Effects of Surface Wetness of Etched Dentin on Bonding Durability of a Total-etch Adhesive System: Comparison of Conventional and Dumbbell-shaped Specimens

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The dentin bonding durability of a total-etch adhesive system (Prime & Bond NT) was investigated by tensile bond test using conventional and dumbbell-shaped specimens. Two bonding techniques were compared. After etching and rinsing, dentin surface was either blotted by cotton ball (wet-bonding) or air-dried (dry-bonding) before bonding agent was applied. The bond strength of wet-bonding specimens was significantly higher than that of dry-bonding for both conventional and dumbbell-shaped specimens after one day. The bond strength of wet-bonded conventional specimens did not decrease after two years of water immersion, but cohesive failure in demineralized dentin was observed after two years. Wet-bonded dumbbell-shaped specimens showed cohesive failure within demineralized dentin after only one day. The bond strength of dry-bonded conventional specimens decreased significantly after one year, and cohesive failure in demineralized dentin was confirmed after three months. As for dry-bonded dumbbell-shaped specimens, they showed cohesive failure in demineralized dentin after one month. The results of the present study showed that the bonding durability of dry-bonded Prime & Bond NT restorations was lower than that of wet-bonded restorations. Further, by using tensile bond test, cohesive failure in demineralized dentin was detected earlier in dumbbell-shaped specimens than in conventional specimens.

Key words: Bonding durability, Dentin, Adhesive system

INTRODUCTION

Resin composites are often used to treat both anterior and molar caries. After the GLUMA system was introduced in mid 1980s, which used a dentin primer, it is now possible to bond resin composites to dentin. Since then, there have been marked advances in adhesive system, where three-step systems consisting of phosphoric acid etching, dentin primer application and bonding resin application have become widely available. Today, acid etching systems are based on two steps consisting of wet-bonding (where drying is minimized after phosphoric acid etching and water rinsing) and application of a priming adhesive that acts as both dentin primer and bonding resin. It has been reported that phosphoric acid etching systems render favorable enamel bond strength. However, if bonding monomer does not sufficiently penetrate into the demineralized dentin layer, the latter remains intact underneath the hybrid layer. This region is then hydrolyzed, and as a result bond strength is reduced over time. The bonding durability of restorative materials has been assessed in vitro in terms of thermocycling, load-cycling, and long-term water immersion. However, Nakabayashi et al. recently introduced a tensile bond strength test using dumbbell-shaped specimens. They reported that the weakest region of resin-dentin bond — which is the remaining demineralized dentin — could be detected within a very short period of time using this test.

The aim of this study was to evaluate the dentin bonding durability of a wet-bonding system (Prime & Bond NT) with wet- and dry-bonding techniques. The dentin bonding durability of wet-bonded and dry-bonded specimens was assessed by tensile bond strength test where conventional specimens were immersed in water for up to two years and dumbbell-shaped specimens were immersed up to one month.

MATERIALS AND METHODS

Table 1 lists the components and compositions of the Prime & Bond NT system used in this study.

Preparation of specimens

Fig. 1 illustrates the preparation procedures of both conventional and dumbbell-shaped specimens.

Table 1 Components of Prime & Bond NT system

<table>
<thead>
<tr>
<th>Material</th>
<th>Component</th>
<th>Composition</th>
<th>Lot No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prime &amp; Bond NT</td>
<td>Etching agent</td>
<td>36% H₃PO₄</td>
<td>9902000952</td>
</tr>
<tr>
<td>(Dentsply Detry GmbH, Konstanz, Germany)</td>
<td>Bonding resin</td>
<td>di- and tri-methacrylates, PENTA, cetylaminehydrofluoride, nanofiller, acetone</td>
<td>9911001334</td>
</tr>
</tbody>
</table>

PENTA: dipentaerythritol pentaacrylate monophosphate
1. Conventional specimens

Extracted bovine teeth — which were promptly frozen and stored — were thawed just before the experiment commenced. After removing the coronal enamel on the labial side using a model trimmer, exposed dentin was polished using up to #600 SiC abrasive paper to form a flat surface. Double-sided adhesive tape with a 3-mm hole was placed on the surface, and a brass mold with 3.5-mm internal diameter and 2-mm height was positioned on the tape to define the bonding area. The resulting surface was treated with 36% phosphoric acid etching gel (Conditioner 36) for 15 seconds and water-rinsed for 10 seconds. Moisture was removed by one of the following two methods: (1) a cotton ball was used to blot the surface to remove excess moisture (i.e., wet-bonding technique, which was the original procedure of Prime & Bond® NT system); or (2) surface was air-dried by blowing from a dental syringe for 5 seconds (i.e., dry-bonding technique). Bonding resin (Prime & Bond® NT) was then applied and left to stand for 30 seconds, and the solvent was evaporated by gentle air-blowing. After light irradiation for 10 seconds using a visible light-curing unit (XL3000, 3M Dental Products, St Paul, MN, USA), a resin composite (Spectrum® TPH, Lot No. 0003000289, Shade A3, Dentsply Detrey GmbH) was filled into the mold and light-cured for 40 seconds. Specimens prepared in this manner were stored in 37°C water until tensile bond strength test.
Table 2 Tensile bond strength (MPa) and failure mode of conventional specimens

