Dentin bond strength of a new adhesive system containing calcium phosphate experimentally developed for direct pulp capping

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The purpose of this study was to evaluate the microtensile bond strength (μ TBS) to human dentin of an experimental bonding agent containing calcium phosphates experimentally developed for direct pulp capping. Different concentrations of four types of calcium phosphates were added to an experimental bonding monomer, and six experimental bonding agents were thus prepared. Clearfil SE Bond/Bond was used as the control. Flat dentin surfaces of human molars were assigned to the experimental adhesive systems and the control. After Clearfil SE Bond/Primer was applied to the dentin surface, each experimental bonding agent was applied and photopolymerized, and then a resin composite paste was placed and photopolymerized. The specimens were subjected to μ TBS testing. Results revealed that there were no significant differences among the μ TBS values of the experimental bonding agents and the control. In other words, the calcium phosphate-containing experimental adhesives did not adversely affect the μ TBS to dentin.

Keywords: Experimental bonding agent, Dentin bond strength, Direct pulp capping

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INTRODUCTION

When incidental pulp exposure occurs, calcium hydroxide is often used as a direct pulp capping agent. This is because its antibacterial effect is instrumental in inducing reparative dentin formation, thereby contributing to pulp healing^{1–3}. However, calcium hydroxide also has some disadvantages, such as the presence of a necrotic layer at the pulp exposure site, tunnel defects in dentin bridge formation⁴, lack of adhesive properties, and insufficient mechanical strength. On these grounds, the search and hunt is on for alternative direct pulp capping materials^{5–17}.

In the pursuit for a direct pulp capping material that circumvents the existing weaknesses, focus was trained on the effects of adhesive resins on the healing and dentin bridge formation of exposed pulp^{12–14}. Medina *et al.*¹² compared the pulp response to seven adhesive systems and their accompanying resin composite systems with that of a commercial calcium hydroxide material when applied to exposed monkey pulp. They concluded that the pulp response to four of seven adhesive systems was not significantly different from the calcium hydroxide application; however, calcium hydroxide could induce earlier and more consistent reparative dentin formation. For other studies^{5–7} which have also investigated direct pulp capping with adhesive systems, the adhesive systems applied to exposed pulp did not cause any pulp irritation, but dentin bridge formation was later than that of calcium hydroxide application. In light of these affirmative results, a new experimental bonding agent

as a direct pulp capping material was developed and it contained calcium phosphate to promote reparative dentin formation^{13,14}.

With the experimental bonding agent containing calcium phosphate to promote dentin bridge, Suzuki and Katoh *et al.*¹³ examined its effect on the wound healing process of rat pulp. Their results showed that the addition of calcium phosphate to the adhesive was indeed efficacious in promoting reparative dentin formation. In addition, Kato *et al.*¹⁴ showed that experimental bonding agents containing different types of calcium phosphate (hydroxyapatite, brushite, whitlockite, and octacalcium phosphate) were similarly effective reparative dentin promoting agents.

Upon examining the polymerized, calcium phosphate-containing bonding agents by using scanning electron microscopy (SEM), electron probe microanalysis (EPMA), and inductively coupled plasma atomic emission spectroscopy (ICP-AES), Katoh *et al.*¹⁸ found that calcium phosphate particles were dispersed in the experimental bonding agents and that calcium and phosphorus ions were eluted from each type of calcium phosphate. In light of these histopathological findings, Katoh *et al.*¹⁸ attributed the efficacious promotion of reparative dentin formation by the experimental calcium phosphate-containing bonding agents to the following contributing factors. Direct contact of calcium phosphate with the exposed pulp promoted calcification, while elution of calcium and phosphorous ions from calcium phosphate resulted in pH change in the local environment of the exposed pulp, hence exerting antimicrobial activity and stimulating dentin bridge

formation.

Apart from promoting dentin bridge formation, the experimental, calcium phosphate-containing bonding agent also holds out the promise of a simplified procedure for direct pulp capping treatment. However, there belies a possibility that the addition of calcium phosphate to a bonding agent may reduce the bond strength to dentin and compromise the integrity at resin-dentin interface.

