Influence of blood contamination before or after surface treatment on adhesion of 4-META/MMA-TBB resin to root dentin

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INTRODUCTION

The diffusion and polymerization of adhesive monomers into the demineralized dentin layer and the formation of a hybrid layer are important to achieve durable adhesion between dentin and adhesive resin. However, in the clinical situation, several factors such as gingival fluid, saliva and blood contaminants reduce the adhesive property. Some studies have reported that blood contamination particularly impaired the adhesion between dentin and resin.

4-methacryloxyethyl trimellitate anhydride/methyl methacrylate-tri-n-butylborane (4-META/MMA-TBB) resin has been widely used for bonding crowns, restorations, orthodontic brackets and temporary splints for periodontal disease because of its remarkable long-term adhesive properties to enamel and dentin. Furthermore, because of its high biocompatibility to dental pulp and periodontal tissue, it has been recently applied for direct pulp capping, retrograde root-end sealing following apicectomy or intentional replantation, sealing of perforation sites and the bonding treatment of vertically fractured roots. These procedures often induce operative hemorrhage and blood contamination of the adherent dentine surface, which result in inferior bonding and increased risk of resin detachment. However, the influence of blood contamination on adhesion of 4-META/MMA-TBB resin to dentin has to be further clarified.

On the other hand, several studies have investigated the influence of blood contamination on adhesion of other restorative resins. Some studies on a conventional three-step adhesive system, which consisted of acid etchant, primer and adhesive, revealed that blood contamination at any step of the adhesive process reduced the bond strength.

The result suggested that residual blood contaminants might interfere with priming and resin impregnation into decalcified dentin.

Another study using the wet bonding system, in which the dentin surface was not dried after acid-etching, reported that blood contamination before primer application did not strongly affect the bond strength after washing because the acidic primer itself could clean the blood and highly penetrate into the low-decalcified dentin.

As for the self-etching adhesive system, it was reported that blood contamination before primer application did not strongly affect the bond strength because of the primer itself. These reports suggest that the influence of blood contamination on resin adhesion differs with dentine adhesive systems, the timing when the contamination occurred in the adhesive process and so on.

In addition, it was shown that with self-adhesive resin cement, which contains acidic phosphate monomer (MDP), blood contamination of the adherent surface can influence the bond strength to dentin. When blood contamination occurred before the collagen fibers were collapsed, the bond strength was less sensitive to contamination before etching.

These results revealed that the influence of blood contamination differed with the adherent surface conditions.

4-META/MMA-TBB resin is a self-cure adhesive resin cement based on MMA with a monomer 4-META and a catalyst TBB. The dentin surface is treated with an aqueous solution of 10% citric acid and 3% ferric chloride that removes the smear layer and, at the same
time, stabilizes the exposed collagen\textsuperscript{23}. These may explain the difference in the influence of blood contamination on adhesion from other resins.

The purpose of this study was to evaluate the influence of blood contamination on the leakage and bond strength of 4-META/MMA-TBB resin to dentin.

MATERIALS AND METHODS

Specimen preparation

Twenty-four extracted bovine incisors were used in this study. 4-META/MMA-TBB resin (SB; Super Bond C&B\textsuperscript{®}, clear type, Sun Medical, Shiga, Japan) was used as the adhesive resin cement (Table 1). Fresh capillary blood obtained from one of the authors was used without anticoagulants as the contaminant. This study was approved by the Medical Research Ethics Commission of Graduate School of Dental Medicine, Hokkaido University.

After superficial soft tissue and cementum were removed from the root of each tooth with a curette-type scaler, the crown was cut off at the cemento-enamel junction with a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA). The root pulp was removed with a barbed broach. The exposed surface was then polished with 600-grit silicon carbide paper to standardize the superficial morphology. Six specimens were obtained from one root. Thirty-six specimens were used for SEM observation of the dentin surface, 60 specimens for the dye leakage test and SEM observation of the adhesive interface, and 48 specimens for the microtensile bond strength test.

