doi:10.1111/j.1365-2591.2008.01396.x

Histological evaluation of direct pulp capping with a self-etching adhesive and calcium hydroxide on human pulp tissue

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Abstract

Lu Y, Liu T, Li H, Pi G. Histological evaluation of direct pulp capping with a self-etching adhesive and calcium hydroxide on human pulp tissue. *International Endodontic Journal*, **41**, 643–650, 2008.

Aim To evaluate human pulp tissue response following direct pulp capping with a self-etching adhesive: Clearfil SE BOND (SB).

Methodology Forty-five sound teeth from 20 subjects were used. Forty-one teeth had their pulp mechanically exposed at the base of a Class 1 cavity preparation and were divided into two groups: group 1, teeth were capped with SB (n = 21), and group 2, with calcium hydroxide cement (CH) (n = 20). Four teeth were maintained intact as an untreated control group. After 7, 30 and 90 days, respectively, 15 teeth were extracted and processed for light microscopic examination. Pulp healing and bacterial microleakage were assessed by haematoxylin and eosin, Masson trichrome and Brown and Brenn stain techniques. The data were analysed statistically by using the Mann–Whitney U test.

Results After the 7-day observation period, the inflammatory reaction in the SB group was slight and significantly less severe than that of the CH group ($P < 0.05$). After the 30- and 90-day observation periods, the inflammatory reaction was slight in both groups, but specimens with dentine bridge formation in the SB group were significantly less common than those in the CH group ($P < 0.05$).

Conclusions Clearfil SB had good biocompatibility with human pulp tissue, but its ability to induce reparative dentine was significantly lower than that of calcium hydroxide.

Keywords: calcium hydroxide, Clearfil SE BOND, direct pulp capping

Received: 9 April 2007; accepted: 3 January 2008

Introduction

During cavity preparation and the removal of carious dentine, it is possible to accidentally expose the dental pulp. Direct pulp capping may be indicated in selected cases for maintaining pulp health and function, and therefore the capping material may be a key factor in deciding the treatment outcome. An ideal direct pulp capping material should control infection, adhere to dentine to prevent microleakage, be clinically simple to handle and promote dentine bridge formation (Tziafas *et al.* 2000).

Calcium hydroxide (CH) has been the most widely used pulp capping agent as a result of its bactericidal effect and its ability to induce dentine bridge formation. When CH is used as a pulp wound dressing, its high pH (pH 12) causes an initial necrotic layer to form on the surface of the tissue, which could also initiate pulp inflammation (Schröder 1985). However, in clinical practice, softening and disintegration of CH can occur during the acid-etching procedure prior to adhesive restoration (Hwas & Sandrik 1984). These effects may result in contamination of the bonding agents and increase the potential for microleakage.

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In light of the drawbacks of CH a number of alternative materials have been evaluated including adhesive resins. Adhesives have now evolved into self-etching products, in which etchants and primers are combined, or etchants, primers and bond are combined (Van Meerbeek *et al.* 2003). Compared with the traditional acids, self-etching products demonstrate higher pH values (about pH 2); thus, the stimulus to the pulp is reduced. Further, self-etching primers treat dentine and enamel with a nonrinsing solution of acidic monomers without removing the smear layer; thus, the movement of dentine tubule fluid is not disturbed (Watanabe *et al.* 1994). Hence, according to the hydrodynamic theory there is a possibility that no pulp irritation will result. In addition, self-etching products have shown good performance in inhibiting gap formation and preventing microleakage (Oliveira *et al.* 2003) which, combined with their considerable antibacterial effect (Cehreli *et al.* 2003), makes them promising as direct pulp capping agents.

This study was designed to evaluate the histological features of human pulp tissue following application of Clearfil SE BOND (SB, a two-step self-etching product, Kuraray, Tokyo, Japan) and CH (Dycal, Dentsply, Weybridge, UK) to exposed pulp on sound human teeth and to assess the feasibility of SB as a direct pulp capping agent.

Materials and methods

Forty-five fully erupted sound third molars scheduled to be extracted for orthodontic reasons were selected from healthy subjects ranging in age from 20 to 25. Eighteen subjects contributed two bilateral mandibular or maxillary third molars, whereas nine subjects contributed a single mandibular or maxillary third molar. The subjects signed consent forms after they had received a thorough explanation concerning the experimental rationale, clinical procedures and possible risks. Four of those teeth received no treatment and were used as control. Permission for conducting the study was given by the Medical Academy Independent Ethical Committee of West China College of Stomatology.

