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Dentine sialoprotein and Collagen I expression after experimental pulp capping in humans using Emdogain®Gel

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Abstract

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Aim To characterize the hard tissue formed in human teeth experimentally pulp capped either with calcium hydroxide or with Emdogain®Gel (Biora AB, Malmö, Sweden) – a derivative of enamel matrix (EMD), using two markers for dentine; dentine sialoprotein (DSP) and type 1 collagen (Col I).

Methodology Affinity-purified rabbit anti-Col I and anti-DSP polyclonal antibodies were used to stain histological sections from nine pairs of contra-lateral premolars that had been experimentally pulp amputated and randomly capped with EMDgel or calcium hydroxide. Twelve weeks after the teeth had been pulp capped, they were extracted, fixed, demineralized and serially sectioned prior to immunohistochemical staining.

Results In the calcium hydroxide treated teeth DSP was seen in the new hard tissue which formed a bridge. DSP was also seen in the newly formed hard tissue in the EMDgel-treated teeth. Proliferated pulp tissue partly filled the space initially occupied by EMDgel and DSP-stained hard tissue was observed alongside exposed dentine surfaces as well as in isolated masses within the proliferated pulp tissue, although the new hard tissue did not cover the pulp exposure. DSP staining was also seen in the cells lining the hard tissue in both groups. Col I staining was seen in the newly formed hard tissue in both groups.

Conclusions The new hard tissue formed after pulp capping with EMDgel or calcium hydroxide contained DSP and Col I, considered to be markers for dentine. Thus, the newly formed hard tissue can be characterized as dentine rather than unspecific hard tissue.

Keywords: calcium hydroxide, collagen type I, dental pulp exposure, dentine, enamel matrix proteins, immunohistochemistry.

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Introduction

Formation of hard tissue after pulp capping has been observed in both clinical and experimental studies and the formation of a hard tissue barrier by activated odontoblasts has been considered a sign of healing (Olsson *et al.* 2006). Healing with functional dentine with an odontoblastic layer is probably needed to protect the pulp in cases when bacteria later enter the wound area due to micro-leakage along the restoration margins. The relatively high failure rates over time observed in long-term studies of pulp capping (Hørsted *et al.* 1985, Barthel *et al.* 2000) may, in part, be due to defective or malfunctioning hard tissue formation during the healing process. During dentinogenesis the dentine extracellular matrix consists of two distinct layers termed predentine and mineralized dentine. Both layers are synthesized and secreted by the odontoblasts, which are highly specialized cells found as a monolayer lining the entire dental papilla. In addition to their synthetic role, odontoblasts may be involved in

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regulating the host response to injury in a similar manner to epithelial cells (Dommisch *et al.* 2008, Goldberg *et al.* 2008).

Attempts have been made to mimic dentinogenesis using bioactive molecules such as amelogenin as pulp capping materials (Jegat *et al.* 2007). An amelogenin-rich fraction of porcine enamel matrix derivative, Emdogain®Gel (EMDgel) (Biora AB, Malmö, Sweden) has been used as a pulp capping material and newly formed hard tissue has been observed (Nakamura *et al.* 2002, Ishizaki *et al.* 2003, Olsson *et al.* 2005, Kiatwateeratana *et al.* 2009). In the study by Olsson *et al.* (2005) considerable amounts of hard tissue in the form of isolated, irregular masses were formed after 12 weeks in the teeth that had been superficially pulp amputated and capped with EMDgel whilst in the teeth capped with calcium hydroxide, the hard tissue was formed as a bridge.