Table 1  Materials used in this study

<table>
<thead>
<tr>
<th>Products/Materials</th>
<th>Composition</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Super-Bond C&amp;B</td>
<td>5% 4-META, MMA</td>
<td>Sun Medical, Shiga, Japan</td>
</tr>
<tr>
<td>Monomer</td>
<td>TBB</td>
<td>PMMA</td>
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<tr>
<td>Catalyst</td>
<td></td>
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<tr>
<td>Polymer</td>
<td>10% citric acid, 3% ferric chloride, water</td>
<td></td>
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<tr>
<td>Green Activator</td>
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<tr>
<td>Bovine teeth</td>
<td></td>
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<tr>
<td>Human blood</td>
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</tbody>
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4-META: 4-methacryloxyethyl trimellitate anhydride; MMA: methyl methacrylate; TBB: tri-$\textit{n}$-butylborane; PMMA: polymethyl methacrylate.

![Fig. 1](image)

Fig. 1  Blood contamination and adhesive procedures.
Blood contamination and adhesive procedures
Specimens were randomly divided into six groups, as described below (Fig. 1).
2. Group Wb: Water-rinse to remove blood contamination before surface treatment.
5. Group RT: Surface re-treatment following water-rinse to remove blood contamination after surface treatment.

The surface treatment of dentin was carried out with an aqueous solution of 10% citric acid and 3% ferric chloride (10-3 solution; activator Green®, Sun Medical, Shiga, Japan) for 5 s, and the surface was rinsed with water for 20 s and air-dried. Blood contamination was carried out as follows: the exposed dentin surface was immediately covered with blood taken from the fingertip with lancet (Singlestick® II, Misawa Medical Industry, Tokyo, Japan) before or after the surface treatment, and the contaminant was left on the surface for 15 s. After 15 s of exposure, the contaminated surfaces were strongly air-blown, rinsed with water for 15 s, or additively re-treated with the 10-3 solution for 5 s after they were rinsed with water to remove the contaminants. The specimens that were not contaminated were used as controls.

SEM observation of the dentin surface
Thirty-six specimens were prepared for observation of the dentine surface after polishing, just after blood contamination or just before applying SB, from each group previously described. These specimens were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) overnight at 4°C, then rinsed 3 times in a 0.1 M sodium cacodylate buffer for 20 min per rinse and in water for 1 min, followed by dehydration in a graded series of ethanol for 20 min. Each specimen was dried in a critical point dryer (HCP-1, Hitachi, Tokyo, Japan), sputter-coated with Pt-Pd (Ion sputter E-1030, Hitachi, Tokyo, Japan) before or after the surface treatment, and the contaminant was left on the ground dentin surface was covered with a smear layer (Fig. 2a). When blood contamination on the ground dentin surface was air-blown before surface treatment with 10-3 solution (group Ab), the dentin surface was covered with a smear layer.

Dye leakage test
Sixty specimens (n=10 per group) were subjected to the dye leakage test. SB was applied on the treated surfaces of each group according to the manufacturer’s instructions using a bulk-mix technique. The diameter of each group was measured by means of a digital micrometer (Mitutoyo CD-15C, Mitutoyo, Kawasaki, Japan). The specimens were then attached to the testing apparatus using a cyanoacrylate adhesive (Model Repair II Blue, Dentsply-Sankin Industry, Ohtawara, Japan) and subjected to microtensile bond testing in a tabletop material tester (EZ Test, Shimadzu, Kyoto, Japan) with a cross-head speed of 1.0 mm/min. MTBS (in MPa) was calculated by dividing the applied maximum force (N) resulting in fracture by the bonded area (mm²).

Microtensile bond strength test
Forty-eight specimens (n=12 per group) were examined for microtensile bond strength (MTBS). A PMMA rod was attached to each specimen with SB. All specimens were stored in distilled water at 37°C for 24 h. Each specimen, approximately 1 mm² in cross-section, was cut perpendicularly to the bonded surface. The bonded area of each specimen was measured by means of a digital micrometer (Mitutoyo CD-15C, Mitutoyo, Kawasaki, Japan). The specimens were then attached to the testing apparatus using a cyanoacrylate adhesive (Model Repair II Blue, Dentsply-Sankin Industry, Ohtawara, Japan) and subjected to microtensile bond testing in a tabletop material tester (EZ Test, Shimadzu, Kyoto, Japan) with a cross-head speed of 1.0 mm/min. MTBS (in MPa) was derived by dividing the applied maximum force (N) resulting in fracture by the bonded area (mm²).

The data were analyzed by One-way ANOVA, Scheffé’s test at a 5% level of significance using a software package (SPSS 10.0J®, SPSS, Chicago, IL, USA).