Cavity preparation and grouping

Each tooth was examined radiographically for periapical and proximal caries. Electric sensibility testing was performed before the experimental procedure and again just before extraction. Calculus and debris were removed from tooth surfaces, which were then swabbed with 3% hydrogen peroxide followed by saline solution. After local anaesthesia and rubber dam isolation, each tooth was polished with a rubber cup and prophylactic paste at low speed, then the site was cleaned with 70% alcohol. Class I cavity preparations, 3 mm long, 3 mm wide and 3.5 mm deep, were prepared in the occlusal surface using a fissure bur (FG-701; SSW, Lakewood, CO, USA) at a high speed under copious water/spray coolant. The cavity walls were vigorously washed and cleaned with sterilized distilled water. Then, a pulp exposure approximately 1 mm in diameter was performed gently in the middle of the cavity using a diamond bur (FG-701, SSW) at a high speed without penetrating into the pulp space. Haemorrhage was arrested using sterile cotton pellets; the surface of the pulp and cavity were irrigated with 2% chlorhexidine solution for 60 s, then washed with physiological saline, and finally dried with sterile cotton pellets. New burs were used for each procedure.

Forty-one teeth were used as experimental teeth and grouped according to the following methods: the 36 molars from the 18 subjects with two bilateral third molars were divided into two experimental groups by flipping a coin. Depending on the result, the pulp exposure of the teeth on the right side was capped with CH (Dycal), and the pulp exposure on the left side was capped with Clearfil SB (Kuraray) or the capping agents for the two sides were exchanged. Single mandibular or maxillary third molars contributed by the five participants were also divided by flipping the coin. Depending on the result, the first participant had the pulp exposure capped with the CH, the following participant had the pulp exposure capped with the SB, the next with the CH and so on. At the end, three participants had their pulps capped with SB and two with CH.

Direct pulp capping

The SB group

SB's primer was applied to the pulpal wound, dentine and enamel of the 21 selected teeth for 20 s, and gently dried using a cotton pellet. Then the SB's bonding agent was applied to the exposed pulp and cavity walls, and light-cured for 10 s according to the manufacturer's instructions.

The CH group

The base and catalyst of CH were mixed in the ratio of 1 : 1(v/v), according to the manufacturer's instructions, and then applied directly to the exposed pulps of the 20 selected teeth, leaving the cavity walls

uncovered as much as possible. Then SB was applied to the cavity walls.

Cavity restoration

All the cavities were restored with Clearfil AP-X composite resin. Each 2 mm increment of composite resin was light-cured for 40 s with a curing light (Curing Light 2500, 3M, St Paul, MN, USA). The restorations were finished with diamond burs (TF-21, Mani, Tochigi-ken, Japan) and polished with a rubber cup (Super-snap Midi 0298, Shofu, Kyoto, Japan).

The same operator prepared and restored all the cavities, and examiners were calibrated before the experiment.

Specimen preparation and examination

On the 7th, 30th and 90th days after direct pulp capping, 15, 14 and 16 teeth were extracted, respectively, under local anaesthesia, and the roots were immediately sectioned midway between the cementum–enamel junction and the apex. Drawing straws amongst the 18 and 5 participants assigned the grouping of observation periods respectively.

After fixation in buffered formalin solution for 96 h, the specimens were decalcified in 0.3 M ethylenediaminetetraacetic acid, dehydrated in increasing concentrations of aqueous ethanol, and embedded in paraffin. Six-micron-thick serial bucco-lingual sections were cut longitudinally through the centre of the exposure site. Sixty sections were taken from each test site, and every third section was stained by one of the three procedures: (i) one set of sections were stained with haematoxylin and eosin for differentiation of connective tissue elements; (ii) with the second set, for better discrimination of the results, Masson trichrome was used to stain dentine collagen, (iii) with the third set, the Brown and Brenn technique was used for bacterial recognition. All sections were viewed under a light microscope ($100\times$, $200\times$ magnification).

The four intact control teeth allowed determination of the effectiveness of the histological processing methods.

For all sections, four histological features were evaluated according to the criteria listed in Tables 1, 2, 3 and 4 by two observers who were not informed of the true nature and purpose of the study. In case of disagreement between the two examiners, the tooth was re-examined and the more severe evaluation was chosen.

