

## Evaluation of human pulp tissue response following direct pulp capping with a self-etching adhesive system containing MDPB

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This study evaluated the human pulp tissue response following direct pulp capping with Clearfil Protect Bond (CPB) self-etching adhesive containing an antibacterial monomer MDPB. The pulps of third molar teeth were exposed by the removal of carious tissue. In an experimental group, CPB was applied to the exposed pulp and dentin. In the control groups, Clearfil SE Bond (CSE) or calcium hydroxide-based cement (CH) was applied to the exposed pulp surfaces. All teeth were filled with resin composite, extracted after 90 days, and the pulp responses were histologically analyzed. No severe inflammation or soft tissue disorganization was observed in CPB and CH groups. CSE group exhibited a disorganized odontoblastic layer and severe inflammatory infiltration. No hard tissue formation was observed in CSE group, and CH formed more of a hard tissue formation than CPB. CPB induced an acceptable healing response when directly applied to exposed pulps with bacterial contamination.

**Keywords:** Direct pulp capping, Clearfil Protect Bond, MDPB, Antibacterial adhesive, Calcium hydroxide

### INTRODUCTION

Pulpal vitality is critical to the maintenance of the structural integrity and normal physiological function of teeth. Direct pulp capping is a conservative therapy frequently performed for preserving pulp vitality when the pulp is exposed during dental treatment. When the pulp tissue is properly protected and preserved with a capping material, the wound may heal uneventfully<sup>1,2</sup>. An ideal direct pulp-capping material should control infection, preserve the vitality of the pulp tissue, stimulate the repair process, adhere to the dentin tightly without permitting leakage, and promote the formation of a hard tissue barrier and a dentin bridge<sup>3,4</sup>.

Calcium hydroxide has been a material of choice for direct pulp capping for several years because it inhibits bacterial growth and exhibits reparative properties<sup>5,6</sup>. However, it has several drawbacks, such as the porosity of its induced hard tissue, its inferior adherence to dentin, and the microleakage produced from its decomposition<sup>7,8</sup>.

Dentin bonding systems have been proposed as an alternative to calcium hydroxide-based materials for direct pulp capping. It is thought that dentin bonding systems effectively seal both dentin and pulp through the formation of a properly hybridized dentin–adhesive interface, allowing complete tissue healing and tertiary dentin formation through the inherent capacity of the dentin–pulp complex<sup>9–11</sup>. Several studies have partially supported this assumption<sup>12,13</sup>. However, contradictory

results were also demonstrated after application of adhesive systems as pulp-capping agents in human and animal teeth<sup>1,6,14</sup>. It has been reported that the success rate of direct pulp capping of exposed pulps following the removal of carious tissue was lower than that of direct pulp capping of traumatically exposed pulps<sup>15</sup>. A potential reason for the failure of pulp healing is the possible presence of bacterial infection in the region of the exposed pulp before the direct pulp-capping procedure<sup>16</sup>. Infected dentin chips introduced into the pulp during caries excavation may also act as nuclei for irreversible inflammation<sup>17</sup>. Nevertheless, the knowledge acquired in recent years about the pulp healing process has highlighted that the pulp healing strategies were effective for pulp exposures during caries removal because of an understanding of the importance of maintaining pulp vitality in conservative dentistry. Therefore, the use of dentin bonding systems with antibacterial activity was expected to generate changes in pulp treatment methods, especially for exposed pulps following caries removal<sup>18,19</sup>.

The antibacterial monomer 12-methacryloyloxy-dodecylpyridinium bromide (MDPB) is a quaternary ammonium compound that copolymerizes with other monomers during curing and is covalently bonded to the polymer network<sup>20</sup>. *In vitro* and *in vivo* studies demonstrated that MDPB has strong bactericidal activity against cariogenic bacteria in cavities and little or no effect on pulpal inflammation<sup>20–22</sup>. In cell culture studies, it has also been found that MDPB is a cytocompatible material with less negative influence on dentinogenesis than bisphenol A-glycidyl methacrylate (Bis-GMA)<sup>23</sup>.