The purpose of this study, therefore, was to evaluate the microtensile bond strengths (μ TBS) to human dentin of experimentally developed, calcium phosphate-containing bonding agents. The null hypothesis of this study was that the addition of a calcium phosphate to the bonding agent would not compromise the bond strength of resin composite to human dentin.

This study was approved by the Ethics Committee of The Nippon Dental University School of Life Dentistry at Niigata (Approval Number: 121).

MATERIALS AND METHODS

Materials used

The materials used in the study are presented in Table 1. Four types of calcium phosphates were used in this study, namely: hydroxy calcium phosphate (hydroxyapatite; Ube Material Industries Ltd.,

Yamaguchi, Japan), dicalcium phosphate dihydrate (brushite; Nacalai Tesque, Inc., Kyoto, Japan), beta-tricalcium phosphate (whitlockite; Taihei Chemical Industrial Co. Ltd., Osaka, Japan), and octacalcium phosphate (Taihei Chemical Industrial Co. Ltd., Osaka, Japan).

A total of six experimental bonding agents were prepared in this study, whereby each type of calcium phosphate was added — in varying concentrations — to an experimental bonding monomer (Kuraray Medical Inc., Osaka, Japan) immediately before applying to the dentin surface. For instance, in the case of 10H, 7 mg of hydroxyapatite powder was mixed with three drops of experimental bonding monomer (63 mg) in a disposable dish using a microbrush. The concentrations of calcium phosphates (wt%) in the respective experimental bonding agents are presented in Table 2. A self-etching adhesive system (Clearfil SE Bond, Kuraray Medical Inc., Osaka, Japan) was used as the control.

Tooth specimens

Thirty-five extracted human molars were used in this study. The teeth were collected after informed consent was obtained from the patients under a protocol approved by the institutional review board of The Nippon Dental University School of Life Dentistry at Niigata. They were cleaned and stored in 0.01%

Table 1 Materials used in the present study

Materials	Manufacturers	Lot No.	Compositions
Experimental monomer	Kuraray Medical	050705	MDP, HEMA, Bis-GMA, PI, Hydrophobic aliphatic dimethacrylate
Clearfil SE Bond Primer Bond	Kuraray Medical	0768AA 1112AA	MDP, HEMA, Water, PI MDP, HEMA, Bis-GMA, Silica filler, PI
Clearfil AP-X	Kuraray Medical	1218AA	Barium glass filler, Silica filler, TEGDMA, Bis-GMA, PI
Clearfil Majesty LV		0202AA	Barium glass filler, Silica filler, TEGDMA, PI
Hydroxyapatite	Ube Material	030606	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$
Brushite	Nacalai Tesque	M7H6575	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$
Whitlockite	Taihei Chemical	04080401	$\text{Ca}_3(\text{PO}_4)_2$
Octacalcium Phosphate	Taihei Chemical	SA3131	$\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$
AD-Gel	Kuraray Medical	00720B	10% sodium hypochlorite
Purelex	Oyalox	3675	6% sodium hypochlorite
Oxydol	Yoshida Pharmaceutical	AP002	3% hydrogen peroxide
Physisalz PL-D	Fuso Pharmaceutical Industries	06H03C	physiological saline solution

MDP: 10-methacryloxydecyl dihydrogen phosphate

HEMA: 2-hydroxyethyl methacrylate

PI: Photoinitiator

Bis-GMA: 2,2-Bis[4-(2-hydroxy-3-methacryloxypropoxy) phenyl]propane

TEGDMA: Triethylene glycol dimethacrylate

Table 2 Concentration of calcium phosphate in each experimental bonding agent (wt%)

Calcium phosphates	Experimental bonding agents					
	5H	10H	5B	5W	5OP	5H5W
Hydroxyapatite	5	10				5
Brushite			5			
Whitlockite				5		5
Octacalcium phosphate					5	

thymol solution at 4°C until use. The occlusal surfaces of the extracted human molars were ground to flat dentin with 120-grit silicon carbide paper (CarbiMet, Buehler Ltd., Lake Bluff, IL, USA) and finished with 600-grit paper using a polishing machine (Lewel Specimen Polisher, Kasai Co. Ltd., Yokohama, Japan) under water irrigation. They were then randomly divided into seven groups of five specimens each.

