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Effects of a new antibacterial adhesive on the repair capacity of the pulp-dentine complex in infected teeth

D. Tziafas[1], E. Koliniotou-Koumpia[2], C. Tziafa[1] & S. Papadimitriou[3]

Departments of [1]Endodontology and [2]Operative Dentistry, School of Dentistry, Aristotle University of Thessaloniki, Thessaloniki; and [3]Department of Clinical Sciences, Veterinary School, University of Thessalia, Karditsa, Greece

Abstract

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Aim To evaluate the effects of a self-etching/priming adhesive system, containing the antibacterial monomer 12-methacryloyloxy-dodecylpyridinium bromide (MDPB), on the repair capacity of the pulp-dentine complex in infected cavities in dog's teeth.

Methodology Class V cavities with a residual dentine thickness ranging from 0.3–0.8 mm were prepared on the buccal surface of permanent teeth in four dogs. Pulpal exposures were performed in half of the cavities. Millipore filters that had been incubated for 3 h in a 10^5 milky suspension of a-streptococci were placed in the cavities, which were then filled temporarily. After 24 h, the filters were removed and both the exposed and non-exposed cavities were washed with sterile saline and assigned to four groups which were treated with either the experimental antibacterial adhesive system, or Clearfil SE bond, Dycal and Teflon discs. Stereotype connective tissue reactions (inflammatory cell response and/or tissue necrosis) and pulp-specific reparative tissue responses (reduction of odontoblasts and tertiary dentine formation) were assessed at post-operative periods of 4 and 8 weeks.

Results Neither severe inflammation nor tissue necrosis was observed, either in the dentinal cavities or pulpal exposures treated with the self-etch adhesive containing MDPB. Rates of tertiary dentine formation in infected dentinal cavities treated with this system were comparable with those observed after dentine treatment with the $Ca(OH)_2$-based material. Dentinal bridging was not seen in pulpal exposures treated with the experimental adhesive.

Conclusions The new antibacterial adhesive system maintained pulp vitality and primary odontoblastic function in infected nonexposed and exposed cavities but interfered with reparative dentine formation in infected pulpal exposures.

Keywords: adhesive systems, antibacterial agents, dentine-pulp complex, pulp repair, tertiary dentine, vital pulp therapy.

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Introduction

It has long been recognized that the presence of bacteria along the cavity walls or within the dentinal tubules may be considered as the critical determinant in pulpal inflammatory responses after restorative procedures (Bergenholtz *et al.* 1982). Thus, residual caries, following cavity preparation, or secondary bacterial invasion can potentially lead to restoration failure (Bergenholtz 2000). It therefore seems reasonable to suggest that restoration longevity might be improved by using restorative materials with antibacterial properties. Adhesive systems containing experimental molecules with bactericidal activities have been investigated.

The monomer 12-methacryloyloxy-dodecylpyridinium bromide (MDPB) provides bacteriostatic proper-

Correspondence: Dimitrios Tziafas, Department of Endodontology, School of Dentistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece (Tel.: 3023 109 99459, 3023 109 99626; fax: 3023 109 99626; e-mail: dtziaf@dent.auth.gr).

ties that act as an inhibitor of bacterial contacts (Imazato *et al.* 1994). Incorporation of this antibacterial agent into a dentine adhesive system resulted in strong antibacterial activity against oral streptococci *ex vivo* (Imazato *et al.* 1995, 1997, 1998). The MDPB-containing dentine adhesive system did not have a significant influence on the cytotoxicity observed when the cured primer had been tested on human pulpal cells *ex vivo* (Imazato *et al.* 1999, 2000, Schmalz *et al.* 2004). The pulpal responses towards the antibacterial dentine adhesive system have been further evaluated histopathologically *in vivo* (Imazato *et al.* 2004); results indicated that the MDPB-containing primer exhibited *in vivo* antibacterial effects, whilst little or no inflammation was observed. Data on the repair capacity of the pulp-dentine complex, as indicated by post-operative dentinogenic activity, after treatment with the new antibacterial adhesive system do not exist.

The aim of the present study was therefore to evaluate pulpal responses, with a particular focus on tertiary dentine formation, after placement of the novel antibacterial adhesive system in infected nonexposed cavities and pulpal exposures in dog's teeth.