In pulp capping studies it has been common to report the occurrence of, for instance, tubular structures within the newly formed hard tissue and then to refer to this as dentine or dentine bridges. Likewise, cells lining the hard tissue have been described as odontoblasts if they have been polarized or columnar. However, it has also been proposed that, in order to define dentine and odontoblasts properly, factors such as morphology, molecular content and, ideally, functions of the cell and tissue should be taken in account. Expression of a wide range of differentiation markers such as members of the SIBLING-family (Small Integrin-Binding LIgand N-linked Glycoprotein) has been suggested to characterize an odontoblast phenotype. Dentine sialoprotein (DSP) is a member of the SIBLING-family and, together with Collagen I (Col I), is suggested to be a relatively specific marker for dentine and late odontoblast differentiation (Butler *et al.* 1992, Narayanan *et al.* 2001, Nakashima *et al.* 2002). One study in humans has been published previously in which the hard tissue formed after pulp capping has been characterized with antibodies against DSP (Min *et al.* 2008). In this study, mineral trioxide aggregate (MTA) (ProRoot, Dentsply, Tulsa, OK, USA) was used as the pulp capping agent, and DSP was expressed in the cells lining the newly formed hard tissue. There are no reports of studies in humans in which the newly formed hard tissue has been characterized based on its content of DSP and Col I after pulp capping with EMDgel or with a paste of calcium hydroxide and saline. In order to see if using EMDgel and calcium hydroxide may be a way of stimulating production of hard tissue similar to dentine, this study aimed to characterize the hard tissue formed in human teeth, experimentally pulp capped with these agents, using two relatively specific markers for dentine; DSP and Col I.

Materials and methods

Characterization of hard tissue using DSP and Col I antibodies was performed on histological sections. The material consisted of 9 contra-lateral pairs of caries-free premolars from 8 patients, aged 12–16 years, scheduled for extraction for orthodontic reasons. The tooth was isolated with a rubber dam and a superficial pulp amputation was performed under aseptic conditions, removing approximately 2 mm of the pulp tissue. The test material Emdogain®Gel [EMD 30 mg mL^{-1} in propylene-glycol-alginate (PGA)] (Biora AB, Malmö, Sweden), was placed on the pulp wound. In the control cases, a paste of calcium hydroxide and saline was applied. A disc of Teflon was placed over the pulp capping material and the cavity was then filled with zinc oxide eugenol cement and restored with glass–ionomer (Ketac-fil; ESPE, Seefeld, Germany). Each subject received treatment with both test and control in a split mouth design after a randomization procedure. The patients made records of any symptoms and were interviewed about pain/discomfort by a blinded examiner. Twelve weeks after the operative procedure the teeth were extracted. The specimens were fixed in neutral buffered formaldehyde (4%) for 7 days, demineralized in EDTA (12.5%) and subsequently embedded in paraffin. After longitudinal serial sectioning (5 μm), every 5th section was stained with haematoxylin and eosin. The criteria for patient selection, operative procedure and sampling have been described in detail before in a previously published study that aimed to investigate the effect of EMDgel on experimentally exposed human pulps and to register postoperative symptoms (Olsson *et al.* 2005).

Immunohistochemical staining

Two central sections (5 μm) from each representative area from the formalin-fixed, paraffin-embedded teeth were dewaxed and rehydrated. In two teeth it was difficult to find one area representing the pulpal status and two different areas were chosen. Thus, together a total of 40 sections from 18 teeth were used for immunohistochemical staining. 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). Non-specific 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 manufacturers instructions (Dako, Glostrup, Denmark). For immunohistochemical localization, sections were then incubated with polyclonal rabbit anti-DSP (a kind gift from Dr. Qin, Department of Endodontics, University of Texas-Houston Health Science Center Dental Branch, Houston, TX, USA), or with polyclonal rabbit anti-Col I antiserum (Nordic Biosite AB, Täby, Sweden), overnight at 4 °C, followed by the StreptABComplex/HRP Duet kit (Dako, Glostrup, Denmark). The anti-DSP and the anti-Col I antisera were diluted 1 : 100 and 1 : 500, respectively, in TBS. The anti-DSP antibody has been described before (Butler *et al.* 1992). Antibody binding was visualized using DAB (0.6 mg mL^{-1}) in TBS containing 0.03% (v/v) hydrogen peroxide for 15 min and sections were counterstained with haematoxylin.

Control sections were treated identically to the test sections except that the primary, or the primary and secondary antibodies, were substituted with TBS only. The positive controls in each section were areas of dentine and predentine in other parts of the tooth. Two teeth from another age-matched patient, not included in the study, were amputated and then immediately extracted without placement of a pulp capping material. These teeth were also used as controls and the sections stained as above.