Bonding procedure

A double-sided adhesive tape (0.12 mm thickness) with a 6-mm-diameter opening was attached to the flat dentin surface to define the bonding area. After the laminated paper was peeled from the attached adhesive tape, a transparent acrylic tube (6 mm diameter, 3 mm height) was placed on the adhesive tape. The dentin surface was treated with 10% sodium hypochlorite (NaClO) gel (AD Gel, Kuraray Medical Inc., Osaka, Japan) for one minute, followed by alternate irrigation with 3% hydrogen peroxide (H₂O₂, Oxydol; Yoshida Pharmaceutical Co., Tokyo, Japan) and 6% NaClO (Purelox, Oyalo Co., Tokyo, Japan). The latter was performed three times as a hemostasis procedure before the application of each experimental bonding agent. The dentin surface was then rinsed with 2 ml of physiological saline solution (Physisal PL-D, Fuso Pharmaceutical Industries Ltd., Osaka, Japan) and gently air-dried.

Each experimental bonding agent was applied to the specimens according to the following bonding procedure. Clearfil SE Bond/Primer (Kuraray Medical Inc., Osaka, Japan) was applied to the dentin surface, and the surface was left undisturbed for 20 seconds, followed by five seconds of intensive air-blowing. Each calcium phosphate powder was dispersed in the experimental bonding monomer, and then the mixture was applied to the dentin surface. The surface was gently air-blown and photopolymerized with a light curing unit (Candelux, J Morita Co., Tokyo, Japan) for 10 seconds.

For the control, Clearfil SE Bond was applied to the specimens according to the manufacturer's instructions.

After photopolymerizing the bonding agent, a flowable resin composite paste (Clearfil Flow FX, Shade A3, Kuraray Medical Inc., Osaka, Japan) was filled into the acrylic tube (approximately 1.0 mm in thickness) and then photopolymerized with a light curing unit for

20 seconds. A resin composite paste (Clearfil AP-X, Shade A3, Kuraray Medical Inc., Osaka, Japan) was further added to the existing composite surface (approximately 2.0 mm in thickness) and filled in excess over the top of the acrylic tube. The flat tip surface of the light curing unit was pressed on the surface of the resin composite paste through a clear plastic sheet to obtain a flat surface, and then the resin composite paste was photopolymerized for 40 seconds. After polymerization, the translucent acrylic tube was removed and the specimens were stored in distilled water at 37°C for 24 hours.

Microtensile bond strength test

After 24-hour water storage, the roots were removed using a diamond point (Bur No. 105R, ISO size 22, Shofu Inc., Kyoto, Japan), and pulp tissue was removed from its coronal parts. The specimens were then serially sectioned in a longitudinal manner into 1-mm-thick slabs using a low-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water cooling. Two slabs were obtained from each specimen, and each slab was sectioned into beams of which the cross-sectional area was approximately 1 mm² using a low-speed diamond saw. Two beams were obtained from each slab, such that four beams were obtained from each specimen. In total, 20 beams were obtained for each experimental group and used for microtensile bond strength testing.

The beam specimens were attached to a testing device (Bencor-Multi-T, Danville Engineering Co., San Ramon, CA, USA) with cyanoacrylate (Model Repair Pink, Dentsply-Sankin, Tochigi, Japan), which was placed into a tabletop material tester (EZ Test, Shimadzu Co., Kyoto, Japan) and then subjected to microtensile testing at a crosshead speed of 0.5 mm/min.

Failure mode analysis

Fractured surfaces of the specimens were examined with a stereomicroscope (Leica EZ4D, Leica Microsystems Ltd., Heerbrugg, Switzerland) at ×35 magnification, and the fracture modes thereby determined. Modes of failure were classified as follows: Adhesive – failure occurred entirely within the adhesive area; Cohesive in resin composite – failure occurred exclusively within the resin composite area; Cohesive in dentin – failure occurred exclusively within the

dentin area; and Mixed – failure continued from the adhesive into either the resin composite or dentin area.

Several representative samples were selected from each group for a precise analysis of the fractured surfaces with a scanning electron microscope (SEM; S-800, Hitachi Ltd., Tokyo, Japan) at an accelerating voltage of 15 kV after being sputter-coated with palladium and platinum.

