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Adhesion of 4-META/MMA-TBB Resin to Heated Dentin: Effects of Pre-treatments with FeCl₃ and/or HEMA

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The purpose of this study was to compare the tensile bond strengths (TBSs) and failure mode of 4-META/MMA-TBB resin to 60℃-heated and unheated bovine dentin, especially to investigate the influence of ferric chloride contained in citric acid pre-conditioning. In addition, the effect of HEMA priming for heated dentin was also evaluated. The TBSs to heated dentin were significantly lower than those to unheated dentin. Adhesive failures were observed in most specimens of the heated and HEMA-non primed group. HEMA application to heated dentin significantly increased the TBSs in each acid conditioning, which were also significantly higher than those of the unheated and ferric chloride-contained citric acid-conditioned group. It was clarified that heating dentin decreased the bond strength without HEMA priming even if the dentin surfaces were acid conditioned with 10-3, while HEMA priming after acid conditioning recovered the bond strength.

Key words: Heated dentin, Dentin bonding, 4-META/MMA-TBB resin

INTRODUCTION

Current concepts on resin bonding to dentin, the hybridization of the resin and demineralized dentin play very important roles in high bond strength1-3). Infiltration of the resin monomer to the dentin substrate without damaging the collagen matrix, and complete resin infiltration to the exposed collagen are necessary1-3). For the resin to adhere to tooth substrates clinically, two major factors contribute to damaging collagen; acid attack during pre-treatment, and heat attack when preparing the cavity.

The prevention and/or recovery of acid-damaged dentin has been widely discussed. Since the first proposition of the formation of hybridized dentin for resin bonding by Nakabayashi et al. in 19824), various adhesive monomers or pre-conditioners have been evaluated for wellness hybridization for resin bonding to dentin. Ferric chloride included in an aqueous solution of 10% citric acid (10-3) is known to achieve a higher bond strength than that without ferric chloride5). The priming effect of 2-hydroxyethyl methacrylate (HEMA) for resin bonding was also reported6-9). HEMA affects the re-expansion of the demineralized collagen matrix or
depresses the surface tension thereby permitting monomer penetration\textsuperscript{7,8}.

On the other hand, the recovery of heat-damaged dentin has seldom been discussed. Mizunuma reported that the bond strength of heated/10-3 treated dentin was significantly lower than that of unheated/10-3 treated dentin, and concluded that the ferric chloride contained in citric acid conditioner was not affect by heated dentin\textsuperscript{10}. However, a comparison of with and without ferric chloride in the citric acid is necessary to clarify the effect of ferric chloride for heated dentin. Furthermore, the effect of HEMA for bonding to heated dentin has not been reported.

The purpose of this study was to investigate the bond strength characteristics of 4-META/MMA-TBB resin to heated and unheated dentin. The null hypotheses tested were that there is no significant difference in the bond strength between 1) heated and unheated dentin, 2) with and without ferric chloride contained in citric acid conditioner and 3) with and without HEMA priming for heated dentin.

MATERIALS AND METHODS

Schematic illustrations of specimen preparations and tensile bond testing are shown in Fig. 1A. Thirty-two extracted bovine teeth, frozen to maintain freshness, were defrosted and cut at the cervix. The coronal sides of the cut surfaces were sequentially abraded under a stream of water with SiC paper from 180- up to 600-grit to prepare

![Schematic illustration of the preparation of mini-dumbbell-shaped bonded specimens.](image-url)
flat dentin surfaces. The ground dentins were then immersed in 60°C distilled water for 15 min. The heating condition followed that reported by Mizunuma(10). After cooling at room temperature, the ground dentin surfaces were attached to the PMMA frame with 3.0×7.0×3.0 mm window to the ground surface with double-sided tape (Nichiban, Tokyo, Japan), then acid-conditioned in the following manner:

Group 1a: 40μl of an aqueous solution of 10% citric acid +3% ferric chloride (10-3) was applied in the attached frame for 15 sec, rinsed and sufficiently dried using a three-way syringe for each 15 sec.

Group 1b: 40μl of 10-3 solution was applied in the attached frame for 15 sec. Immediately after rinsing and sufficiently drying, 40μl of an aqueous solution of 35% HEMA (Nakarai Tesque, Kyoto, Japan) was applied for 10 min. After 10 min, the conditioner was removed with mild air blowing.

Group 2a: 40μl of an aqueous solution of 10% citric acid (10-0) was applied in the attached frame for 15 sec, rinsed and sufficiently dried with three-way syringe for each 15 sec.