Two calibrated observers individually evaluated the reactivity of each antibody using a light microscope (Leitz Aristoplan, Leica, Gothenburg, Sweden). In cases of disagreement consensus was reached by discussion. At the moment of observation, the observers knew if the sections had been stained for DSP or Col I, but the sections were coded in a blind manner regarding the pulp capping material (EMDgel or calcium hydroxide). The degree of inflammation has formerly been classified according to criteria modified after Heyeraas *et al.* (2001) from studies of nearby sections stained with hematoxylin and eosin. These former recordings and haematoxylin and eosin stained sections were available to the observers during the evaluation of the immunohistochemically stained sections.

Staining of microorganisms

In order to investigate the possible presence of microorganisms in the wound area, a modified Brown and Brenn technique (Churukian & Schenk 1982) was used to stain adjacent sections and these were evaluated in a light microscope (Leitz Aristoplan, Leica, Gothenburg, Sweden).

Results

Pulp capping with calcium hydroxide

In the teeth where calcium hydroxide was used as the pulp capping agent, new hard tissue was formed as a bridge covering the pulp exposure and no proliferation of the pulp tissue into the excavation cavity was seen (Fig. 1a). The hard tissue bridge was continuous with the predentine and odontoblast layer in the pulp and was on average, 150 μm thick. However, in some teeth, a wedge was observed at the junction between the dentine wall and the bridge, where the thickness was considerably less than in the central part of the bridge (Fig. 1b). A layer of columnar cells was present in the pulp tissue adjacent to the hard tissue bridge (Fig. 1b).

Staining with the DSP-specific antibody revealed the protein both in the primary dentine and the predentine of all the calcium hydroxide treated teeth, although staining was more marked in the predentine (Table 1). In each section, the presence of staining in the primary dentine and predentine acted as a positive control and the intensity was used as a reference standard for evaluation of the amount of staining in the newly formed hard tissue. Patchy areas of staining, of a similar intensity to that in the predentine were seen in the newly formed hard tissue bridge in all the teeth pulp capped with calcium hydroxide (Table 1, Fig. 1c). In addition, more intense DSP staining was seen in the layer of cells lining the hard tissue bridge which most likely correspond to hard tissue producing cells. Elongated structures with similarities to dentine tubules were seen extending into the hard tissue in the vicinity of these cells (Fig. 1c). The Col I specific antibody showed diffuse staining throughout the whole newly formed hard tissue bridge as well as in the cells lining it (Fig. 1d). Staining was also seen in the extracellular matrix within the pulp tissue. A similar pattern was seen in all teeth where observations were possible (Table 1).

Pulp capping with EMDgel

In all the EMDgel-treated teeth, pulp tissue proliferated to partly fill the space initially occupied by the gel. Hard tissue had formed as isolated masses in the proliferated pulp tissue as well as alongside the exposed primary dentine surfaces, although the total amount of hard

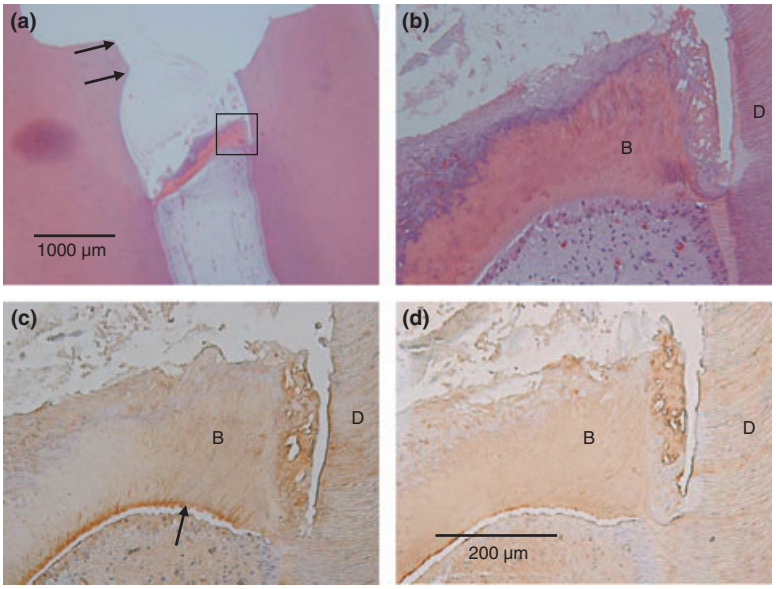

Figure 1 Staining of a calcium hydroxide treated tooth 12 weeks postoperatively. (a) The lower arrow points to the level of the pulp amputation whereas the upper arrow points to where the Teflon disc was placed. The area between the pulp and the Teflon disc was filled with calcium hydroxide. (b) Detail of (a) at a higher magnification (100×) showing the new hard tissue formed as a bridge (B) covering the pulp exposure. Haematoxylin and eosin staining. DSP staining (brown) (c) and Col I staining (d) was detected in the dentine (D) as well as in the bridge (B).