Statistical analysis

Data of the μ TBS tests were statistically analyzed

using one-way ANOVA, followed by Bonferroni/Dunn *post hoc* test to compare the μ TBS values among the six experimental groups and the control at a 95% confidence level.

RESULTS

Table 3 shows the μ TBS test results of the experimental adhesive systems and the control and their respective failure modes. The lowest mean value of μ TBS was exhibited by 5B at 45.1 MPa, while the highest mean

Table 3 Microtensile bond strength results and failure modes for each group

Group	μ TBS* (Mean $\pm$ SD, MPa)	Failure modes**			
		Cfd	Cfr	Mf	Af
5H	47.2 $\pm$ 10.7 ^a	8 (40%)	3 (15%)	2 (10%)	7 (35%)
10H	49.2 $\pm$ 7.7 ^a	6 (30%)	1 (5%)	1 (5%)	12 (60%)
5B	45.1 $\pm$ 5.6 ^a	2 (10%)	0	5 (25%)	13 (65%)
5W	46.4 $\pm$ 9.5 ^a	10 (50%)	0	1 (5%)	9 (45%)
5OP	47.6 $\pm$ 6.4 ^a	4 (20%)	0	1 (5%)	15 (75%)
5H5W	48.7 $\pm$ 8.7 ^a	7 (35%)	2 (10%)	2 (10%)	9 (45%)
Control	52.7 $\pm$ 8.7 ^a	1 (5%)	2 (10%)	3 (15%)	14 (70%)

* Values with the same superscript letters indicate no significant difference ($p > 0.05$)

** Cfd: Cohesive failure in dentin, Cfr: Cohesive failure in resin composite, Mf: Mixed failure, Af: Adhesive failure

Fig. 1 SEM photographs of debonded surfaces for each failure mode: (a) adhesive failure; (b) cohesive failure in dentin; (c) cohesive failure in resin composite; (d) mixed failure. Original magnification: $\times 35$ (upper row); $\times 1000$ (lower row).

value of μ TBS was exhibited by the control at 52.7 MPa. On the overall, all the experimental adhesive systems exhibited lower mean values of μ TBS than the control. However, ANOVA revealed that there were no significant differences among the μ TBS values of the experimental adhesive systems and the control ($F=1.7$, $p=0.12$).

Failure mode analysis results revealed that the predominant mode of failure was adhesive failure (56.4%), followed by cohesive failure in dentin (27.2%). The incidence of cohesive failure in dentin was almost the same as that of adhesive failure in the experimental groups of 5H, 5W, and 5H5W. Amongst all the failure modes, the incidence of cohesive failure in resin composite ranked the lowest at 5.7%.

Figure 1 shows the representative SEM photographs of the debonded surfaces for each failure mode after μ TBS testing. Numerous dentinal tubules could be seen on the debonded surfaces at the dentin side in the lower photographs of (a), (b), and (d).

DISCUSSION

Based on the results obtained, the null hypothesis that the addition of a calcium phosphate to a bonding agent would not compromise the dentin bond strength of resin composite was accepted. This result might be explained as follows. Our previous study using SEM, EPMA, and ICP-AES showed that the calcium phosphate particles were not dissolved in the resin monomer, but rather were well dispersed within the polymerized experimental bonding agents¹⁸. Since the particles were not dissolved, the shapes and approximate mean sizes of the dispersed calcium phosphate particles could be clearly identified by SEM evaluation. For hydroxyapatite, the particles were of a spherical shape with a length of 10 μ m; for brushite, it was a mass of small, plate-shaped particles with a length of 30 μ m; for whitlockite, the particles were fine granular deposits with a length of 2 μ m; and for octacalcium phosphate, it was a mass of fine, needle-shaped particles with a length of 10 μ m¹⁸.

On the impact of incorporating calcium phosphate particles in the experimental bonding agents, they might have altered the ability of monomer diffusion into the dentin surface due to viscosity change although the bonding agents' chemical properties are not affected. Consequently, reduced monomer penetration might then result in the slightly lower dentin bond strengths of all the experimental adhesive systems as compared to the control. Nonetheless, the dentin bond strengths of all the experimental calcium-phosphate containing bonding agents were deemed sufficient at 24 hours postoperatively. However, it is possible that the bond strength would gradually decrease with the passage of time. This could occur because the physical properties of the bonding layer might become diminished due to the elution of calcium and phosphorous ions from calcium phosphate.