Group 2b: 40μl of 10-0 solution was applied in the attached frame for 15 sec. Immediately after rinsing and sufficiently drying, 40μl of an aqueous solution of 35% HEMA was applied for 10 min. After 10 min, the conditioner was removed with mild air blowing.

After the conditioning, the conditioned surfaces were bonded to PMMA square rods (8.0×8.0×8.0 mm) using 4-META/MMA-TBB resin (Super-Bond C&B; Sun Medical Co., Moriyama, Japan) as the adhesive system. The samples were allowed
to stand at room temperature for 60 min and then stored in water at 37°C for 24 hrs. The bonded teeth were then serially sectioned vertically to make 2.0-mm thick bonded dentin slabs, using a low-speed diamond saw (Isomet™; Buehler, Lake Bluff, IL, USA). Each bonded slab was trimmed to a mini-dumbbell-shaped test specimen with a 3.0×2.0 mm cross-section at the adhesive interface (Fig.1B) using a diamond point (FG #211 regular; Shofu, Kyoto, Japan) in a high-speed air turbine handpiece with copious air-water spray. The prepared specimens were affixed to a disposable PMMA jig, and tensile strengths were measured using a universal testing machine (Tensilon RTC-1150-TSD; Orientec Co., Tokyo, Japan) at a cross-head speed of 0.5 mm/min. There were eight samples in each group, and failed specimens before TBS testing were regarded as 0 MPa with an explicit note of the numbers of pre-testing failures. The data were recorded and subjected to two-way analysis of variance (ANOVA) and Fisher’s Protected-LSD test at the 5% level to determine statistical significance using a commercially available statistical package (StatView® 5.0 J; SAS Institute, Cary, NC, USA).

For Group 1c and 2c, the same procedure was followed as in the Group 1a and 2a, respectively, but the ground dentin was not heated (cited from our previous study). After the tensile bond testing, each fractured specimen was examined using both a light stereomicroscope (Microscope system MS-803, Moritex, Tokyo, Japan) and a scanning electron microscope (SEM: JSM-6340F; JEOL, Tokyo, Japan) to determine the exact locus of the fracture.

RESULTS

Results of the tensile bond strengths (TBS) for comparison of the influence of the heating of dentin are shown in Table 1. Two-way ANOVA revealed a significant difference between pairs of means for the factors “heating of dentin” at $p<0.0001$ and “acid conditioner” at $p<0.0001$. The TBS of 10-3 treated-unheated dentin (Group 1c) was 24.6±6.0 MPa. In contrast, that of 10-3 treated-heated dentin (Group 1a) was

<table>
<thead>
<tr>
<th>Conditioner</th>
<th>Heated (PTF)</th>
<th>$p$-value</th>
<th>Unheated*(PTF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-3</td>
<td>12.0±6.2$^a$(0)</td>
<td>$p&lt;0.0001$</td>
<td>24.6±6.0$^a$(0)</td>
</tr>
<tr>
<td>p-value</td>
<td>$p&lt;0.0001$</td>
<td></td>
<td>$p&lt;0.0001$</td>
</tr>
<tr>
<td>10-0</td>
<td>0.1±0.3$^{*b}$(7)</td>
<td>$p=0.0816$</td>
<td>4.3±1.8$^b$(0)</td>
</tr>
</tbody>
</table>

$n=8$; the bonded specimens were soaked in water at 37°C for 1 day. Mean values designated with same superscript letter are not significantly different ($p>0.05$)
*Unheated groups were cited from Kameyama et al. 13
**Seven of eight specimens were broken before tensile testing; the TBS of remaining one was 0.9 MPa.
PTF: The number of pre-testing failure
Table 2 Influence of HEMA on tensile bond strengths (TBSs) of heated dentin

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>n</th>
<th>Mean±S.D. (PTF)</th>
<th>p-value</th>
<th>Statistical groups**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1a (10-3)</td>
<td>8</td>
<td>12.0±6.2 (0)</td>
<td>p=0.0001</td>
<td>a</td>
</tr>
<tr>
<td>Group 1b (10-3+HEMA)</td>
<td>8</td>
<td>30.4±8.2 (0)</td>
<td></td>
<td>b</td>
</tr>
<tr>
<td>Group 2a (10-0)</td>
<td>8</td>
<td>0.1±0.3 (7)</td>
<td></td>
<td>c</td>
</tr>
<tr>
<td>Group 2b (10-0+HEMA)</td>
<td>8</td>
<td>24.7±6.4 (0)</td>
<td>p=0.0001</td>
<td>b</td>
</tr>
</tbody>
</table>

