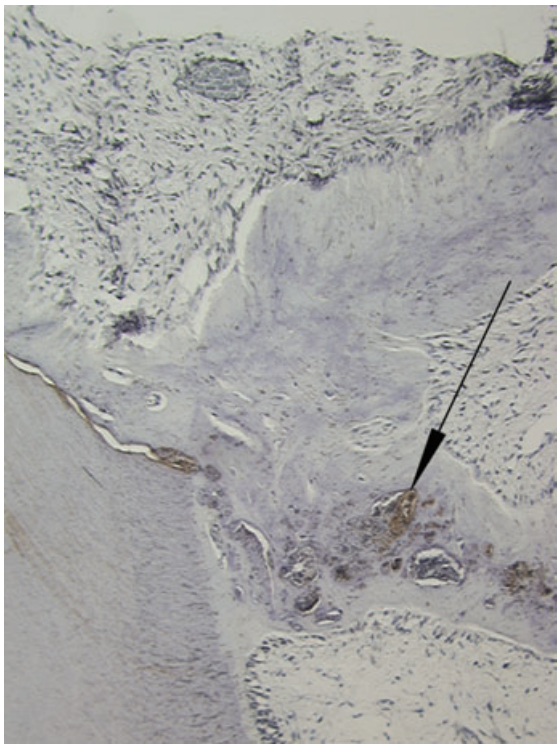

Figure 6 Micrograph of premolar 12 weeks after treatment with EMDgel (subject no. 4a). Immunohistochemical staining for EMD (arrow) in close contact with the newly formed hard tissue, original magnification ×100.

tissue formation. When evaluating new pulp capping materials calcium hydroxide is considered to be the control material of choice (Federation Dentaire International CoDM, Instruments, Equipment and Therapeutics 1980). Choosing such a control material makes it possible to evaluate the surgical method as the appearance in the histological sections treated with calcium hydroxide is quite characteristic when performed correctly. In this study one can argue that it would have been better to choose the PGA vehicle without EMD as a negative control to find evidence for the hard tissue promoting effect of EMD. In a previous study where the PGA vehicle was used as a negative control when performing pulp amputations in rat molars, hard tissue formation was observed in the test group as well as in the control group (Igarashi *et al.* 2003). However, the amount of hard tissue was larger in the group treated with EMD in a PGA vehicle than in that with PGA vehicle alone (Igarashi *et al.* 2003). The appearance of the pulp tissue reactions in the published sections from the animal models with EMD in a PGA vehicle was somewhat different from the pulp reactions seen in the present material. New hard tissue was not only formed in the wound area but also along the margins of the residual pulp, stressing the possible variance in hard tissue formation in animals and in humans (Nakamura *et al.* 2001, 2002, Igarashi *et al.* 2003, Ishizaki *et al.* 2003). The results from the immunohistochemical staining in the present study, where EMD was detected in close proximity to the newly formed hard tissue, supported the hypothesis that EMD promotes hard tissue formation in exposed human pulp.

The experimental model left a space between the pulp wound and the Teflon disc to be filled with the pulp capping material. This model allowed pulpal proliferation in the EMDgel-treated teeth as the gel does not form a solid material and thus leaves a space for the pulp to proliferate. Such pulp tissue proliferation may have been enhanced by the EMD (Lyngstadaas *et al.* 2001). Consequently the formulation for EMD with PGA as vehicle may not be suitable when performing pulp capping procedures. The use of a solid material as a vehicle would perhaps be more suitable for the formation of a hard tissue barrier.

The surgical procedure was performed blindly until the point when the test or control material was placed, i.e. when the randomization envelope was opened to reveal the treatment assignment for the tooth. As the nature of the two capping materials was totally different the operator knew what material was placed on the pulp wound. However, the semi-blinded operator never assessed the symptoms. These assessments were made by a blinded examiner who had no information about the materials used. The assessment of the histological sections was also performed blindly.

The inflammatory reaction seen in the EMDgel-treated teeth was more pronounced than in the calcium hydroxide-treated teeth. One explanation for this might be a microbial invasion from the oral environment through leakage along the margins of the temporary restorations (Balto 2002, Tewari & Tewari 2002, Zaia *et al.* 2002). Such microbial invasion would affect the EMD-treated pulps more than the calcium hydroxide-treated, as calcium hydroxide gives an unfavourable environment for microbial growth. Another explanation might be an increase in attachment and growth rate similar to that observed in human periodontal ligament cell cultures exposed to EMD. These cells showed increased intracellular cAMP signalling and autocrine production of TGF-β1, IL-6 and PDGF AB (Lyngstadaas *et al.* 2001). These growth factors are all known as mediators in processes such as healing, inflammation and tissue homeostasis and neogenesis. It

is possible that the observations in the cell culture are also applicable to the human pulp.