Materials and methods

Four healthy dogs, all 2 years of age, with intact dentitions were used. The experimental study was carried out in accordance with the Ethical Guidelines laid down by the Research Committee of the Aristotle University of Thessaloniki (European Communities Directive of 24 November 1986–86/609/EEC), for the care of animals in experimental procedures and approved by the Ethical Committee of the School of Dentistry, Aristotle University of Thessaloniki, Greece. All measures were taken to minimize pain or discomfort of the animals.

Each animal was sedated with an intramuscular injection of 1 mg kg^{-1} xylazine. General anaesthesia was induced with an intramuscular injection of 6 mg kg^{-1} theopentone. Before the beginning of all experimental procedures the trachea was intubated and general anaesthesia was maintained using halothane (1.5–2.5%) in oxygen, delivered through a semi-closed breathing circuit.

Experimental procedures

Permanent first and second molars, second and third premolars, canines and third incisors of both jaws were selected. All teeth were scaled and polished with a rubber cup on the day of the operative procedure. Teeth were isolated with rubber dam and cleaned with an iodine solution (5%), whilst saliva was controlled with high-speed evacuation.

Eighty-eight Class V cavities (approximately 3.00 mm wide, 3.00 mm long, 1.5–2.0 mm deep) were prepared on the buccal surface of teeth using a tungsten carbide pear-shaped bur (ISO, no. 330 L SS; White, Lakewood, NJ, USA) at ultra-high speed with copious water spray. The active tip of the bur was limited to 1.4 mm. A new bur was employed on every fourth cavity to avoid excessive heating. The preparations were cut 0.5–1 mm above the free gingiva, parallel to the cemento-enamel junction (CEJ). Cavities were exposed to sterile saline and excess moisture was removed with sterile cotton pellets.

In 44 cavities (in canines, first and second molars) pulpal exposures were further performed in the middle of the cavity floor using a round carbide bur 0.8 mm in diameter (ISO no. 1; Shofu Inc., Kyoto, Japan) at high speed and under water cooling. A new bur was used for each tooth. The pulp exposures produced were approximately the same size (0.8–1.0 mm). The cavities were washed with sterile saline and dried with cotton pellets, light pressure was applied to control haemorrhage.

Sterile Millipore filters that had been incubated for 3 h, in a 10^5 cells mL^{-1} of milky suspension of haemolytic Streptococci Viridans (clinical sp.), obtained from a positive human root canal culture and grown in blood agar, were placed in contact with the floor of exposed and nonexposed cavities, which were further filled with the temporary filling material Cavit G (3M ESPE AG, Seefeld, Germany).

After 24 h, the fillings and Millipore filters were removed and cavities were washed repeatedly with sterile saline. The cavities were randomly assigned to four groups (three experimental and one control) of either nonexposed or exposed cavities and treated as follows

1. Fourteen nonexposed and 14 exposed cavities with the MDPB-containing antibacterial adhesive system (ABF; Kuraray Medical Inc., Okayama, Japan).

2. Fourteen nonexposed and 14 exposed cavities with the Clearfil SE Bond (Kuraray Medical Inc., Okayama, Japan).

3. Eight nonexposed and eight exposed cavities with the $Ca(OH)_2$-based material Dycal (Caulk Lab, Milford, DE, USA) and

4. Eight nonexposed and eight exposed cavities with Teflon discs.

Cavities were restored with Clearfil APX (Kuraray Medical Inc.). Cavities in which Teflon discs had been placed were filled with amalgam. In all cases, the manufacturers' instructions for adhesive and restorative procedures were followed strictly. The materials were cured with a visible light source (Astralis 5 Vivadent Ets, Bendererstrasse2; Schaan, Liechtenstein) in accordance with the manufacturers' recommended times.

The pulpal tissue responses were assessed at post-operative periods of 4 and 8 weeks. At the termination of the experimental periods, the animals were sacrificed by using an overdose of pentobarbital sodium, the teeth were extracted, and their roots immediately sectioned at the apical third of the root. Teeth were fixed in 10% neutral-buffered formalin solution for 2 weeks and demineralized using Morse's solution (50% formic acid + 20% sodium citrate) for 2 months. Finally, teeth were embedded in paraffin and serially sectioned at 7 μm thickness. All sections coming through the cavity floor or pulp exposure site were stained either with Mayer's hematoxylin–eosin stain or using modified Brown-Brenn's technique.