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Incorporation of MDPB into a dentin adhesive system resulted in strong antibacterial activity against oral streptococci *ex vivo* as well as antibacterial effects before and after the curing process of the bonding agent<sup>24</sup>. Clearfil Protect Bond (CPB) is a self-etch adhesive system containing an antibacterial MDPB monomer in its self-etching primer. Self-etching primers treat the dentin surface with a non-rinse solution of acidic monomers that modifies and/or dissolves the smear layer but does not remove its residues; thus, the movement of dentinal fluid is not disturbed and therefore there is a possibility that no pulp irritation will result<sup>25</sup>. All these positive properties of CPB make this agent a promising material to be used on exposed vital pulp tissues.

The aim of this study was to evaluate the histopathological changes and repair performance of human dental pulp following 90 days of direct capping with the self-etch adhesive system containing MDPB (CPB), and to compare the results with a conventional self-etch adhesive system and a calcium hydroxide-based cement in human molars with deep occlusal caries lesions. In previous pulp-capping studies, only intentionally exposed pulps of healthy intact teeth have been studied. This study differs from other studies in that the pulpal response after direct pulp capping with bonding agents was evaluated in the small pulp perforations which occurred during caries removal.

## MATERIALS AND METHODS

The experimental protocol was conducted according to the ethical guidelines for clinical research in humans in the Selcuk University Faculty of Dentistry. The study protocol and consent forms were approved by the Institutional Review Board of the University (Protocol # 2008/1-3). The patients received thorough explanations concerning the clinical procedures and possible complications. Written informed consent was obtained from all participants before initiation of the treatment.

### *Experimental procedures*

The teeth were selected from patients aged 23–35 years and one tooth of each patient was used. Third molars with cavitated deep occlusal carious lesions, with radiolucent image extending into the pulpal quarter of the dentine, scheduled for extraction, with sensible (vital) pulps and no symptomatic/irreversible pulpitis, were included in the study. Each tooth was examined radiographically for caries location and depth and possible periapical pathologies (Fig. 1). Teeth were excluded that exhibited signs or symptoms of irreversible pulpitis such as prolonged unbearable pain or pain at night disturbing sleep, lack of response to electrical pulp testing, percussion pain, periodontal inflammation, radiographic evidence of calcification of the pulp chamber or canals, internal/external resorption, or furcal/periapical radiolucency. A sample size was determined by a statistical power analysis (G\*Power version 3.1.9.2 software, Kiel University, Kiel, Germany) as seven teeth per group, which would give



Fig. 1 Radiographic image of a third molar with deep caries.

more than 80% power to detect significant differences with a 0.58 effect size at a significance level of 0.05. The patients were assigned to the study groups using a computer-generated randomization list (simple equal randomization) to receive their pulp capping procedures with one of the study materials.

A standardized operative procedure was followed and performed by a single operator. Calculus and debris were removed from the tooth surfaces, which were then swabbed with 3% hydrogen peroxide followed by saline solution. After local anesthesia, each tooth was polished with a rubber cup and prophylactic paste at low speed. The occlusal preparation areas were then wiped out with sterilized cotton pellets saturated in 70% ethyl alcohol and the outlines of Class I occlusal cavities were prepared using a fissure diamond bur (ISO size 012, MANI, Tochigi, Japan) at high speed under copious water/spray coolant. The caries lesions were removed by assessing the color and hardness of the lesion with visual and tactile examination. A sterile carbide round bur (No. 1.204.018, Komet, Lemgo, Germany) was used in a slow-speed hand piece to remove carious dentin in the cavity. The soft caries tissue in cavity floor removed until a feeling of resistance was obtained to a hand excavator, while cavity margins were left hard. After all soft infected caries tissues have been removed, the remaining thin dentin at the bottom of the cavity was removed with the same bur with low-speed to create approximately 1 mm diameter of pulp perforation without forcefully penetrating the pulp chamber and not creating any further trauma with the pressure. Bleeding was arrested using sterile cotton pellets impregnated with physiological saline, and the cavity was dried with sterile cotton pellets. The isolation of the operation area was made with the cotton rolls and high volume evacuation.

### *Direct pulp capping*

The adhesive systems used in this study are shown in Table 1. The teeth with pulp exposures were randomly restored with one of the study protocols described below.