Fracture analysis
After the MTBS test, the fractured surfaces of the dentin side were observed using SEM to evaluate morphological differences. The specimens were air-dried, mounted on an aluminum stub, Pt-Pd sputter-coated for 180 s, and then examined using an FE-SEM at a voltage of 10 kV.

RESULTS
SEM observation of dentin surface after blood contamination
The ground dentin surface after being polished was covered with a smear layer (Fig. 2a). When blood contamination on the ground dentin surface was air-blown before surface treatment with 10-3 solution (group Ab), the dentin surface was covered with...
numerous residues, round-shaped components like erythrocytes and filamentous form components resembling fibrin (Fig. 2b). When the surface was rinsed with water (group Wb), blood contaminants were mostly removed but dentinal tubules were obstructed by smear plugs similar to the ground dentin surface (Fig. 2c).

In group NC, the dentinal tubules were wide open on the ground dentin surface after the surface treatment (Fig. 2d). When the blood contamination was air-blown, and treated with 10^{-3} solution (group Ab), none of the contaminants remained and the dentinal tubules were slightly narrow (Fig. 2e). When the contaminated dentin surface was rinsed with water and treated with 10^{-3} solution (group Wb), the dentinal tubules were open, but smaller than that of group NC (Fig. 2f).

When the blood contamination was carried out after surface treatment and then air-blown (group Aa), the dentin surface was covered with a thick layer of blood contaminants (Fig. 2g). After the blood was rinsed with water (group Wa), blood corpuscle elements were
removed, but filamentous residues were still observed and a large number of dentinal tubules were closed (Fig. 2h). However, on subsequent re-treatment of the surface (group RT), the dentinal tubules were opened and only a few, residual blood contaminants were observed (Fig. 2i).

**Dye leakage assessment**

The leakage values of group Aa and Wa were significantly higher than that of group NC \( (p<0.05) \). The results are expressed as mean±standard deviation (SD). Statistical differences in each group were analyzed using one-way ANOVA, Games-Howell test \( (n=10 \text{ per group}) \).

**SEM observation of the adhesive interface**

There were no spaces and hybrid layer at the adhesive interface in groups Ab, Wb, RT and NC. However, a gap was formed at the interface in groups Aa and Wa (Fig. 4).

**Microtensile bond strength measurement**

The MTBS of group Wa was significantly lower than that of NC group \( * \). Not detected because all the specimens debonded during preparation for the MTBS test. Same letter revealed no significant difference \( (p<0.05) \). The results are expressed as mean±standard deviation (SD). Statistical differences in each group were analyzed using one-way ANOVA, Scheffé’s test \( (n=12 \text{ per group}) \).

**Fracture analysis**

According to fractographical analysis (Fig. 6), in group Ab and Wb, the incidence of cohesive failure was similar to that of mixed failure and only a few specimens showed adhesive failure. In group Aa, only cohesive failures were found. In group Wa, the incidence of cohesive failure was almost the same as that of mixed failure and...
there were no cases of cohesive failure. Most specimens in groups RT and NC showed cohesive failure within the resin (Fig. 7).

DISCUSSION

In this study, the influence of blood contamination on adhesion of 4-META/MMA-TBB resin was evaluated, because although it has been recently applied to many surgical procedures where blood contamination often occurs, not much investigation had been carried out. Fresh peripheral blood without anticoagulants was used as the contaminant in this study, whereas blood with anticoagulant was used in some experiments. This is because blood coagulation might be an important factor in determining the influence that blood contamination has on bonding. We evaluated the influence of blood contamination before and after surface treatment with 10-3 solution in this study, because the adhesion property may be affected by acid etching with

10-3 solution that exposes dentinal collagen fibers.

When blood contamination occurred before surface treatment with 10-3 (group Ab and Wb), MTBS was slightly lower compared with that of group NC (no significance was recognized) and high incidences of not only cohesive failure but also mixed failure were recognized in the fracture analysis in the present study. SEM images of group Wb showed that most of the blood contaminants were removed by rinsing with water and dentinal tubules were smaller than that of group NC following treatment with 10-3 solution. In group Ab after air-blowing, numerous blood contaminants were observed on the SEM images. However, after treatment with 10-3 solution, SEM images showed none of the contaminants and the dentinal tubules were open enough and slightly smaller than that of group Wb. Furthermore, the leakage values of group Ab and Wb did not show significant differences compared with that of group NC, and no spaces and hybrid layer were observed at the adhesive interface in the present study. These results indicate that the 10-3 solution can reach and demineralize the dentin surface even if residues remain over the dentin surface, and that blood contamination on the untreated dentin surface was almost completely eliminated, together with smear layer, by surface treatment with 10-3 solution. Therefore, blood contamination before surface treatment may have less influence on leakage and bond strength.