Histological assessment

The stereotypic connective tissue reactions and the pulp-specific reparative tissue response to the combined effect of cavity preparation, infection and restoration were evaluated according to the following criteria:

Inflammatory cell response
Inflammatory cell infiltration of the pulp tissue was classified as: *none*, absence of inflammatory cells; *slight*, a few scattered inflammatory cells; *moderate/severe*, the presence of masses of inflammatory cells in the coronal pulp or abscess formation.

Tissue disorganization
Disorganization of pulp tissue was classified as: *no*, physiological appearance of the pulp-dentine interface and central pulp tissue; *slight disorganization*, a reduction in cells in the odontoblastic layer beneath the cavities, but central pulp normal (in nonexposed teeth); *partial necrosis*, disorganized odontoblast layer and tissue parenchyme in at least half of the coronal pulp; *total necrosis*, pulp tissue necrosis in the coronal pulp.

Tertiary dentine formation
The presence of tertiary dentine was evaluated as: *no*, unchanged morphology of dentine–predentine–odontoblast layer in nonexposed cavities or the absence of a continuous zone of post-operatively formed tertiary dentine matrix; *yes*, the presence of a continuous zone of post-operatively formed tertiary dentine matrix beneath the axial wall in nonexposed cavities or bridging the exposure site in exposed teeth. The presence of a calcio-traumatic line distinguished tertiary dentine from the remaining circumpuplal dentine.

Presence of bacteria
Presence of stained bacteria in the pulp space or along the cavity walls/within the cut dentinal tubules was characterized as *pulp* or *dentine positive* bacterial detection respectively.

All stained sections were evaluated and the remaining dentine thickness was measured between the cavity floor and the line of interface between pre-operative circumpulpal dentine and post-operatively formed tertiary dentine. The minimum remaining dentine thickness was estimated for every specimen. The 20 adjacent sections were analysed twice on blind basis by two independent observers. Inter-observation variation was only noticed in scoring tissue disorganization of nonexposed teeth. In these cases the severe score was finally recorded.

Ordinal data were statistically analysed by the Kruskal–Wallis and the Mann–Whitney *U*-tests, whilst binary data were analysed by the use of Fisher's exact test.

Results

Nonexposed cavities

The minimum remaining dentine thickness was compared amongst the four groups. In Table 1 the

Table 1 Nonexposed cavities. Mean value of the minimum remaining dentine thickness (RDT) and standard deviation (SD) in specimens treated with the test materials

Groups of teeth	*n*	Mean value of RDT (μm)	SD
Teflon	8	0.46	0.19
Dycal	8	0.41	0.17
C-SE Bo	14	0.43	0.15
MDPB-ad	14	0.46	0.17

C-SE Bo, Clearfil SE bond; MDPB, 12-methacryloyloxy-dodecylpyridinium bromide.

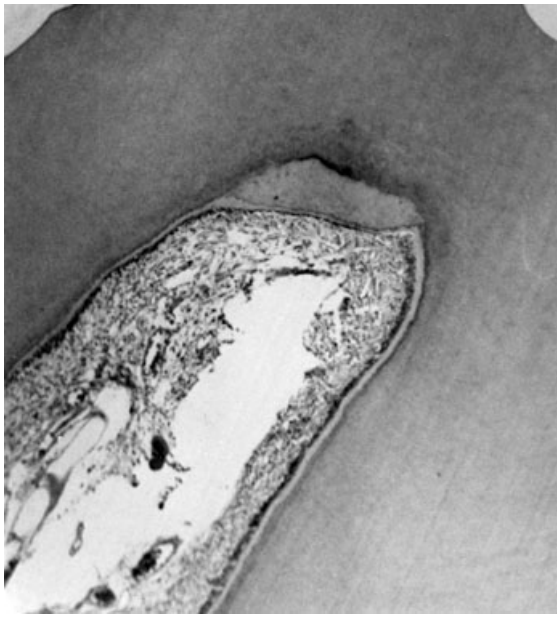

Figure 1 Treatment of non-exposed cavity with the $Ca(OH)_2$-based material dycal. Tertiary dentin formation 8 weeks post-operatively (haematoxylin–eosin, original magnification 40×).