Statistical method

The data were analysed statistically by using the Mann–Whitney *U* test (SPSS10.0 software, SPSS, Chicago, IL, USA) to identify differences between the CH and the SB groups, according to the pre-determined histological grading criteria of Tables 1, 2, 3 and 4. The significance level was set at $P < 0.05$.

Table 1 Inflammatory cell response

Grade	Characterization
0	None or a few scattered inflammatory cells present in the pulpal area corresponding to pulp exposure, characteristic of normal tissue
1	Slight inflammatory cell infiltration with polymorphonuclear or mononuclear leukocytes
2	Moderate inflammatory cell infiltration involving the coronal pulp
3	Severe inflammatory cell infiltration involving the coronal pulp or abscess present

Table 2 Tissue damage

Grade	Characterization
0	Normal tissue
1	Odontoblast layer disorganization, but central pulp normal
2	Total disorganization of the pulp tissue morphology
3	Pulp necrosis

Table 3 Hard tissue formation

Grade	Characterization
0	Absent
1	Modest hard tissue deposition beneath the exposed area
2	Moderate hard tissue deposition beneath the exposed area
3	Heavy hard tissue deposition beneath the exposed area appearing as a complete dentin bridge

Table 4 Stained bacteria

Grade	Characterization
0	Absent
1	Presence of stained bacteria along the cavity lateral walls
2	Presence of stained bacteria along the cavity lateral walls and axial walls
3	Presence of stained bacteria along the cavity walls within the cut dentin tubules or over the pulp tissue

Results

No specimen was lost during the study. In both the CH and the SB groups, the electric sensibility testing revealed similar results to that of the intact control

group. No patient reported postoperative pain or hypersensitivity. Radiography revealed no periapical pathosis.

In histological evaluation, the inter-observer agreement was satisfactory, with only two cases having to be re-examined. The results are listed in Table 5 and summarized as follows:

7-day observation period

CH

In six specimens (86%), the initial reaction was a necrotic zone associated with slight to moderate inflammatory reaction, characterized by the infiltration of neutrophils and mononuclear cells at the exposure site. One (14%) showed a severe inflammatory reaction associated with discrete necrosis. All specimens had disorganization of the pulp tissue, dilated and congested blood vessels, and lack of a dentine bridge at the exposure site. Bacteria were not observed (Fig. 1).

SB

One specimen (14%) had moderate inflammation with neutrophils and mononuclear infiltration at the exposure site. The other pulps manifested slight inflammation. All presented dilated and congested blood vessels, and lack of reparative dentine. No bacteria were observed (Fig. 2).

During this period, the inflammatory reaction of the SB group was significantly less than that of the CH group ($P < 0.05$).

Table 5 Grading of the histological features of the sections

Observation period		7 day		30 day		90 day		
Group		SB	CH	SB	CH	SB	CH	Control
Number of specimens		7	7	7	6	7	7	4
Feature	Grade							
Inflammatory response	0	0	0	0	0	5	6	4
	1	6*	2*	6	5	2	0	0
	2	1*	4*	1	1	0		0
	3	0	1	0	0	0	1	0
Tissue disorganization	0	0	0	0	0	0	0	4
	1	7	6	7	6	7	7	0
	2	0	1	0	0	0	0	0
	3	0	0	0	0	0	0	0
Reactional dentin	0	7	7	6*	2*	4	1	4
	1	0	0	0	0	2	0	0
	2	0	0	1	3	1	1	0
	3	0	0	0	1	0*	5*	0
Stained bacteria	0	7	7	7	5	7	6	4
	1	0	0	0	1	0	0	0
	2	0	0	0	0	0	1	0
	3	0	0	0	0	0	0	0

SB, SE BOND; CH, calcium hydroxide
* Indicates significant difference between SB group and CH group over a specific time period; $P < 0.05$.

30-day observation period

CH

Four specimens (67%) had a thin layer of partially calcified dentine matrix, accompanied by a weak inflammatory reaction mediated by a few mononuclear cells. Cell inclusion was seen in one example of reparative dentine, which was the so-called osteoid dentine bridge. All pulps were characterized by slight inflammation and oedema (Fig. 3). One specimen

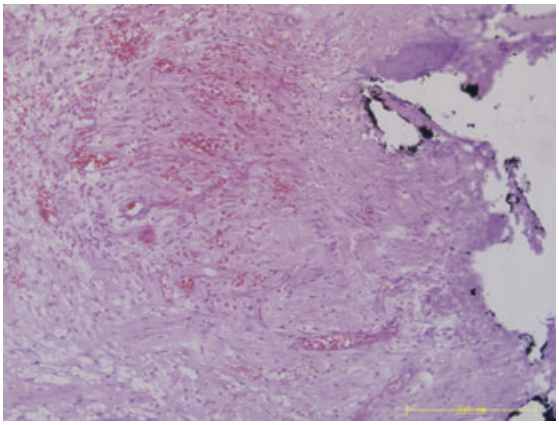

Figure 1 Histological reaction of pulp to Dycal in the 7-day period (200 ×).

