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Histological evaluation of direct pulp capping of rat pulp with experimentally developed low-viscosity adhesives containing reparative dentin-promoting agents

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ABSTRACT

Objectives: This study examines the wound healing process in exposed rat pulp when capped with experimental adhesive resin systems.
Methods: Experimental adhesive resin system for direct pulp capping was composed of primer-I (PI), -II (PII), and -III (PIII) and an experimental bonding agent (EBA). PI was Clearfil® SE Bond®/Primer (CSP) containing 5.0 wt% $CaCl_2$, PII was PI containing 10 wt% nanofiller (Aerosil® 380), and PIII was CSP containing 5.0 wt% of compounds of equal moles of synthetic peptides (pA and pB) derived from dentin matrix protein 1. EBA was Clearfil® SE Bond®/Bond (CSB) containing 10 wt% hydroxyapatite powders. Three experimental groups were designed. PI was assigned to experimental Groups 1 and 3. PII was assigned to experimental Groups 2 and 3. PIII and EBA were assigned to all experimental adhesive groups. Control teeth were capped with calcium hydroxide preparation (Dycal®), and CSP and CSB were applied to the cavity. The rats were sacrificed after each observation period (14, 28, 56, and 112 days). The following parameters were evaluated: pulp tissue disorganization, inflammatory cell infiltration, reparative dentin formation (RDF), and bacterial penetration.
Results: There were no significant differences among all the groups for all parameters and all observation periods ($p > 0.05$, Kruskal–Wallis test). All groups showed initial RDF at 14 days postoperatively and extensive RDF until 112 days postoperatively. Groups 2 and 3 demonstrated higher quantity of mineralized dentin bridge formation compared with Group 1.
Conclusions: Addition of nanofillers to the primer was effective in promoting high-density RDF.
Clinical significance: Experimentally developed adhesive resin systems induce the exposed pulp to produce almost the same quantity of reparative dentin as calcium hydroxide. However, we need further studies to elucidate whether the same results could be obtained in humans.

1. Introduction

Previous studies have investigated the effects of various materials such as hydroxyapatite [1], tri-calcium phosphate (TCP) [2], and mineral trioxide aggregate (MTA) [1,3,4] on direct pulp capping. Several recent studies have reported that MTA is highly efficient in inducing reparative dentin formation (RDF) without inflammatory responses in the pulp [1,3,4]. However, its limitations include difficulty in handling, poor adhesion to tooth substrate, and high cost. The ideal pulp capping material should exhibit adequate adherence to the dentin around the pulp exposure site so as to prevent bacterial invasion into the pulp tissue.

The bioactive efficacy of dental adhesive materials as well as their effectiveness in adhering to tooth substrate has been studied previously [5–7]. Some studies have suggested that adhesive resins should not be applied to the exposed pulp as they have poor biocompatibility, are cytotoxic, cause bacterial leakage, and affect dentin bridge formation [8–13]. Additionally, adhesive systems using the "total etch" technique have been shown to have disastrous effects during direct pulp capping in primates [14]. However, many researchers have attempted to use adhesive resins as a direct pulp capping agent [15–19] as it requires simple

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procedure and provides effective bonding to prevent bacterial invasion. In several previous studies including our study [20–22] the pulp was directly capped with several adhesive resins and almost normal tissue morphology without severe reactions was observed throughout the observation period. However, teeth capped with adhesive resins showed delayed RDF compared with those capped with calcium hydroxide [20–22]. Therefore, we examined the effects of using experimentally developed adhesive resin systems containing reparative dentin-promoting agents for direct pulp capping. To improve the efficacy of adhesive resin systems in inducing RDF at the exposed pulp site, we collaborated with Kuraray Noritake Dental Inc. and fabricated an experimental adhesive system containing calcium chloride ($CaCl_2$), synthetic peptides (pA and pB) derived from dentin matrix protein 1 (DMP1), and hydroxyapatite (OHAp). This system can be used for direct pulp capping [23–25], and the procedure for dentin bridge formation is as follows: first, the primer containing $CaCl_2$ is applied to the exposed pulp, to supply calcium ions for hard tissue formation. Second, application of the other primer, containing pA and pB, to nucleate formation of OHAp by binding with calcium ions. Finally, the bonding agent containing OHAp is applied as a scaffold for RDF.

When this adhesive system was applied to exposed rat pulp, the addition of $CaCl_2$, pA, and pB to the primers and incorporation of OHAp in the bonding agent were fairly effective in promoting dentin bridge formation for 14–28 days postoperatively [25]. However, the dentin bridge formed by this system showed some problems. In particular, the reparative dentin was observed at a position that was deeper than the pulp exposure site. The dentin bridge also exhibited a specific layered structure, with the pulp tissue existing between the layers. We speculated that the dentin-promoting agents, $CaCl_2$, pA, and pB, may have diffused into the depths of the pulpal exposure site because of penetration of the primers and caused formation of a layered dentin bridge. Therefore, we created an experimental low-viscosity primer (LVP), containing $CaCl_2$ and nanofillers, to control primer penetration at the exposed pulp surface.