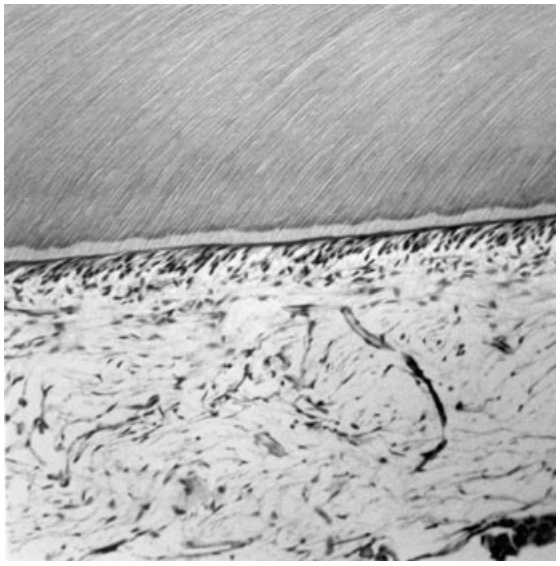

Figure 2 Treatment of non-exposed cavity with the experimental antibacterial adhesive material. No odontoblastic changes beneath the cavity and absence of tertiary dentin formation 4 weeks post-operatively (haematoxylin–eosin, original magnification 100×).

mean and standard deviation of the minimum remaining dentine thickness in each group of teeth is seen. No statistical difference was observed amongst the groups.

No positive bacterial reaction was identified in the pulp in any of the specimens. Dentine positive bacterial reaction was detected superficially, along the cavity walls or within the cut dentinal tubules (no more than 30 μm in depth) in all of the teeth in which Teflon discs were applied (controls), as well as in two teeth treated with the antibacterial adhesive and in one tooth treated with Clearfil SE Bond.

No moderate to severe inflammation or complete tissue necrosis was found in any of the specimens (Figs 1–4). Pulp tissue reactions are given in Table 2. Results submitted to Kruskal–Wallis and to Mann–Whitney *U*-tests showed that there were no differences between the teeth treated with the antibacterial dentine adhesive and other test materials, as far as inflammatory cell response and tissue disorganization in both observation periods were concerned. Furthermore, with regard in tertiary dentine formation the Fisher's exact test showed that:

1. There were no differences between groups of teeth treated with the antibacterial adhesive and Dycal in both observation periods,

2. There was a significantly higher rate of tertiary dentine formation between teeth treated with the antibacterial adhesive and those treated with Clearfil SE bond, after a period of 8 weeks.

Pulpal exposures

Positive pulpal bacterial reaction was only detected in three out of eight teeth capped with Teflon discs. Dentin positive bacterial reaction was also detected along the cavity walls or within the cut dentinal tubules in all teeth capped with Teflon discs in both observation periods, as well as in one tooth treated with the antibacterial adhesive and one tooth with Clearfil SE Bond in 8-week period.

Pulp tissue reactions are given in Table 3. Reparative dentine formation in contact with the capping material was found only in teeth treated with Dycal (Fig. 5). None of the teeth from the other groups showed any evidence of reparative tissue formation bridging the exposure site in any of the observation periods (Figs 6–8). Tertiary dentine formation was found along the circumpulpal dentine around the exposure site in all teeth treated with the antibacterial adhesive system and Dycal (Figs 5 and 6).