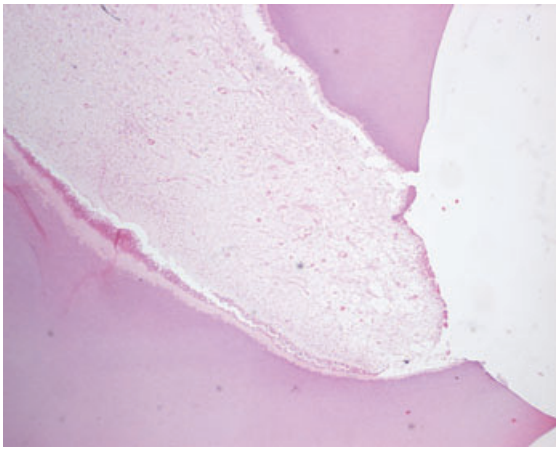

Figure 2 Histological reaction of pulp to SE BOND (SB) in the 7-day period (200 ×).

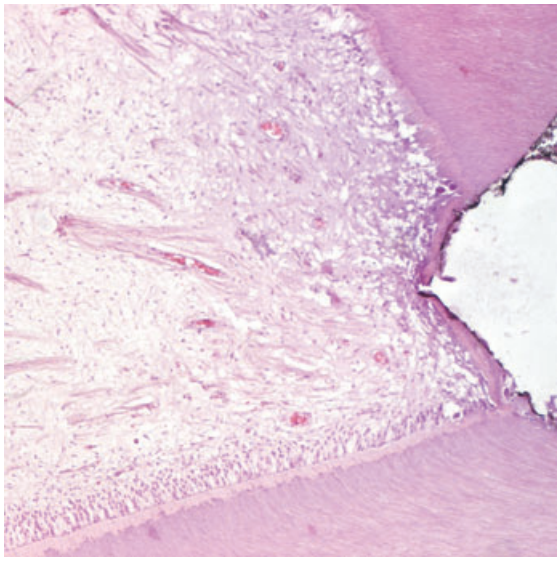

Figure 3 Histological reaction of pulp to Dycal in the 30-day period (200 ×).

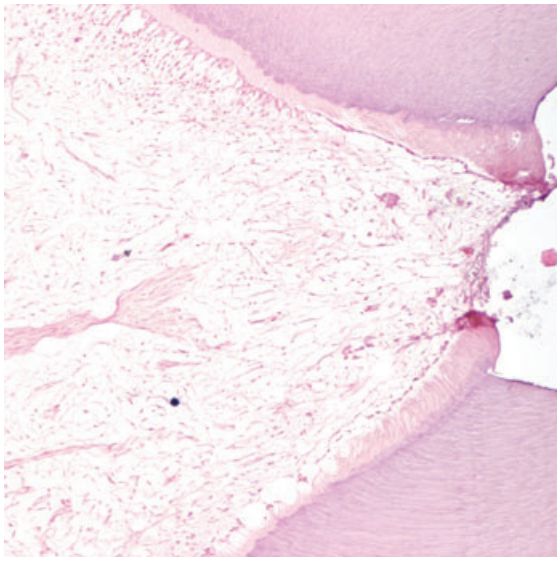

Figure 4 Histological reaction of pulp to SB in the 30-day period (200 ×).

(17%) demonstrated stained bacteria on the lateral cavity wall.

SB

One specimen (14%) demonstrated a thin layer of partially calcified dentine matrix and normal pulp. Six (86%) were devoid of reparative dentine; five (71%) of them demonstrated only slight inflammatory reaction at the exposure site (Fig. 4), whereas one showed moderate inflammation characterized by resinous fragments engulfed by macrophages and giant cells, accompanied by mononuclear cell infiltration. Stained bacteria were not seen.

90-day observation period

CH

Six specimens (86%) demonstrated a complete dentine bridge with normal pulp (Fig. 5); two of them were an osteoid dentine bridge. One (14%) had pulp necrosis, no tertiary dentine formation across the exposure, and stained bacteria were observed at the lateral and axial walls.