CPB group ( $n=7$ ): The pulps of the teeth were capped

Table 1 Composition, pH and batch numbers of the adhesive systems used

Adhesive manufacturer	Composition	pH	Batch number
CSE (Kuraray Noritake Dental, Tokyo, Japan)	Primer: HEMA, MDP, hydrophilic dimethacrylate, N, N-diethandiol-p-toluidine, CQ, water	Primer: 1.9	00195A
	Adhesive: HEMA, Bis-GMA, MDP, hydrophilic dimethacrylate, N, N-diethandiol-p-toluidine, CQ, silanized colloidal silica	Adhesive: 2.8	00193A
CPB (Kuraray Noritake Dental)	Primer: HEMA, MDP, hydrophilic dimethacrylate, MDPB, water	Primer: 1.9	0012A
	Adhesive: HEMA, Bis-GMA, MDP, hydrophilic dimethacrylate, N, N-diethandiol-p-toluidine, CQ, silanized colloidal silica, surface treated sodium fluoride	Adhesive: 2.8	0020A

Bis-GMA: Bisphenol A glycidyl dimethacrylate, CQ: D, 1-camphorquinone, HEMA: 2-hydroxyethyl methacrylate, MDP: 10-methacryloxydecyl dihydrogen phosphate, MDPB: 12-methacryloyloxydodecyl-pyridinium bromide, CSE: Clearfil SE Bond, CPB: Clearfil Protect Bond (Clearfil Protect Bond is also commercialized under the name Clearfil SE Protect)

Table 2 Criteria used to assess pulp response

Scores	Inflammatory cell response
0	A few or no scattered inflammatory cells present at the pulp exposure site or below the hard-barrier formation, characterizing normal tissue
1	Slight inflammatory cell infiltration with polymorphonuclear or mononuclear leukocytes below the pulp exposure site
2	Moderate inflammatory cell infiltrate below the pulp exposure site
3	Severe inflammatory cell infiltrate involving the whole radicular pulp, characterizing pulpal necrosis or abscess
Scores	Soft tissue organization
0	Normal or almost normal tissue morphology below the pulp exposure site
1	Slight disorganization immediately below the pulp exposure site or adjacent to the hard-tissue formation
2	Moderate disorganization involving 2/3 of the pulp tissue below the pulp exposure site or adjacent to the hard-tissue formation
3	Total disorganization associated with pulp breakdown
Scores	Hard tissue formation
0	Hard tissue formation to completely close the exposure site
1	Moderate hard tissue formation beneath the pulp exposure site (the exposed area is not completely closed)
2	Slight hard tissue formation beneath the pulp exposure site (most of the exposed site is not closed)
3	Absence of hard tissue formation

with Clearfil Protect Bond (Kuraray Noritake Dental, Tokyo, Japan)

CSE group ( $n=7$ ): The pulps of the teeth were capped with Clearfil SE Bond (CSE; Kuraray Noritake Dental).

Both adhesives were used according to the manufacturer's instructions. The primer of CPB or CSE was applied to the cavity floor, including the exposed area and the lateral cavity walls, for 20 s, followed by gentle air-drying for 5 s. The bonding resin was subsequently applied and light-cured for 10 s. Then, the cavity was filled with a resin composite (Clearfil Photo Posterior, Kuraray Noritake Dental) and cured for 20 s with a light-curing unit (Optilux 500, Kerr/Demetron,

Danbury, CT, USA).

CH group ( $n=7$ ): A calcium hydroxide hard-setting cement (Dycal, Dentsply, Milford, DE, USA) was applied on the pulpal exposure site and left for 2 min to harden. The cavity was then restored with Clearfil SE Bond and Clearfil Photo Posterior as described above.

#### *Specimen preparation and examination*

The pulp-capped teeth were extracted after 90 days. The patients were questioned about the presence of postoperative sensitivity throughout the study period, and pulp sensitivity was confirmed before the teeth were extracted. The extractions were performed under local anesthesia. Immediately after extraction, the root tips of

half of the teeth were horizontally sectioned with a high speed hand piece under intensive water spray to allow penetration of the fixative solution. The teeth were fixed in 10% neutral-buffered formalin solution for 7 days. The specimens were then demineralized in 9% EDTA for 5 months.

Finally, the teeth were embedded in paraffin blocks and serially sectioned through the pulp at a thickness of 7 µm. All sections coming through the cavity floor were stained with hematoxylin-eosin stain to assess the response of the pulp tissue. All sections were evaluated blindly for histologic features: inflammatory cellular response, soft tissue disorganization, and hard tissue deposition. The intensity of the pulp response was evaluated by light microscope (Nikon Eclipse E400, Nikon, Tokyo, Japan) using the criteria defined in Table 2.