Results submitted to Kruskal–Wallis and to Mann–Whitney *U*-tests showed

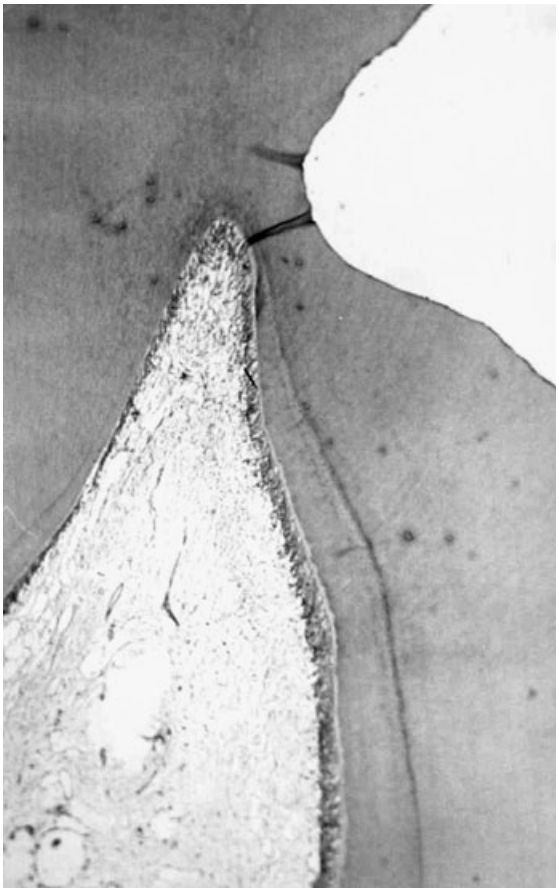

Figure 3 Treatment of nonexposed cavity with the experimental antibacterial adhesive material. Thick zone of tertiary dentin formation 8 weeks post-operatively (hematoxylin–eosin, original magnification 40×).

1. Significantly less inflammatory response in the antibacterial adhesive group compared with Teflon disc control group ($P < 0.001$) or Clearfil SE Bond ($P = 0.001$). No tooth capped with the antibacterial adhesive had moderate to severe inflammatory cell infiltration. Also,

2. Significantly less extent of tissue disorganization observed with the antibacterial adhesive and Dycal groups compared with Teflon discs or Clearfil SE Bond ($P < 0.001$). No tooth capped with the antibacterial adhesive or Dycal had complete tissue disorganization.

Discussion

One of the most significant problems in restorative dentistry is the possible biological risks involved in using resin-based restorations in close proximity to the pulp tissue (Costa *et al.* 2000). Research monitoring pulpal responses in various animal systems has raised discussion concerning their ability to adequately predict the response of human pulp. Although human studies are the 'gold standard' in current *in vivo* tests, ethical, legal, economic and practical issues are even more complex in these instances than for animal

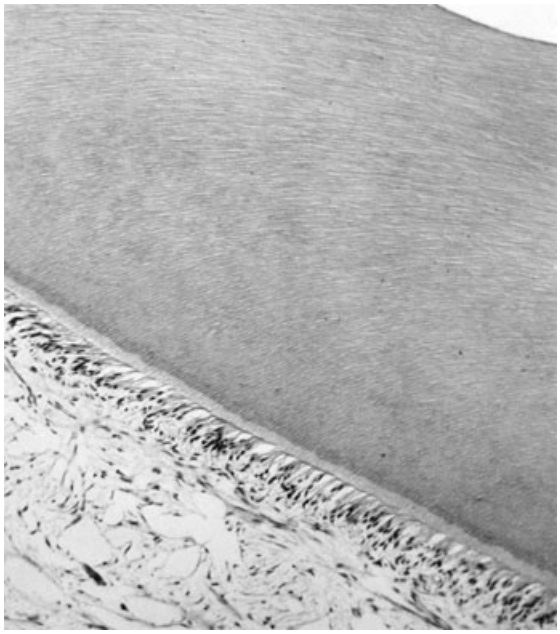

Figure 4 Treatment of non-exposed cavity with the adhesive material Clearfil SE Bond. Slight odontoblastic changes beneath the cavity and absence of tertiary dentin formation 8 weeks post-operatively (haematoxylin–eosin, original magnification 100×).

Table 2 Pulp tissue reactions in non-exposed cavities. Frequency of scores for each group of teeth, 4/8 weeks after treatment with the test materials

Groups of teeth	Histological findings								
	Inflammatory cell response			Tissue disorganization				Tertiary dentine formation	
	no	sl	mo/se	no	sl	pa	co	No	Yes
Teflon	4/4	0/0	0/0	0/1	3/2	1/1	0/0	4/2	0/2
Dycal	4/4	0/0	0/0	3/3	1/1	0/0	0/0	0/0	4/4
C-SE Bo	7/7	0/0	0/0	2/1	3/2	2/4	0/0	7/4	0/3
MDPB-ad	7/7	0/0	0/0	3/1	3/5	1/1	0/0	2/0	5/7

no, none; sl, slight; mo/se, moderate/severe; pa, partial; co, complete; C-SE Bo, Clearfil SE bond; MDPB, 12-methacryloyloxy-dodecylpyridinium bromide.