SB

One specimen (14%) had a complete dentine bridge at the exposure site (Fig. 6). Two specimens (29%) manifested a thin and discrete layer of reparative dentine,

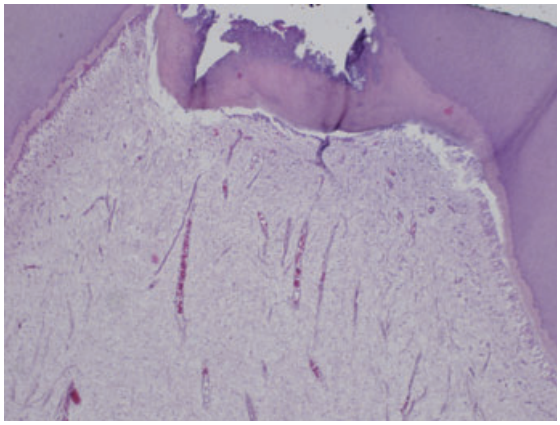

Figure 5 Histological reaction of pulp to Dycal in the 90-day period (200 ×).

accompanied by a dense concentration of collagenous fibres in the underlying zone. Two specimens (29%) had normal histological features, but dentine bridge formation was absent. Two specimens (29%) demonstrated degeneration of the pulp cells at the exposure site, whereas the remaining pulp tissue remained normal. No globules of resinous material, macrophages, or giant cells were observed in any specimens. Stained bacteria were not seen.

In the 30- and 90-day observation periods, specimens with dentine bridge formation in the SB group were significantly less common than those in the CH group ($P < 0.05$)

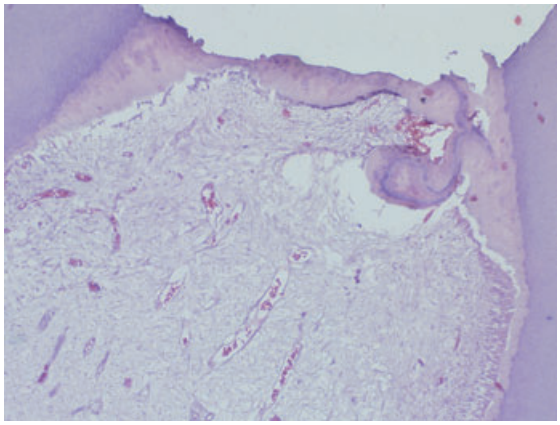

Figure 6 Histological reaction of pulp to SB in the 90-day period (200 ×).

Control group

All the specimens in this group exhibited an ordered odontoblast layer, zone of Weill, cell-rich zone and central pulp characteristic of normal pulp tissue, which demonstrated that the histological processing methods used in the present study were effective.

Discussion

It was believed that the primary cause of pulp damage was direct toxic effects from dental materials (Suarez *et al.* 1970). However, animal studies have demonstrated that restorative materials previously reported as 'toxic' do not cause pulp inflammation or pulp necrosis when placed directly on the exposed pulp (Costa *et al.* 2003), if bacteria were sealed-off at the margins.

The present study demonstrated that in the 7-day observation period, most pulps of the SB group had slight to moderate inflammation, whereas in the 30- and 90-day periods, pulps were inclined to show normal histological features. Short-term inflammation is considered by many to be caused by traumatic injury during cavity preparation (Costa *et al.* 2001a), so the toxic effect of SB appeared moderate and acceptable. In the 7-day observation period of the CH group, most pulps had a necrotic layer at the exposure site, which has been shown to be related to its high pH (Schröder 1985).

In the 7-day observation period, all pulps manifested extravasated erythrocytes. Haemorrhage control is an important procedure determining the outcome of direct pulp capping. According to Schröder (1978), lack of adequate haemostasis before placement of the medication adversely affected the treatment, because a blood clot could form a barrier that prevented contact between the capping material and the exposed pulp, and such clots may act as a substrate for microorganisms, thus leading to pulp infection (Kopel 1992). No blood clot formation at the exposure site was observed in the SB group over 7 days. SB is a two-step adhesive, with priming and bonding without water rinsing. In this study, the surface of the exposed pulp tissue turned visually white when the primer was applied. This might be because of coagulation of the proteins from the superficial pulpal tissue, which would suppress the exudates. Therefore, the SB's primer might be effective in preventing bleeding. Although many studies have indicated that NaOCl is an effective haemostatic agent, it was not used in the present study. NaOCl has been reported to be toxic to pulp tissue (Costa *et al.* 2001b) and can remove the collagen fibrils from dentinal surfaces, thus preventing the formation of a healthy hybrid layer, which could result in low-bond strength (Ozturk & Ozer 2004). Consequently, 2% chlorhexidine solution was applied for 60 s to disinfect the prepared cavities; a method developed by Pameijer & Stanley (1998). The present clinical findings also showed that 2% chlorhexidine solution was effective in controlling bleeding.