*Statistical analysis*

The mean scores assigned to the sub items for each of the evaluation criteria were analyzed with the nonparametric Kruskal-Wallis test. Between-group comparisons were performed with the Mann-Whitney U-test.  $p < 0.05$  was considered significant.

**RESULTS**

The pulp tissue reaction scores of the sectioned teeth are presented in Table 3. Except for four teeth removed from the CSE group due to the severe pain experienced by patients 1 week after the pulp-capping treatment, all teeth exhibited pulpal sensitivity and did not show any clinical or radiographic signs and/or symptoms of irreversible pulpitis or pulp necrosis/apical periodontitis.

**CPB group:** One specimen (14.2%) formed a complete hard tissue formation at the exposure site (Fig. 2). One specimen (14.2%) demonstrated moderate hard tissue formation beneath the pulp exposure site. Two specimens (28.6%) with slight inflammation (Figs. 3a, b) and two specimens (28.6%) with moderate inflammation demonstrated slight hard tissue formation. One specimen (14.3%) showed moderate inflammation, moderate pulp tissue disorganization, and no hard tissue formation. Six specimens in this group (85.7%) demonstrated slight pulp tissue disorganization at the exposure site. No globules of resinous material, macrophages, or giant cells were observed in any tooth specimen.

**CSE group:** Four teeth (57.1%) were extracted

after one week due to severe pain experienced by the patients. Therefore, in this group, only three samples were available for histopathological analysis. In three teeth of the group (42.8%), the pulp tissue displayed a significant disorganization zone and severe inflammatory infiltration under the exposure sites. No signs of hard tissue formation were found at the wound surfaces (Fig. 4).

**CH group:** In this group, three specimens (42.8%) demonstrated complete hard tissue formation with normal pulp (Fig. 5). One specimen (14.3%) had moderate inflammation and no hard tissue formation across the exposure. The other three specimens demonstrated incomplete hard tissue formation, a slight inflammatory response, and slight or no pulp tissue disorganization.

CH formed significantly more hard tissue formation than CPB ( $p < 0.05$ ). No significant differences were observed between the other assessed criteria in CPB and CH groups for the observation period used in this study ( $p > 0.05$ ). There were statistically significant differences both between the CSE and CH groups and also between the CSE and CPB groups for all assessed criteria ( $p < 0.05$ ).

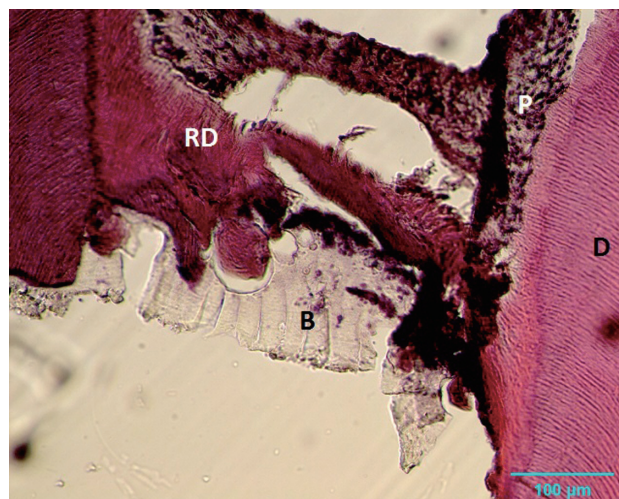


Fig. 2 CPB group: A complete hard tissue formation can be seen (hematoxylin-eosin, original magnification ×200, RD: reparative dentin, D: dentin, P: pulp, B: bonding agent).

Table 3 Grading of the histological features of the sectioned teeth

Materials	n	Inflammatory cell response				Soft tissue organization				Hard tissue formation			
		0	1	2	3	0	1	2	3	0	1	2	3
CPB	7	2	2	3	0	0	6	1	0	1	1	4	1
CSE	3	0	0	0	3	0	0	0	3	0	0	0	3
CH	7	2	4	1	0	4	2	1	0	3	2	1	1