*TBSs of seven pre-test failed specimens were regarded as 0 MPa.
**Mean values designated with the same letter are not significantly different (Fisher’s PLSD; p>0.05)
PTF: The number of pre-testing failure

Table 3 Failure patterns after tensile bond tested samples

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Adhesive</th>
<th>Adhesive/Resin</th>
<th>Resin/Adhesive</th>
<th>Resin</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-3</td>
<td>Group 1c</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>10-0</td>
<td>Group 2c</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>heated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-3</td>
<td>Group 1a</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>10-0*</td>
<td>Group 2a</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10-3+HEMA</td>
<td>Group 1b</td>
<td>0</td>
<td>1</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>10-0+HEMA</td>
<td>Group 2b</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

*Including the specimens which were broken before testing

significantly lower (12.0±6.2 MPa, p=0.0031). On the other hand, the influence of heating to dentin was not significant in the 10-0 treated dentin (p=0.1590). However, seven of eight specimens were broken before TBS testing only in Group 2a, despite that the other five groups did not show the pre-test failure. There was a significant interaction between the independent variables “heating of dentin” and “acid conditioner” (p=0.0104).

Results of the tensile bond strengths (TBS) for comparison of the influence of the addition of HEMA priming are shown in Table 2. Two-way ANOVA also revealed significant differences in the factors “addition of HEMA priming” at p<0.0001 and “acid conditioner” at p=0.0003. Addition of HEMA priming for heated dentin affected the TBS higher in both 10-3 and 10-0, but the TBS of 10-3+HEMA (Group 1b) was significantly higher than that of 10-0+HEMA (p=0.0467). No significant interaction was revealed between the independent variables “acid conditioner” and “addition of HEMA priming” (p=0.1593).

SEM views of fractured surfaces in each group are shown in Fig. 2, and fracture modes are summarized in Table 3. In the unheated-dentin groups, the fracture pattern was different in 10-3 and 10-0 conditioning (Figs. 2A and 2C); a mixed failure of resin and the adhesive interface was mainly observed in Group 1c (Fig. 2C), but adhesive failure was observed in all specimens of Group 2c (Fig. 2F). Similar findings to Group 2c were also observed in Group 2a (Fig. 2D). In Groups 1a and 2b, most specimens showed a mixed failure in resin, the adhesive interface and the hybrid layer (Fig. 2A). In Group 1b, a mixed failure in the resin and hybrid layer was
Fig. 2A SEM micrograph of the fractured dentin-side surface of a bonded specimen (Group 1a; 13.1 MPa). Most of the surface shows a failure at the top of the hybrid layer (HL). Arrow: Scratches of SiC paper. Adhesive failure between resin and dentin is partially shown (arrowhead).

Fig. 2B SEM micrograph of the fractured dentin-side surface of a bonded specimen (Group 1b; 31.0 MPa). Cohesive failure in cured resin (R) was entirely observed.

Fig. 2C SEM micrograph of the fractured dentin-side surface of a bonded specimen (Group 1c; 23.9 MPa). Both cohesive failure in resin (R) and failure in demineralized dentin (DD) are mixed.

Fig. 2D SEM micrograph of the fractured dentin-side surface of a bonded specimen (Group 2a; 0.9 MPa). Adhesive failure between resin and dentin (A) is shown.

Fig. 2E SEM micrograph of the fractured dentin-side surface of a bonded specimen (Group 2b; 23.8 MPa). Most of the surface shows cohesive failure in the resin (R). However, partial adhesive failure is shown (arrow).

Fig. 2F SEM micrograph of the fractured dentin-side surface of a bonded specimen (Group 2c; 4.4 MPa). Adhesive failure between resin and dentin (A) is shown, and is similar to Fig. 2D.
observed and adhesive failure was slightly observed (Fig. 2B).

DISCUSSION

One of the purposes of this study was to compare the dentin bonding to heated and unheated dentin. The first hypothesis was rejected since ANOVA clearly demonstrated the significant influence of heating dentin \( p<0.0001 \). Some reasons are suggested for the decrease in the TBS; a decrease in the mechanical properties of heated dentin, weakness of demineralized collagen by heat and inhibition of monomer penetration. The decrease in the TBS probably did not affect the decrease in the mechanical properties of heated dentin, because of the invisible cohesive failure in the mineralized dentin. Tonami et al. reported that the tensile strength of boiled dentin (approx. 98°C, 45 min) was about 70 MPa, which supports our suggestion\(^{13}\). Although the failure in the hybridized dentin (HL) was partially observed in Groups 1a, 2a and 2b, most failure modes in the bonded specimens for the heated dentin were observed between the adhesive interface (A) and/or the demineralized and resin-unprotected dentin (DD) (Figs. 2A, 2D and 2E). Thus, the decrease in the TBS in the heated dentin was probably due to the inhibition of monomer penetration and weakness of demineralized collagen rather than the weakness of mineralized dentin itself.