Table 3 Pulp tissue reactions in exposed cavities. Frequency of scores for each group of teeth, 4/8 weeks after treatment with the test materials

Groups of teeth	Histological findings							
	Inflammatory cell response			Tissue disorganization			Tertiary dentine formation	
	no	sl	mo/se	no	pa	co	No	Yes
Teflon	0/0	0/0	4/4	0/0	4/1	0/3	4/4	0/0
Dycal	2/4	1/0	1/0	0/0	4/4	0/0	0/0	4/4
C-SE Bo	0/0	1/0	6/7	2/1	3/3	2/3	7/7	0/0
MDPB-ad	3/7	4/0	0/0	1/3	6/4	0/0	7/7	0/0

no, none; sl, slight; mo/se, moderate/severe; pa, partial; co, complete; C-SE Bo, Clearfil SE bond; MDPB, 12-methacryloyl-oxy-dodecylpyridinium bromide.

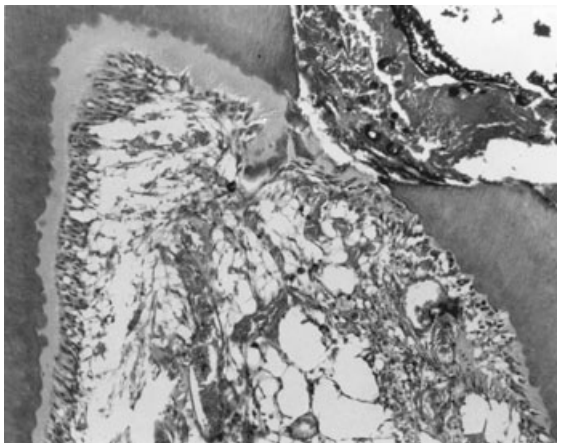

Figure 5 Treatment of pulpal exposure with the $Ca(OH)_2$-based material Dycal. Slight inflammatory cell infiltration, partial disorganization of the central pulp and reparative dentine bridge formation 4 weeks post-operatively (haematoxylin–eosin, original magnification 100×).

testing (Bouillaguet & Wataha 2004). Thus, *in vivo* tests performed on animal models play a considerable role in evaluating the biological risks of materials, especially when tissue-specific issues (such as the differentiation of odontoblastic lineage cells and the expression of dentinogenic activity) are concerned.

It is clear from the literature that the presence of residual bacteria along the cavity walls or within the cut dentinal tubules is the most significant factor in determining pulpal reaction in nonexposed cavities under resin-based restorative materials (Mjor & Tronstad 1972, Tziafas & Kolokuris 1987, Camps *et al.* 2000, Bergenholtz 2001). In the present experimental

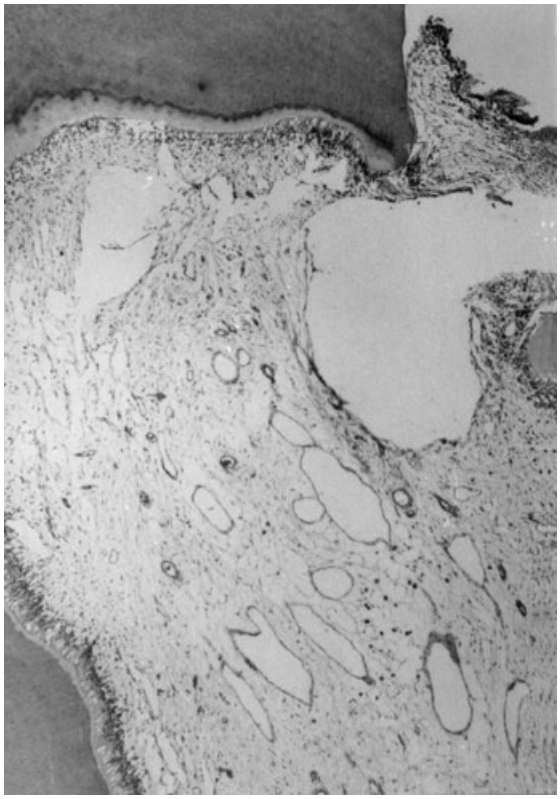

Figure 6 Treatment of pulpal exposure with the experimental antibacterial adhesive material. No inflammatory cell infiltration, no pulp tissue disorganization and absence of any sign of reparative tissue formation (haematoxylin–eosin, original magnification 100×).