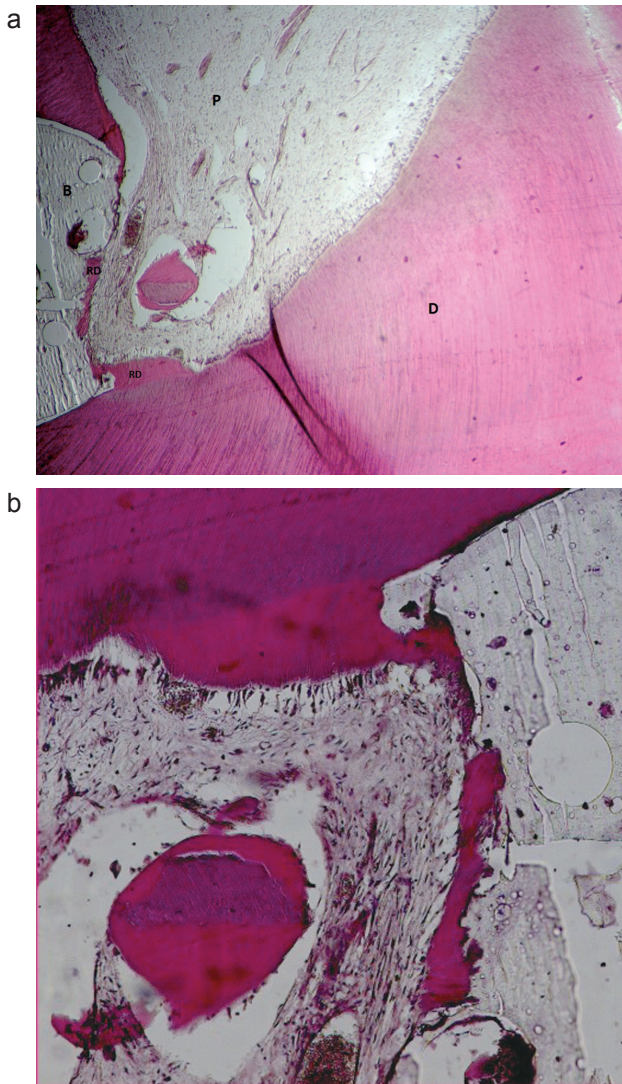


Fig. 3 a, b: CPB group: Slight hard tissue formation is present, with slight inflammatory cell infiltration and slight disorganization in the pulp tissue immediately below the exposure site (hematoxylin-eosin, original magnification a;  $\times 40$ , b;  $\times 200$ , RD: reparative dentin, D: dentin, P: pulp, B: bonding agent).

## DISCUSSION

It is well known that bacterial infection is strongly relevant to the failure of pulp-capping procedures<sup>26,27</sup>. Perforation of the pulp during the removal of infected carious lesion and then bacterial contamination of the tissue result in the production of acids and toxins, which can cause a pulpal reaction<sup>16,28</sup>. Therefore, pulp-capping materials with antibacterial effects are expected to enhance success in the treatment of deep carious lesions.

CSE and CPB are adhesives with a similar composition. The most important compositional

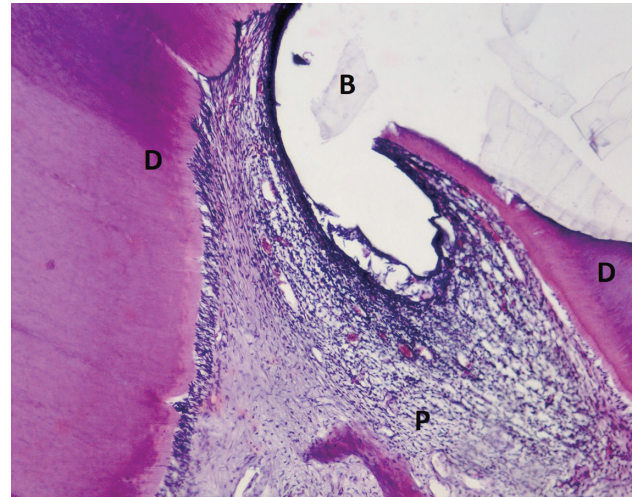


Fig. 4 CSE group: Severe inflammatory cell infiltration and significant disorganization in the pulp tissue is evident below the exposure site. No signs of hard tissue formation are present at the wound surfaces (hematoxylin-eosin, original magnification  $\times 40$ , D: dentin, P: pulp, B: bonding agent).