On the other hand, when blood contamination occurred after surface treatment followed by blown air or water-rinse (group Aa and Wa), the leakage value was significantly higher compared with that of group NC. SEM images in group Aa showed that the surface was covered with a thick layer of blood components resembling blood corpuscles and fibrin. In group Wa, MTBS was also significantly lower compared with group NC, the incidence of adhesive failure increased compared with that of group NC and SEM images showed that most blood corpuscles were eliminated but filamentous
residues remained and the dentinal tubules were narrow or closed even after rinsing. Shiraishi confirmed that the protein and lipid derived from blood remained on the dentin surface even after rinsing blood contaminants with water for 15 s using the leucomalachite green test and observation by confocal laser scanning microscopy\(^9\). In the present study, blood contaminants were rinsed with water for 15 s in the same way and the influence of blood contamination on dentin bonding was similar to what they had observed. These findings indicate that it is difficult to remove the blood contaminants completely after surface treatment and obtain efficient adhesion only by rinsing with water, because blood components (i.e., filamentous residues) might be strongly attached to the superficial layer of exposed collagen and the contaminant may inhibit monomer infiltration into the dentin and polymerization. Leite et al. observed that collagen network exposure by citric acid solution would lead to a more stable fibrin network and blood cell attachment to the dentin surface\(^23\). Therefore, blood contamination after surface treatment remarkably reduces the bond strength and sealing ability.

In addition, when the surface was re-treated with 10-3 solution after rinsing the blood contaminants with water, open dentinal tubules and almost none of the filamentous or corpuscle residues were observed on SEM images. The leakage, MTBS, high incidence of cohesive failure, and the adhesive interface of group RT were similar to that of group NC. These results reveal that good adhesion was achieved after re-treatment with 10-3 solution, although some studies have reported that re-etching reduced the bond strength\(^4\)\(^-\)\(^28\). These inconsistent results are attributed to several factors such as the different acid (phosphoric acid, maleic acid, citric acid) used, the total period of etching, and the bonding system applied. 10-3 solution consists of 10% citric acid (pH 0.86) and 3% ferric chloride. Citric acid is known to have protein removal ability and anticoagulant action with its calcium chelating property and is used in clinical medicine for blood sampling, and central dialysis fluid delivery systems for hemodialysis (heat citric acid disinfection method)\(^27\). The anticoagulant might be efficacious in removing the blood components. With an increase in the etching time, the depth of demineralised dentin is increased, resulting in reduction of the adhesive property\(^23\)\(^,\)\(^24\)\(^,\)\(^29\). In this study, the period of surface treatment with 10-3 solution was set to 5 s. The total treatment time of 5–10 s was the manufacturer’s recommendation. This made penetration of 4-META/ MMA-TBB resin possible through the entire demineralized dentin reaching the underlying intact dentin and initiating polymerization\(^24\)\(^,\)\(^29\), and thus demineralized dentin without immersed 4-META/ MMA-TBB resin may not remain. As mentioned, re-treatment with 10-3 solution (5 s) used in this study is thought to be a reasonable method to restore the adhesive properties.

The result of this study showed that the adhesive properties were maintained by rinsing the blood with water if the blood contamination occurred before surface treatment. In contrast, when the treated surface was contaminated with blood, the surface had to be rinsed with water, followed by re-treatment with 10-3 solution. However, blood coagulation may affect the elimination of contaminants. Further investigations on the influence of the duration of blood contamination and the durability of bonding are necessary.

**CONCLUSION**

If blood contamination occurred before surface treatment with 10-3 solution, washing the blood away reduced the effect of contamination on adhesion of 4-META/ MMA-TBB resin to dentin. On the other hand, blood contamination after surface treatment significantly decreased the adhesion even if the blood was washed away. It is recommended that the contaminated surface be thoroughly rinsed with water and retreated with 10-3 solution.

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