Postoperative symptoms were less frequent in the EMDgel-treated teeth than in those treated with calcium hydroxide. An explanation of the frequent early symptoms in the calcium hydroxide-treated teeth might be that the calcium hydroxide was applied with a gentle pressure against the pulp wound. This procedure might have traumatized the pulp tissue in addition to the known chemical burn effect that may possibly provoke symptoms (Schröder & Granath 1971). Although the calcium hydroxide-treated teeth frequently had mild to moderate symptoms, the pulps exhibited no or slight inflammation at 12 weeks postoperatively. The findings indicate that pulp capping procedures in young individuals with healthy pulps may be associated with mild or moderate symptoms shortly after the pulp capping procedure without the pulp having signs of persisting inflammation in a later stage. The clinical symptoms that were revealed at the examination prior to extraction in the EMDgel-treated teeth correlated well to the presence and degree of inflammation. The subject who reported intake of analgesic 6 weeks after treatment with calcium hydroxide exhibited necrosis in the major part of the pulp at the time of extraction. However, there was a hard tissue bridge in this tooth, indicating that the pulp necrosis developed some time after the pulp had responded with hard tissue formation.

Considerable amounts of hard tissue were formed in the pulp tissue after the capping with EMDgel. The hard tissue was formed alongside the exposed dentine surfaces and in patches in the proliferated pulp tissue. EMD is released from the PGA-gel and precipitates when the acidity is neutralized and the temperature is increased, e.g. by tissue fluids (Gestrelius *et al.* 1997a). The EMD precipitated on the exposed areas including the walls of the exposed dentine surfaces, as observed in the specimens immunohistochemically stained for EMD, supposedly giving the hard tissue appearance as seen in Figs 3 and 4. It is not unlikely that a longer observation time would have given a more favourable result with a hard tissue barrier. More investigations and further refinement of the vehicle are necessary in order to utilize the potential of EMD to stimulate a hard tissue barrier in the exposed human pulp.

Conclusions

Postoperative symptoms were less frequent in EMDgel-treated teeth and EMDgel induced greater amounts of hard tissue in a markedly different pattern compared with the teeth treated with calcium hydroxide. However, the operative procedure and the formulation with EMD in a PGA vehicle do not seem to be effective for the formation of a hard tissue barrier and as a consequence for dental pulp capping.

Acknowledgements

This study was supported by Biora AB, Malmö, Sweden, by the European Society of Endodontology and by the Swedish Dental Society.

References

Balto H (2002) An assessment of microbial coronal leakage of temporary filling materials in endodontically treated teeth. *Journal of Endodontics* **28**, 762–4.

Barthel CR, Rosenkranz B, Leuenberg A, Roulet JF (2000) Pulp capping of carious exposures: treatment outcome after 5 and 10 years: a retrospective study. *Journal of Endodontics* **26**, 525–8.

Cvek M (1978) A clinical report on partial pulpotomy and capping with calcium hydroxide in permanent incisors with complicated crown fracture. *Journal of Endodontics* **4**, 232–7.

Decup F, Six N, Palmier B, et al. (2000) Bone sialoprotein-induced reparative dentinogenesis in the pulp of rat's molar. *Clinical Oral Investigations* **4**, 110–9.

Federation Dentaire International CoDM, Instruments, Equipment and Therapeutics (1980) Recommended standard practices for biological evaluation of dental materials. *International Dental Journal* **30**, 140–88.

Gestrelius S, Andersson C, Johansson AC, et al. (1997a) Formulation of enamel matrix derivative for surface coating. Kinetics and cell colonization. *Journal of Clinical Periodontology* **24**, 678–84.

Gestrelius S, Andersson C, Lidström D, Hammarström L, Somerman M (1997b) In vitro studies on periodontal ligament cells and enamel matrix derivative. *Journal of Clinical Periodontology* **24**, 685–92.

Gestrelius S, Lyngstadaas SP, Hammarström L (2000) Emdogain-periodontal regeneration based on biomimicry. *Clinical Oral Investigations* **4**, 120–5.