To evaluate pulp reaction over long periods, previous studies focused on two aspects: tissue repair and marginal sealing effect. In terms of tissue repair, it seems to represent that odontoblast-like cell differentiation, fibroblastic proliferation, fibrodentine deposition and hard tissue barrier formation were dependent on healthy pulp tissue but not on the capping material (Olmez *et al.* 1998). Previous studies performed in human teeth reported that displaced resinous particles were released from the bonding materials into the pulp space, and these particles triggered a foreign body response characterized by the presence of mononuclear inflammatory infiltration as well as appearance of multinuclear giant cells and macrophages (Hebling *et al.* 1999) The persistent inflammatory response prevented pulp healing and dentine bridge formation. In the present study, only one specimen had an inflammatory response induced by debris of resinous capping material, and most pulps demonstrated normal features. In addition, dentine bridge formation associated with differentiation of odontoblast-like cells was observed in one tooth over 30 days and three teeth over 90 days. The results demonstrated that in most cases, SB did not release many particles into the pulps, and the pulps were not adversely affected. It is

interesting to explore why fewer SB granules entered into pulp compared with other adhesive systems reported in previous studies. During polymerization of bonding resin, inward fluid shifts in dentine tubules have been reported to occur because of the heat generated by polymerization; at the same time lack of curing at the deepest levels of resin tags resulted in unpolymerized resin particles remaining (Ferracane 1994). Consequently, the inward fluid flow carried unpolymerized resin particles into pulp through dentine tubules. In the self-etching system, the acidic primer infiltrates the collagen fibres as it simultaneously decalcifies the inorganic component to the same depth, then the acidic primers are neutralized by inorganic components of dentine such as calcium or phosphate released during demineralization. It is the self-limiting mechanism that leads to limited depth of dissolution, so the innermost part of the hybrid layer was completely polymerized (Bergenholtz 2000), leaving a limited amount of unpolymerized monomer. It can be speculated that the observed pulpal histological features could be attributed to the adhesion characteristics of SB.

Marginal sealing is another key factor determining the final result of direct pulp capping. Bergenholtz (2000) discussed the correlation between pulpal breakdown and bacterial contamination, whereas Cox *et al.* (1998) reported that the pulp could form some hard tissue barrier if a proper biological seal was provided so that bacteria did not gain access to the wound. In the present study, bacteria were not found along the lateral cavity wall or in the pulp space in the SB group, which demonstrated that SB had a satisfactory sealing effect. In the CH group, bacterial stain was observed in one 30-day specimen and in one 90-day specimen. During the acid etching procedure, prior applied CH cement can become soft and disintegrate, so the bonding agents and cavity wall will become contaminated, and the potential for microleakage increased. This was also reported by McComb (1983).

Two specimens of the SB group had localized degeneration of pulp tissue adjacent to the capping agent. The mechanism of degeneration cannot be deduced from this study, so whether pulp vitality could be maintained is unknown. Also attention should be paid to the evidence that cases with dentine bridge formation in the SB group were significantly fewer than those in the CH group ($P < 0.05$). Considering the fact that the resin–dentine bonds undergo degradation *in vivo* over time, risk of pulp infection by invaded microorganisms may be greater in cases without a hard tissue barrier than in cases with a dentine bridge. Hence, to determine whether SB can be used for direct pulp capping requires further study with a longer observation period and a larger sample size.

Conclusion

Clearfil SB displayed satisfying biocompatibility and sealing effect, but its ability to induce reparative dentine was significantly weaker than Dycal. studies with a longer observation period, and larger samples should be carried out to ascertain the feasibility of applying Clearfil SB as a direct pulp capping agent.

Acknowledgements

Dr Ralph Duckworth for his help when writing this paper. This study is supported by the ''National Key Technologies R&D Program of the Tenth-five Plan, The Ministry of Science and Technologies of China''.

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