Although ANOVA clearly demonstrated the significant influence of heating dentin \( p<0.0001 \), there was significant interaction between the independent variables “heating of dentin” and “acid conditioner” \( p=0.0104 \). However, there was also no significant difference between Groups 2a and 2c \( p=0.0816 \). These findings suggest that the influence of heating on bond strength was different depending on the acid conditioner. However, the difference between Groups 2a and 2c might be clear because of the difference in the number of pre-failed specimens.

The TBSs of 10-3 were significantly higher than those of 10-0 regardless of heating to dentin \( p<0.0001 \). The tendency of that was also demonstrated even if the heated dentin was HEMA-primed after the acid conditioning \( p<0.0001 \). Thus, the second hypothesis was also rejected. The addition of 3% ferric chloride in 10% citric acid conditioner has been well known to promote high bond strength\(^{5}\). This finding was suggested to arise because demineralization with acid treatment causes the denaturing of collagen and the collapse of the matrix thereby permitting the inhibition of monomer penetration. The ferric chloride contained in the citric acid prevents the denaturing of collagen, thereby preventing the collapse of the demineralized collagen network\(^{1,4,5}\). However, recent studies revealed that the collapse of demineralized collagen was similar between 10-3 and 10-0\(^{14-16}\). In the present study, the heated collagen was already denatured before acid treatment, and the reason for the difference between 10-3 and 10-0 may not be due to the explanation given above. It is suggested that the results due to the soluble dentin protein by heat generation prevented the monomer penetration, and ferric chloride only changed insoluble anionized dentin phosphoprotein\(^{17,18}\). Thus, the difference in the TBS between 10-3 and 10-0 might be due to the difference in the soluble protein.
We also determined the effect of HEMA priming for heated dentin. The third hypothesis was also rejected since HEMA priming affected the higher TBS to the heated dentin in addition to the unheated dentin ($p<0.0001$). As described above, HEMA affects the re-expanding of the demineralized collagen matrix or lowers the surface tension thereby permitting the monomer penetration$^8)$. HEMA priming after acid treatment increased the bond strength and was slightly higher than that of our previous findings of unheated/HEMA-treated dentin (10-3: 29.3±4.7 MPa, 10-0: 18.1 ±6.3 MPa)$^{19)$. While the cohesive failure in the hybridized dentin was partially observed, the failure in the demineralized dentin was almost not observed in most of Groups 1b and 2b (Figs. 2B and 2E). These results suggest that even if the dentin was heated, the increase in monomer penetration brought about the high bond strength adjacent to that of unheated dentin.

Acid etching causes degradation of the dentin collagen, collapse of the collagen network and changes insoluble to soluble$^{20,21)$. EDTA treatment instead of acid conditioning was reported to be useful because neutral-EDTA has a milder demineralizing ability and significantly smaller influence on dentinal collagen, however, it did not work well for bonding of 4-META/MMA-TBB resin$^{17)$. Shimizu reported that EDTA containing ferric chloride (EDTA 3-2) achieved a high bond strength while EDTA without ferric chloride (EDTA 5-0) was almost unbonded$^{17)$. The reason for this was recently hypothesized as follows; the existence of soluble noncollagenous dentin phosphoproteins (NCPs) interfere with the monomer penetration into the demineralized dentin, and the Fe$^{3+}$ contained in citric acid or EDTA strongly bind to NCPs, and changes it to an insoluble form$^{18)$. In contrast, insoluble dentin collagen was changed to soluble under the immersion of 60°C water$^{10,21)$. The present results that showed low bond strength to heated dentin, might be due to the change in the demineralized collagen to a strongly soluble form as a result of both heat and acid attack, thereby interfering with the monomer penetration.

CONCLUSIONS

In this study, we investigated the bond strength characteristics of heated dentin, and concluded the following;
1. The influence of heat to dentin decreased the bond strength without HEMA priming, however the HEMA priming after acid conditioning recovered the bond strength.
2. Ferric chloride contained in citric acid conditioner was less effective for heated dentin.

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