To date, there have been many reports on the

effects of NaClO application after acid-etching on bond strength to dentin¹⁹⁻²⁷. On one hand, Prati *et al.*¹⁹ and Saboia *et al.*²⁰ reported that NaClO treatment removed the unprotected collagen layer after acid-etching and increased the dentin bond strength of some adhesive systems. On the other hand, Nikaido *et al.*²¹ and Perdigao *et al.*²² reported that NaClO treatment damaged the organic components of dentin and accounted for a lower bond strength to dentin. On the application of 10% NaClO to the dentin surface followed by alternate irrigation with 3% H₂O₂ and 6% NaClO before primer application, no reports are yet available on the effects of this procedure on dentin bond strength.

In this study, the hemostasis procedure entailed treating the dentin surface with 10% NaClO gel for one minute, followed by three-time alternate irrigation with 3% H₂O₂ and 6% NaClO before the application of each experimental bonding agent. Based on the result obtained in this study, this pretreatment of the dentin surface did not adversely affect the dentin bond strength. The μ TBS value of Clearfil SE Bond to dentin with this pretreatment was 52.7 MPa in this study, which was significantly higher than the 29.2 MPa obtained for the same adhesive without pretreatment in a previous study²⁸. This significant difference in μ TBS value hence suggested that pretreating the dentin surface with 10% NaClO gel followed by alternate irrigation with 3% H₂O₂ and 6% NaClO might be effective in increasing the dentin bond strength of self-etching primer systems.

Although NaClO is known to be effective in removing organic components, Perez-Heredia *et al.*²⁷ reported that 2.5% NaClO had a slight decalcifying effect²⁷. Therefore, it might be suggested that the smear layer on the dentin surface was removed after 10% NaClO gel application due to the latter's ability to dissolve both organic and inorganic components. Consequently, removal of the smear layer with 10% NaClO gel application resulted in the formation of a resin-impregnated layer and resin tags in the dentinal tubules after applying the self-etching primer system, thereby leading to an increase in dentin bond strength.

In dentin adhesion experiments, distilled water is commonly used as the irrigation fluid. In the present study, physiological saline solution was used to simulate the clinical treatment of exposed pulp. When the dentin surface was air-blown after irrigation with physiological saline solution, sodium chloride residues remained on the dentin surface. However, it seemed that the influence of residual substances was negligible as the μ TBS value obtained in this study was quite comparable to the values reported in another study²⁹ where distilled water was used for irrigation. Most probably, the sodium chloride remnants dissolved in the aqueous self-etching primer which was subsequently applied on the dentin surface, and then removed by air dispersion. Further, due to its fairly low concentration (0.9%) in the physiological saline solution, the sodium chloride remnants did not

interfere with the components of the self-etching primer and affect the latter's efficacy. For these two reasons, physiological saline solutions could be considered to be safe for use in clinical situations.

According to failure mode analysis, adhesive failure occurred most frequently for the experimental groups of 10H, 5B, 50P and the control. However, the incidence of adhesive failure was almost the same as that of cohesive failure in the experimental groups of 5H, 5W, and 5H5W. Further, the failure modes after μ TBS testing showed no correlations with the μ TBS values — which was in agreement with the results of previous studies. It has also been pointed out that the direction of force applied on the beam specimens influenced the measured values in a μ TBS test³⁰. Accordingly then, the position of the beam specimen to the pull-out test apparatus might have influenced the failure mode results.

Our previous study showed that calcium phosphate-containing bonding agents were efficacious in promoting reparative dentin formation. In the present study, it was shown that these selfsame bonding agents exhibited adequate bond strength. In light of these encouraging results, further follow-up investigations should be carried out to bridge the gap in realizing the potential use of these bonding agents as direct pulp capping materials.

CONCLUSION

Based on the results of this study, it was concluded that the incorporation of calcium phosphates in experimental bonding agents did not affect their μ TBS values to dentin.

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