Table 1 Evaluation of the staining of each antibody (DSP or Col I) in the EMDgel or calcium hydroxide treated teeth. In two teeth, one treated with EMDgel and one with calcium hydroxide, it was difficult to find one section representing the pulpal status and two different sections were chosen. In all; a total of 40 sections from 18 teeth were used for immunohistochemical staining. The primary dentine in each section was used as reference standard (= moderate). The table presents number of sections with moderate or strong staining of total number of sections in which recordings were possible

	EMDgel (n = 10)	Calcium hydroxide (n = 10)
Moderate to strong DSP staining within		
The newly formed hard tissue	10/10	10/10
Areas corresponding to predentine	8/8[a]	9/9[b]
Cells lining the newly formed hard tissue	9/9 [c]	9/9[b]
Moderate to strong Col I staining within		
The newly formed hard tissue	10/10	10/10
Areas corresponding to predentine	8/8[a]	10/10
Cells lining the newly formed hard tissue	5/7[d]	6/6[e]

[a] In 2 of the 10 sections, no areas corresponding to predentine could be observed.
[b] One tooth showed total necrosis, though a hard tissue bridge could be observed.
[c] In one section, no cells lining the newly formed hard tissue could be observed.
[d] In 3 of the 10 sections, no cells lining the newly formed hard tissue could be observed.
[e] In 4 of the 10 sections, no cells lining the newly formed hard tissue could be observed. One tooth showed total necrosis, though a hard tissue bridge could be observed.

tissue varied considerably between different teeth (Figs 2a and 3a). The hard tissue formed alongside exposed dentine surfaces did not appear to be attached to the dentine walls in the space initially occupied by the gel, but the areas corresponding to the predentine in the newly formed hard tissue were nevertheless continuous with the predentine and odontoblastic layer in the pulp (Figs 2a and 3a).

As for the calcium hydroxide treated teeth, staining with the DSP-specific antibody showed the protein to be present in both the primary dentine and the predentine of all the EMD-treated teeth, although staining was again more marked in the predentine (Figs 1c and 2c). DSP was also present in diffuse areas in the newly formed hard tissue within the proliferated pulp and alongside the exposed dentine surfaces in all nine teeth examined (Table 1). In eight of the nine teeth, the isolated hard tissue masses within the proliferated pulp tissue were surrounded by numerous cells stained with DSP, most likely corresponding to hard tissue producing cells (Figs 1c and 2c). The elongated appearance of these cells differed from the cells present in the pulp below the amputation site which had an appearance consistent with that normally seen in pulp tissue from young people. Moderate to strong Col I-staining was seen in both the dentine and predentine of the EMD-treated teeth and, as for DSP, staining was stronger in the predentine layer (Table 1, Figs 1d and 2d). Again, Col I was present in the hard tissue masses in the proliferated pulp tissue and in the cells lining it, as well