The purpose of this study was to examine the wound healing process in exposed rat pulp tissue, especially the formation of reparative dentin, when capped with the experimental adhesive system. The null hypothesis of this study was that the LVP containing $CaCl_2$ and nanofillers would not affect wound healing and dentin bridge formation on the exposed rat pulp.

2. Materials and methods

2.1. Experimental animals

The sample consisted of a total of 48 rats (Sprague-Dawley male rats, 6 weeks old and about 180 g in weight), including those that died during the experiment. The rats were fed solid food (MF, Oriental Yeast Co., Tokyo, Japan) and water for 2 weeks in the cages of the breeding house affiliated with our university. A total of 80 noncarious, upper (maxillary) first molars were treated with direct pulp capping when the rodents were 8–9 weeks old and weighed 300–400 g. Five teeth were assigned to each experimental group. The following teeth were excluded: those with large cavities and pulp exposures, teeth with no bleeding, and those with suspected fractures. This study was approved by the Laboratory Animal Committee of The Nippon Dental University, School of Life Dentistry at Niigata (receipt and permission number: 117).

2.2. Experimental groups and observation periods

Table 1 summarizes the composition of the materials used in this study. Clearfil® SE Bond®/Primer (CSP, Lot #00297A, Kuraray Noritake Dental Inc., Tokyo, Japan) and Clearfil® SE Bond®/Bond (Lot #01468A, Kuraray Noritake Dental Inc.) served as bases for the experimental self-etching primer and bonding agent, respectively. The experimental direct pulp capping adhesive resin system was composed of primer-I (PI), -II (PII), and -III (PIII) and the experimental bonding agent (EBA) (Table 2). PI was CSP containing 5.0 wt% $CaCl_2$ powder (Lot #100616, Kuraray Noritake Dental Inc.). PII was an LVP made up of CSP containing 10 wt% nanofiller (Aerosil® 380, hydrophilic fumed silica, specific surface area of 380 m^2/g, Nippon Aerosil Co., Tokyo, Japan) and 5.0 wt% $CaCl_2$. PIII was CSP containing 5.0 wt% of compounds of equal moles of pA and pB (Kuraray Noritake Dental Inc.), which are synthetic peptides derived from DMP1. EBA was made up of CSB containing 10 wt% OHAp powders (Lot #100617, Kuraray Noritake Dental Inc.). The $CaCl_2$ and OHAp were mixed in the appropriate materials just before application in the cavities. A summary of the experimental groups is given in Table 3. In experimental Group 1, PI was applied first in the cavity, followed by PIII. In experimental Group 2, PII was applied first followed by PIII, and in experimental Group 3, PI and PII were applied first and were then followed by application of PIII. EBA was applied in all experimental groups. In the control group,

Table 1
Composition of the different materials used in this study.

Material		Abbreviation	Lot #	Composition	Manufacturer
Clearfil® SE Bond®	Primer	CSP	00297A	HEMA;hydrophilic dimethacrylate; MDP; *N*,*N*-diethanol-*p*-toluidine; *d*,*l*-camphorquinone; water	Kuraray Noritake Dental Inc.
	Bond	CSB	01468A	Bis-GMA, HEMA, *d*,*l*-camphorquinone, hydrophobic aliphatic dimethacrylate, MDP, amine	
Calcium chloride powder		$CaCl_2$	100616	–	Kuraray Noritake Dental Inc.
Synthetic peptide derived from dentin matrix protein 1		pA and pB	–	pA (residues 386–390), M_w = 578.51 pB (residues 414–422), M_w = 1036.92	Kuraray Noritake Dental Inc.
Hydroxyapatite powder		OHAp	100617	$Ca_{10}(PO_4)_6(OH)_2$	Kuraray Noritake Dental Inc.
Low-viscosity primer		LVP	0100616-3	CSP containing 10 wt% nanofiller (Aerosil® 380)	Kuraray Noritake Dental Inc.
Clearfil® AP-X		APX	1342A	Bis-GMA, TEG-DMA, barium glass, silanated colloidal silica, *d*,*l*-camphorquinone	Kuraray Noritake Dental Inc.
Dycal®		DY	100607	Base paste: ester glycol salicylate, calcium phosphate, Ca tungstate, ZnO Catalyst paste: ethylene toluene sulfon amide, $Ca(OH)_2$, ZnO_3 Ti_2O, Zn stearate	Dentsply International Inc.

Bis-GMA, bisphenol A glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; M_w, molecular weight; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; TEG-DMA, triethylene glycol dimethacrylate.