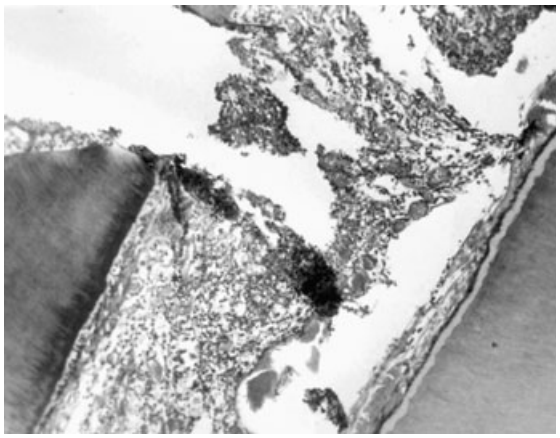

Figure 7 Treatment of pulpal exposure with the adhesive material Clearfil SE Bond. Severe inflammatory cell infiltration and complete tissue necrosis; no tertiary dentine formation could be seen 4 weeks post-operatively (haematoxylin–eosin, original magnification 40×).

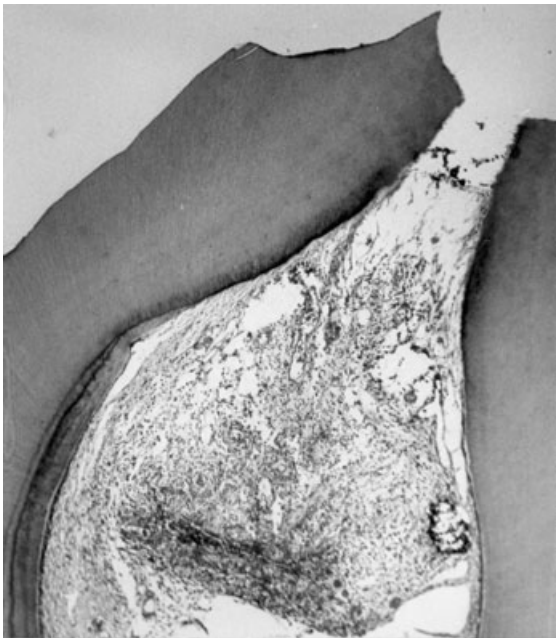

Figure 8 No treatment of pulpal exposure (control teeth capped with Teflon). Severe inflammatory cell infiltration and complete tissue necrosis; no tertiary dentin formation could be seen 4 weeks post-operatively (haematoxylin–eosin, original magnification 40×).

model, conditions where the smear layer and the associated dentinal interface were contaminated with a standardized bacterial infection were created. All the teeth, which were filled with Teflon disks and amalgam, had a positive bacterial reaction along the cavity walls and superficially in the cut dentinal tubules, at both post-operative periods, indicating that the placement of Millipore filters, that had previously been soaked, through suspension, in a-streptococci, in the prepared cavities for 24 h, created conditions where a control bacterial infection could be developed after restorative procedures. Models for the experimental infection of tooth cavities, including methods for leaving prepared (Cox *et al.* 2001) or previously-etched (Akimoto *et al.* 1998) dentinal cavities open to the oral environment, or inoculation of bacterial suspension for 30 min in the previously etched dentine, in which bacteria were further recovered from the subjacent dentinal substrate (Imazato *et al.* 2004), have been reported previously.

Inflammatory cell response was not seen in the nonexposed cavities in which Teflon discs were applied, even after 8 weeks. Imazato *et al.* (2004) using that approach, found mild to moderate inflammation in the untreated cavities after periods of 7, 30 and 75 days. It seems that the present model of experimental cavity infection creates conditions appropriate for the evaluation of the antibacterial properties of dental materials for use in long-term usage studies.