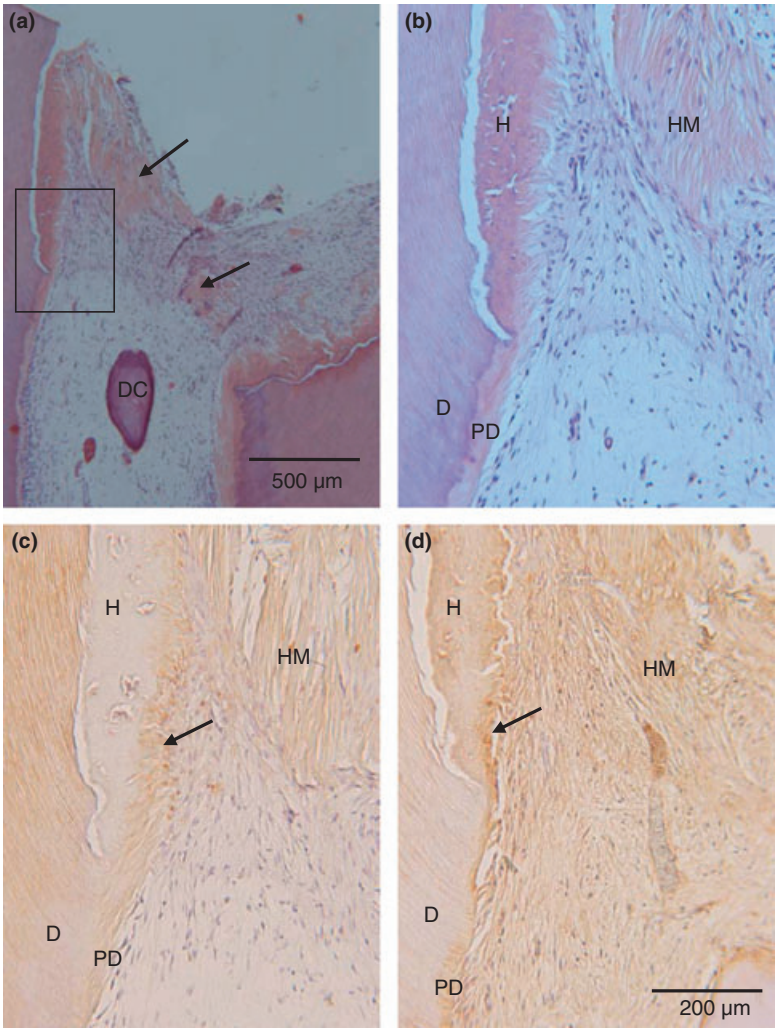

Figure 2 Staining of an EMDgel-treated tooth 12 weeks postoperatively. (a) The pulp has proliferated into the space initially occupied by the gel. New hard tissue is observed alongside the exposed dentine surfaces and in isolated masses (arrows) within the proliferated pulp tissue. A dentine chip (DC) is seen in the central part of the pulp. The inflammation was classified as severe (marked cellular infiltration including local abscess formation). Haematoxylin and eosin staining, original magnification 40×. (b) Detail of (a) at a higher magnification (100×) showing the newly formed hard tissue alongside the exposed dentine (H) and in isolated masses (HM). The dentine (D) and predentin (PD) below the pulp amputation level were used as positive controls and reference standard. (c) DSP staining (brown) is seen in the dentine (D) and predentine (PD) as well as in the newly formed hard tissue. The staining is marked in the newly formed hard tissue lining the exposed dentine in areas corresponding to predentine and odontoblasts (arrow). (d) Strong Col I staining (brown) was detected in the dentine and newly formed hard tissue, especially in the areas corresponding to predentine (arrow).

as in the pulp tissue, in five of the seven teeth where such cells were observed (Table 1).

Thus, in both the calcium hydroxide and EMDgel-treated teeth, the newly formed hard tissue had the characteristics of dentine regarding its content of DSP and Col I. In all the teeth capped with calcium hydroxide, this tissue was formed as a bridge covering the pulp exposure whereas in all the EMDgel-treated teeth, islands of hard tissue were formed within the proliferated pulp tissue. The histological appearance of the two groups was thus so different that despite the small sample size (9 teeth in each group) the observed patterns can be considered to be representative for the respective treatment methods.

The majority of the calcium hydroxide treated teeth showed no or only mild inflammation after 12 weeks, as judged by the occurrence of inflammatory cells and fibroblasts present in the pulp tissue beneath the hard tissue bridge (Fig. 1b). One tooth, however, had signs of total pulp necrosis even though a hard tissue bridge was present. In the EMDgel-treated teeth areas with moderate (increased number of fibroblasts, some inflammatory cells and increased number of capillaries) – to – severe (marked cellular infiltration including local abscess formation) inflammation could be observed in the proliferated pulp tissue in some of these teeth (Figs 2a,b and 3a,b). In one tooth an abscess was also observed. However, even in the teeth where the haematoxylin and eosin-stained sections showed moderate-to-severe inflammation, the newly formed hard tissue and the staining patterns seen for DSP and Col I were similar to those with mild or no