Granath LE, Hagman G (1971) Experimental pulpotomy in human bicuspids with reference to cutting technique. *Acta Odontologica Scandinavica* **29**, 155–63.

Hørsted P, Søndergaard B, Thylstrup A, El Attar K, Fejerskov O (1985) A retrospective study of direct pulp capping with calcium hydroxide compounds. *Endodontics and Dental Traumatology* **1**, 29–34.

Hammarström L (1997) Enamel matrix, cementum development and regeneration. *Journal of Clinical Periodontology* **24**, 658–68.

Heijl L, Heden G, Svärdström G, Östgren A (1997) Enamel matrix derivative (EMDOGAIN) in the treatment of intrabony periodontal defects. *Journal of Clinical Periodontology* **24**, 705–14.

Heyeraas KJ, Sveen OB, Mjør IA (2001) Pulp-dentin biology in restorative dentistry. Part 3: Pulpal inflammation and its sequelae. *Quintessence International* **32**, 611–25.

Huskisson E (1983) Visual Analogue Scales. In: Melzack R, ed. *Pain Measurements and Assessment. New York: Raven Press, pp. 33–7.*

Igarashi R, Sahara T, Shimizu-Ishiura M, Sasaki T (2003) Porcine enamel matrix derivative enhances the formation of reparative dentine and dentine bridges during wound healing of amputated rat molars. *Journal of Electron Microscopy (Tokyo)* **52**, 227–36.

Inai T, Kukita T, Ohsaki Y, Nagata K, Kukita A, Kurisu K (1991) Immunohistochemical demonstration of amelogenin penetration toward the dental pulp in the early stages of ameloblast development in rat molar tooth germs. *The Anatomical Record* **229**, 259–70.

Ishizaki NT, Matsumoto K, Kimura Y, Wang X, Yamashita A (2003) Histopathological study of dental pulp tissue capped with enamel matrix derivative. *Journal of Endodontics* **29**, 176–9.

Lyngstadaas SP, Lundberg E, Ekdahl H, Andersson C, Gestrelius S (2001) Autocrine growth factors in human periodontal ligament cells cultured on enamel matrix derivative. *Journal of Clinical Periodontology* **28**, 181–8.

Mejàre I, Cvek M (1993) Partial pulpotomy in young permanent teeth with deep carious lesions. *Endodontics and Dental Traumatology* **9**, 238–42.

Nakamura Y, Hammarström L, Lundberg E, et al. (2001) Enamel matrix derivative promotes reparative process in the dental pulp. *Advances in Dental Research* **15**, 105–7.

Nakamura Y, Hammarström L, Matsumoto K, Lyngstadaas SP (2002) The induction of reparative dentine by enamel proteins. *International Endodontic Journal* **35**, 407–17.

Nakashima M (1994) Induction of dentin formation on canine amputated pulp by recombinant human bone morphogenetic proteins (BMP)-2 and -4. *Journal of Dental Research* **73**, 1515–22.

Nyborg H (1955) Healing processes in the pulp on capping. *Acta Odontologica Scandinavica* **13**, 1–130.

Papagerakis P, MacDougall M, Hotton D, Bailleul-Forestier I, Oboeuf M, Berdal A (2003) Expression of amelogenin in odontoblasts. *Bone* **32**, 228–40.

Rutherford RB, Wahle J, Tucker M, Rueger D, Charette M (1993) Induction of reparative dentine formation in monkeys by recombinant human osteogenic protein-1. *Archives of Oral Biology* **38**, 571–6.

Schröder U (1972) Evaluation of healing following experimental pulpotomy of intact human teeth and capping with calcium hydroxide. *Odontologisk Revy* **23**, 329–40.

Schröder U, Granath LE (1971) Early reaction of intact human teeth to calcium hydroxide following experimental pulpotomy and its significance to the development of hard tissue barrier. *Odontologisk Revy* **22**, 379–95.

Spahr A, Lyngstadaas SP, Slaby I, et al. (2002) Expression of amelin and trauma-induced dentin formation. *Clinical Oral Investigations* **6**, 51–7.

Tewari S, Tewari S (2002) Assessment of coronal microleakage in intermediately restored endodontic access cavities. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **93**, 716–9.

Zaia AA, Nakagawa R, De Quadros I, et al. (2002) An *in vitro* evaluation of four materials as barriers to coronal microleakage in root-filled teeth. *International Endodontic Journal* **35**, 729–34.