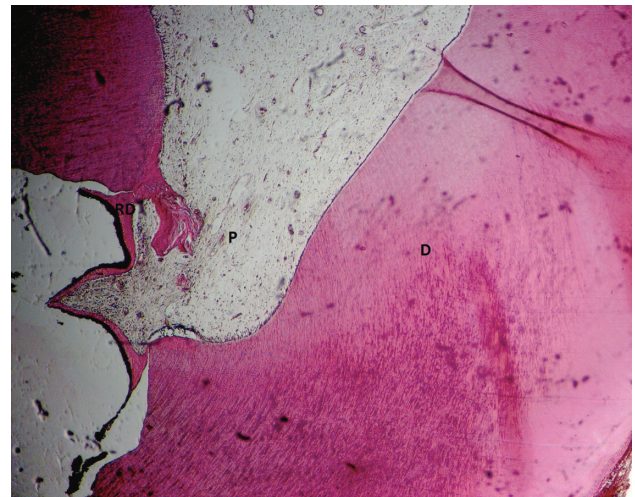


Fig. 5 CH group: Protrusion of pulp tissues into the cavity can be seen at the periphery of the exposed area. Complete hard tissue formation and normal pulp tissue is evident (hematoxylin-eosin, original magnification  $\times 40$ , RD: reparative dentin, D: dentin, P: pulp).

differences are the inclusion of the antibacterial monomer MDPB and sodium fluoride in CPB. All self-etching adhesives have a short-term mild antibacterial effect caused by the acidic monomer that is incorporated in the primer<sup>29</sup>. However, such antibacterial effects caused by low pH are limited, because the acidity of the self-etching primer is neutralized by the buffering capacity of the dissolved calcium ions from the tooth tissue<sup>30</sup>.

Furthermore, the antibacterial activity of self-etching adhesives is reduced after light activation because the acidic monomers are polymerized<sup>31</sup>. Imazato *et al.* reported that in the absence of MDPB, the self-etching primer did not exert any antibacterial effects against *S. mutans* or lactobacilli. This finding demonstrated that the antibacterial effect of CPB mainly depended on the presence of MDPB<sup>20,32</sup>. Another study by Gondim *et al.* clearly demonstrated that CPB exhibited greater antibacterial activity than CSE<sup>33</sup>.

In the cavitated deep carious lesions, the selective removal to soft dentine in which soft carious tissue left over the pulp is recommended because the complete caries removal of the soft infected dentin until the firm hard tissue increases the risk of pulp perforation<sup>34</sup>. The present study however, evaluated the benefit of using CPB as a direct pulp-capping treatment for the teeth with pulps exposed as a result of complete caries removal in cavitated deep lesions to simulate the accidental pulp exposures occurs during complete removal of infected carious tissue. Neither severe inflammation nor severe soft tissue disorganization were observed in any of the pulps capped with CPB after 90 days, although direct pulp capping with CSE caused severe inflammatory reactions. Our findings suggest that the severe pulp reaction observed in the CSE group was due to bacterial invasion of the pulp, which may be caused by the contaminated bur used in the removal of infected carious tissue and perforation of the pulp. On the other hand the antibacterial monomer MDPB in the primer of CPB may have eradicated such bacterial infection to allow normal healing of the pulpal tissue.

A hermetic seal of dentin to prevent bacterial microleakage is also desirable to enhance the long-term success of pulp-capping treatment<sup>35</sup>. The most important aspect in the application of dentin bonding systems is their ability to properly seal the dentinal margins<sup>36</sup>. Self-etching adhesives such as CPB and CSE demineralize dentin only partially and to a limited depth, so that residual hydroxyapatite is still attached to the collagen<sup>37</sup>. Hydroxyapatite has chemical bonding potential with the adhesion-promoting monomer 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) included in both CSE and CPB. The binding of 10-MDP with hydroxyapatite is extremely strong and hydrolytically stable because of the production of a Ca salt, which is barely soluble<sup>38</sup>. Additionally, retaining hydroxyapatite around the collagen offers better protection against hydrolysis, thereby preventing degradation of the bond<sup>39</sup>. Therefore, CPB exhibits superior and more stable bonding, which assists in preventing bacterial microleakage<sup>40</sup>. Additionally, the antibacterial component immobilized by the polymerization of MDPB exhibits inhibitory effects against bacteria<sup>41</sup>, and CPB has been reported to show antibacterial effects even after curing<sup>42</sup>. Such effects may have contributed to the better results observed for direct pulp capping with CPB than CSE.