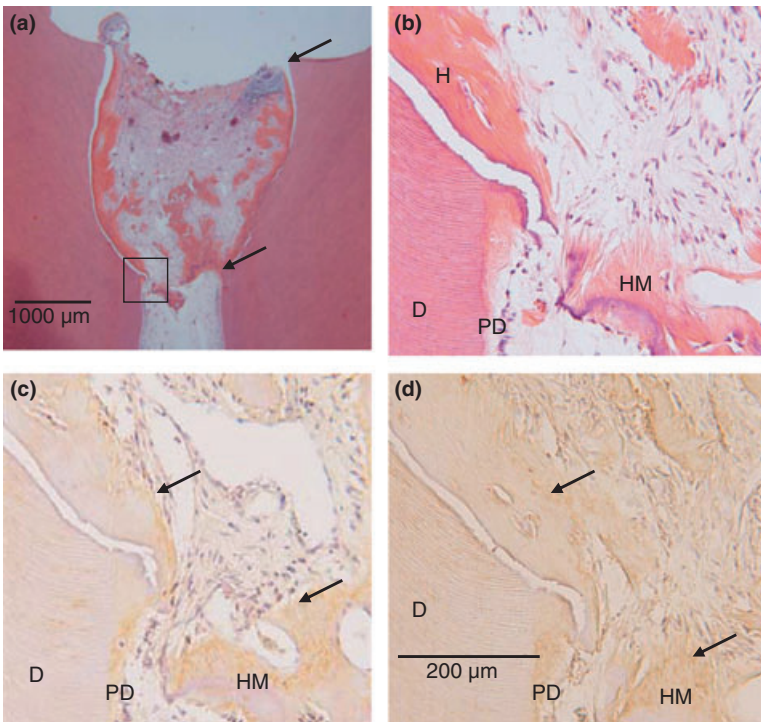

Figure 3 Staining of an EMDgel-treated tooth 12 weeks postoperatively. (a) The pulp has proliferated into the space initially occupied by the gel. New hard tissue is observed alongside the exposed dentine surfaces and in isolated masses within the proliferated pulp tissue. The lower arrow points to the level of the pulp amputation whereas the upper arrow points to where the Teflon disc was placed. The area between the pulp and the Teflon disc was filled with EMDgel. The inflammation was classified as moderate – (increased number of fibroblasts, some inflammatory cells and increased number of capillaries. (b) Detail of (a) at a higher magnification (100×) showing new hard tissue alongside the exposed dentine surfaces (H) and in masses (HM) in the proliferated pulp tissue. Haematoxylin and eosin staining. (c) DSP staining (brown) is seen in the dentine (D), predentine (PD) as well as in the newly formed hard tissue, especially in areas corresponding to predentine (arrows). (d) Col I staining (brown) was detected in the dentine and newly formed hard tissue, especially in the areas corresponding to predentine (arrows).

inflammation. Postoperative symptoms were reported more often in the calcium hydroxide treated teeth than in the teeth treated with EMDgel (Olsson *et al.* 2005).

Immunohistochemical controls and staining of microorganisms

DSP and Col I staining was also observed in the primary dentine and predentine of the two positive control teeth that were extracted immediately after performing the pulp amputation (data not shown). No immunoreactivity was observed in the negative control sections (Fig. 4). No bacteria could be detected in the wound area or alongside the dentine walls, after staining of sections with the modified Brown and Brenn technique and thus no association could be made between the presence of bacteria and the degree of inflammation.

Discussion

The aim of pulp capping is that following the operative procedure, the pulp exposure should heal through the formation of reparative dentine which is capable of acting as a new barrier to protect the pulp from the oral environment. This process requires the recruitment and differentiation of odontoblast-like cells and synthesis of new dentine-like hard tissue. DSP, a member of the SIBLING-family, and Col I, are considered to be relatively specific markers for dentine and odontoblasts. Although, members of the SIBLING-family are also expressed to a certain extent in bone and periodontium (Butler & Ritchie 1995, Qin *et al.* 2002, Baba *et al.* 2004) there is a consensus amongst researchers dealing with formation of dentine that SIBLING proteins, such as DSP, in combination with Col I are