The present data showed that inflammation or complete tissue disorganization was not found in nonexposed cavities treated either with the antibacterial adhesive, Clearfil SE bond or Dycal. Differences between the three groups of treated teeth regarding the pulp-specific reparative tissue reactions were limited in the 4-week observation period. A distinct difference in tertiary dentine formation was seen after 8 weeks between the teeth treated with the two bactericidal materials, MDPB-containing dentine adhesive or Dycal, and those where Clearfil SE bond or Teflon discs had been placed. It is clear that an important issue besides those of tissue architecture and inflammation, is the application of criteria to assess the repair capacity of the pulp-dentine complex which takes into consideration the combined effects of mechanical, bacterial and chemical trauma.

The present experiments showed that pulp vitality and odontoblastic function appear to be maintained after treatment of deep nonexposed cavities with the antibacterial self-etch adhesive system. Tertiary dentine formation in response to this antibacterial adhesive was found to be comparable with that seen after dentine treatment with the $Ca(OH)_2$-based material. Since the two adhesive systems tested differ only in their incorporation of the antibacterial monomer MDPB, it might be suggested that the benefit of the adhesive system containing MDPB, in stimulating tertiary dentine formation under the treated dentin area, might be attributed to its antibacterial properties.

Optimal compatibility of the antibacterial adhesive with the pulp tissue was further confirmed by the present direct pulp capping experiments. Maintenance of pulp vitality and the biosynthetic function of primary odontoblasts surrounding the exposure site was seen clearly in infected exposed cavities treated with the $Ca(OH)_2$-based material and the antibacterial adhesive. Antibacterial activity of the adhesive system containing MDPB could explain the different histopathological features observed between teeth treated with the two adhesive systems. The good tissue compatibility of the newly introduced antibacterial adhesive, supports the above-mentioned data for nonexposed cavities. However, it does not mean that the adhesive system containing MDPB might be considered to be the ideal pulp-capping material.

The present direct pulp-capping experiments clearly showed that tertiary (reparative) dentine formation

bridging the exposure site was only found in response to the $Ca(OH)_2$-based material. Hard tissue formation was not seen in any of the teeth where the antibacterial adhesive, Clearfil SE bond or Teflon discs had been placed. Adhesives have been proposed as an alternative to $Ca(OH)_2$-based materials in direct pulp capping treatment. The formation of a properly hybridized dentine-adhesive interface has been considered to seal both dentine and pulp effectively, allowing complete tissue healing and tertiary dentine formation (Cox *et al.* 1998). Several experimental studies have supported this assumption (Cox *et al.* 1998, Pameijer & Stanley 1998, Tarim *et al.* 1998, Costa *et al.* 1999, Kitasako *et al.* 1999, Hafez *et al.* 2000). Nevertheless, other contradictory experimental data (Hebling *et al.* 1999, Carvahlo *et al.* 2000, Schuurs *et al.* 2000, Horsted-Bindslev *et al.* 2003, Koliniotou-Koumpia & Tziafas 2005), showed that dentine adhesives interrupt the potential of pulpal cells to express their dentinogenic activity. The present results support the later statement, at least in the case of infected pulp exposures. In general, since ideal treatment of clinical exposures would be one that could control wound infection at the same time as stimulating mineralized tissue formation (Bergenholtz 2001), it seems obvious that acidic and resinous materials might not be considered appropriate capping materials in vital pulp therapy.

Conclusions

1. Application of the antibacterial MDPB-containing self-etch adhesive in deep nonexposed cavities or in direct contact with the pulp was not correlated with severe inflammatory cell response or disorganization of the pulp tissue.

2. Pulp vitality and primary odontoblastic function maintained after treatment of infected nonexposed or exposed cavities with the antibacterial adhesive system. Rates of tertiary dentine formation in infected dentinal cavities treated with this system seem to be comparable with those observed after dentine treatment with a $Ca(OH)_2$-based material. The antibacterial adhesive system used as pulp capping material in infected pulp exposures interfered with tertiary (reparative) dentin bridge formation.

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