CH has been used as the material of choice for direct pulp capping for many years because of its bactericidal effect and hard tissue formation ability. However, pulp-

capping materials lack effectiveness if leakage occurs at the interface between the dentin and the pulp-capping material or around the restorative material<sup>43</sup>. Therefore, the solubility of CH, its lack of chemical or mechanical adhesiveness, its potential accelerated degradation during the adhesion bonding process<sup>44</sup>, and its inability to provide effective long-term protection against microleakage are all of concern. For direct pulp-capping treatment, rapid formation of a pulp-protective barrier against bacterial contamination is desirable to minimize the damage to the pulp, but it takes at least a couple of weeks for calcium hydroxide to form a natural dentin bridge<sup>43</sup>. Moreover, 90% of the reparative dentin, usually formed as a localized response to CH, contains multiple tunnel defects that may allow the passage of bacteria and their toxins to the pulp<sup>45</sup>.

Nevertheless, the ability of calcium hydroxide to allow pulp repair and hard tissue formation has been reported in previous histological studies<sup>46,47</sup>. Similarly, in our study, full hard tissue formation was more frequently observed in teeth capped with CH than in teeth capped with CPB. The formation of the hard tissue after the application of calcium hydroxide is mediated *via* the alkaline phosphatase enzyme, stimulated by hydroxyl ions at pH 10.2 and calcium-dependent pyrophosphatase<sup>48</sup>. The alkaline environment appears to favor the further differentiation of pulp cells into odontoblast-like cells, which synthesize and deposit the dentin matrix, giving rise to the hard tissue<sup>49,50</sup>. It has been suggested that fluoride at low concentrations can be a useful therapeutic agent where hard tissue formation is desired. Nakade *et al.*<sup>51</sup> stated that fluoride at micromolar concentrations can stimulate the proliferation and alkaline phosphatase activity of human dental pulp cells. Fluoride at this level stimulates thymidine incorporation into DNA in dental pulp cells, with optimal effects around 50  $\mu\text{Mol}$ , and increases the alkaline phosphatase activity in dental pulp cells by  $177\pm 12\%$ . Extracellular matrix synthesis (Type I collagen) has also been shown to be increased by  $150\pm 8.7\%$ <sup>51</sup>. Therefore, it can be speculated that CPB releases fluoride in an amount that could stimulate hard tissue formation when used as pulp-capping material.

Tziafas *et al.*<sup>18</sup> investigated the pulpal response of dog teeth after pulp-capping treatment with a calcium hydroxide-based material or CPB. Before application of each pulp-capping material, a suspension of streptococci was placed in the cavities and incubated for 3 h to achieve bacterial contamination of the exposed pulps. The results indicated that the cavities treated with calcium hydroxide-based material demonstrated reparative dentin formation with bridging at the exposure site, while hard tissue formation was not observed in any of the teeth treated with CPB. They speculated that the MDPB-containing antibacterial adhesive system maintained pulp vitality and primary odontoblastic function in infected exposed cavities, but interfered with reparative dentin formation in infected pulp. These findings are consistent with the results of some early studies that found that dentin adhesives



interrupted the potential of pulpal cells to express their dentinogenetic activity and resulted in an inflammatory reaction, predominantly with a chronic inflammatory cell infiltration<sup>46,47</sup>. Despite the negative findings of some authors, after direct pulp capping with CPB in non-carious human teeth, Parthasarathy *et al.* observed partial hard tissue deposition<sup>13</sup>, and we observed the formation of a complete hard tissue bridge in our previous study<sup>9</sup>. Similarly in uninfected dog teeth, Akhavan *et al.* also demonstrated odontoblast layer generation and hard tissue formation in some teeth following direct pulp capping with different bonding systems including CSE<sup>52</sup>. In agreement with the statement that bacterial infection is one of the most important reasons for the failure of pulp-capping procedures, the difference between our results and those of Tziafas *et al.*<sup>18</sup> is probably due to the different conditions of bacterial exposure and infection level. Specifically, the dog pulp in the study by Tziafas *et al.*<sup>18</sup> was artificially infected by inoculating the pulp with a single species of streptococcus, while we conducted a clinical study to apply CPB to pulp exposed by caries removal. Although it would be desirable to study more clinical cases, the present results obtained in our human clinical study support the use of direct pulp-capping materials with antibacterial effects to preserve the vitality of pulp exposed during caries removal.

## CONCLUSION

Within the limitations of the present *in vivo* study, the results showed that CPB containing the antibacterial monomer MDPB maintained pulp vitality and induced partial hard tissue deposition in exposed areas after capping of pulpal exposures formed during the removal of carious dentin tissue in deep carious lesions.

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