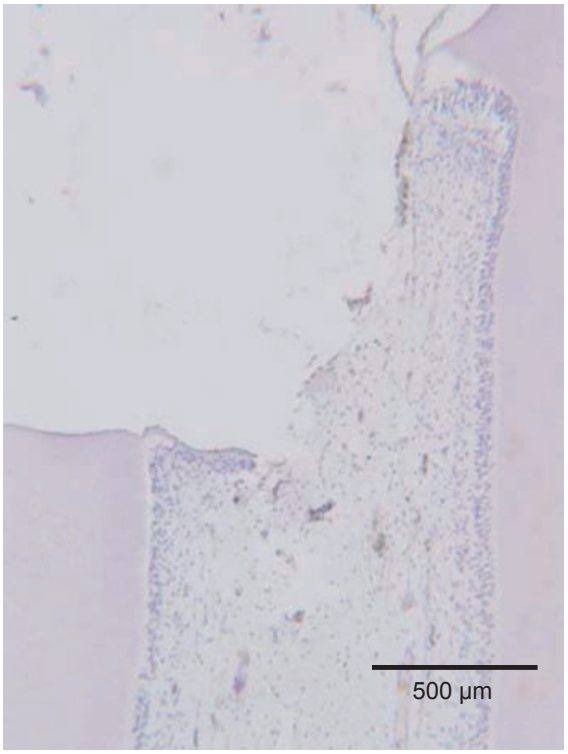

Figure 4 Negative control section that had been treated identically to the test sections except that the primary and secondary antibodies were substituted with TBS only. No immunoreactivity was observed in the dentine or predentine. The tooth was from an age-matched patient, not included in the study, which had been amputated and then immediately extracted without placement of a pulp capping material.

suitable phenotypic markers for this tissue (Chen *et al.* 2008, Hao *et al.* 2009. In the present study, DSP staining was seen in the newly formed tissue corresponding to predentine and dentine in both the calcium hydroxide and EMDgel treated teeth. This staining was more intense in the predentine than in the dentine, possibly due to the higher level of mineralization and lower protein content within the dentine. A similar staining pattern was seen for Col I, the dominant structural protein in mineralized tissues. Thus, using the two most widely used markers for dentine, the tissue formed in both the calcium hydroxide and EMDgel treated teeth can be characterized as dentine. Recently, DSP has also been detected in hard tissue formed after pulp capping in humans where MTA was used as a capping material with Dycal (Dentsply Caulk, Milford, DE, USA) as a control (Min *et al.* 2008). In a study similar to the present one carried out in miniature swine, DSP and Col I were used to characterize dentine after EMDgel or Dycal were applied to experimental pulp exposures (Nakamura *et al.* 2004). Staining was detected in the newly formed hard tissue in both groups. However, the histological findings were totally different from those obtained in the present study in that the newly formed hard tissue formed after placing the calcium hydroxide cement seemed to be less effective in bridging the wounds than EMDgel. This may be due to the different species used or differences in the performance of the pulp capping procedure.

Although in the present study the tissue formed in the calcium hydroxide and EMD treated teeth can be considered to be similar in that it contained dentine proteins, the morphology of the new hard tissue deposited in the wound area in response to the pulp capping materials differed considerably. In the teeth capped with calcium hydroxide, the hard tissue was formed as a continuous bridge covering the exposed pulp whereas in the EMD teeth, hard tissue formation was initiated as islands within the pulp tissue which proliferated into the area initially occupied by the EMD gel. This difference may, in part, be due to the formulation with EMD in a PGA-vehicle since this material is rather voluminous and does not leave a solid base covering the pulp amputation as does calcium hydroxide or MTA. Thus, our findings suggest that after 12 weeks, the bridge-like structure of the hard tissue formed in the calcium hydroxide treated teeth may be capable of providing a structural barrier for protection of the pulp whereas this would be unlikely for the hard tissue islands formed in response to EMDgel. Closure of the entire pulp exposure in response to EMD has been observed in a previous study performed in miniature swine (Nakamura *et al.* 2002) and it can be speculated that this would have been the case in humans if a longer observation period were used. However, Kiatwateeratana *et al.* (2009) have recently performed a study in humans using EMDgel with a longer observation period than in the present study. This showed that the pattern of the hard tissue formation was similar to that seen here and even after 6 months the hard tissue masses did not close the pulp exposure. Thus, from a structural point of view, the hard tissue formed after exposure to calcium hydroxide would appear to have a better potential to provide protection for the exposed pulp tissue than that formed in response to EMDgel treatment.

In addition to their role in the synthesis of hard tissue, odontoblasts have been proposed to play a role in host defence through the expression of pro-inflammatory mediators in response to bacterial exposure (Dommisch

et al. 2007, 2008, Goldberg *et al.* 2008). Since, in the long term it is probable that bacteria may reach the pulp capping site due to micro-leakage along the restoration margins or secondary carious lesions it appears to be important for the barrier function that the newly differentiated odontoblast-like cells lining the hard tissue respond in the same way as primary odontoblasts. One reason for late failures observed in clinical studies after pulp capping (Hørsted *et al.* 1985, Barthel *et al.* 2000) may be that the odontoblast-like cells do not respond to bacterial insults with formation of reactionary dentine and triggering of the immunologic and inflammatory responses as seen with primary odontoblasts. Thus, it seems important that the hard tissue formed after pulp capping not only has the structural characteristics of dentine, but also the functional properties of primary dentine. In this study, odontoblast-like cells expressing DSP were seen in areas corresponding to predentine after capping with calcium hydroxide or EMDgel. In the calcium hydroxide treated teeth these were arranged as a layer associated with the hard tissue bridge and in this regard they seem to have a similar morphology to primary odontoblasts. In the EMDgel-treated teeth, odontoblasts surrounded the discrete hard tissue masses and thus even if the cells themselves are capable of initiating a response to the presence of microorganisms, the separate hard tissue masses did not form a closure of the pulp exposure and thus did not meet the demands placed on functional reparative dentine.

After the 12-week study, the level of inflammation seen in the EMDgel-treated teeth was greater than in the calcium hydroxide treated ones. Since only teeth free from caries were included in the study, at the time of the pulp capping procedure all pulps must be considered to have been free from inflammation. Thus, the response could be due to the pulp capping materials themselves or oral microorganisms that reached the wound area through micro-leakage along the restoration margins. Eugenol may cause an inflammatory reaction when placed in direct contact with pulp tissue (Cowan 1966) and in previous studies of pulp capping with EMD, inflammation has been described as resulting from eugenol in the material used to seal the amputation site. In the present study a Teflon disc was placed over the pulp capping material in order to avoid contact with the sealing material, although it cannot be ruled out that some of this material could have caused the inflammation observed in some of these teeth. In an attempt to study if the presence of microorganisms could be linked to the inflammation, a modified Brown and Brenn staining method was used. In spite of the fact that some of the sections showed areas of necrotic pulp, in which one would have expected to detect bacteria with the technique used here, no bacteria were seen. The reason for this is not known but it cannot be ruled out that bacteria have been removed during the processing of the sections (Wijnbergen & van Mullem 1991). Thus, no direct cause of the inflammation could be established in this study. Although it is probable that pulp inflammation would interfere with the formation of hard tissue, in the present material the amount of newly formed hard tissue stained with DSP and Col I could not be related to the degree of inflammation in the adjacent parts of the pulp. This unexpected finding is in contrast to other authors such as Kiatwateeratana *et al.* (2009) who found less hard tissue formation in EMDgel-treated teeth that exhibited severe pulp inflammation. A possible explanation for the fact that newly formed hard tissue and signs of inflammation was observed in the same sections was that hard tissue formation was initiated prior to the onset of the inflammation, and that the inflammation was a response to late invasion of microorganisms into the wound area.

Conclusions

After pulp capping with EMDgel and calcium hydroxide, pulpal cells are capable of differentiating and producing new hard tissue that contains DSP and Col I although the hard tissue formed in the presence of EMDgel appeared to have a lower potential for protection of the pulp tissue as the hard tissue did not cover the pulp exposure. Further studies are required to determine how the presence of bacteria in the wound affects the hard tissue formation process as well as to verify that the newly formed hard tissue and odontoblast-like cells have the same functions as seen in primary dentine and odontoblasts, such as an ability to respond to bacterial insult.

Acknowledgements

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