<table>
<thead>
<tr>
<th></th>
<th>1 day</th>
<th>1 month</th>
<th>3 months</th>
<th>6 months</th>
<th>1 year</th>
<th>2 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet</td>
<td>13.4±2.3 (BR,A)</td>
<td>11.8±4.5 (BR,A)</td>
<td>11.0±3.0 (BR,A)</td>
<td>9.4±2.5 (BR,A)</td>
<td>9.9±4.2 (BR,A)</td>
<td>8.6±1.8 (BR,A,DD)</td>
</tr>
<tr>
<td>Dry</td>
<td>3.7±1.0 (A)</td>
<td>3.0±2.5 (A)</td>
<td>1.7±1.7 (A,DD)</td>
<td>1.5±1.3 (DD)</td>
<td>0.8±0.9 (DD)</td>
<td>0.9±1.8 (DD)</td>
</tr>
</tbody>
</table>

n=10, mean±SD, Significant difference: *p<0.01, **p<0.05
BR: cohesive failure in bonding resin, A: adhesive failure, DD: cohesive failure in demineralized dentin

Table 3 Tensile bond strength (MPa) and failure mode of dumbbell-shaped specimens

<table>
<thead>
<tr>
<th></th>
<th>1 day</th>
<th>2 weeks</th>
<th>1 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet</td>
<td>29.7±4.8 (BR,A,DD)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dry</td>
<td>7.4±1.8 (A)</td>
<td>6.7±3.5 (A)</td>
<td>4.0±3.9 (A,DD)</td>
</tr>
</tbody>
</table>

n=10, mean±SD, Significant difference: *p<0.01
BR: cohesive failure in bonding resin, A: adhesive failure, DD: cohesive failure in demineralized dentin

2. Dumbbell-shaped specimens

Dentin surfaces polished using to #600 SiC abrasive paper were prepared in the same manner as the conventional specimens described above. A plastic mold with 4.0-mm internal diameter and 2.0-mm height was placed on the polished surface using double-sided adhesive tape. A resin composite was filled into the mold and treated in the same manner as the conventional test specimens. A resin-dentin slab of 2.0-mm width was then cut using a low-speed diamond wheel saw (Model 650, South Bay Technology Inc., San Clemente, CA, USA) and trimmed using a superfine diamond point (Smooth-cut K3ff, GC Dental Industrial Co., Tokyo, Japan) to prepare a dumbbell-shaped specimen with adhesive area of 1.5×2.0 mm.

The prepared specimens were affixed with 4-META/MMA-TBB resin (Superbond C&B, Sun Medical Co., Moriyama, Japan) to a disposable PMMA jig and stored in 37°C water until tensile bond strength test.

Measurement of bond strength

Tensile bond strength tests were conducted with a universal testing machine (IM-20, Intesco, Tokyo, Japan) at a cross-head speed of 0.5 mm/min after 1 day, 1 month, 3 months, 6 months, 1 year, and 2 years of water immersion for conventional specimens; and after 1 day, 2 weeks, and 1 month for dumbbell-shaped specimens. A brass rod was attached perpendicularly to each conventional specimen to serve as a handle during tensile bond strength measurement. As for dumbbell-shaped specimens, the fractured surfaces were individually measured using a micrometer (Mitsutoyo, Tokyo, Japan) to confirm the bonding area. Mean bond strength and standard deviation were then calculated (n=10). Bond strength at different immersion periods was compared by one-way ANOVA and Scheffe’s multiple comparison test for both wet- and dry-bonded specimens. A t-test was used to compare the wet- and dry-bonded specimens at each immersion period.

SEM observation of fractured surface

After tensile testing, fractured surfaces were gold-coated and analyzed by SEM (JSM-5610LV, JEOL, Tokyo, Japan) at ×2,000 magnification.

RESULTS

Tables 2 and 3 show the results of tensile bond strength tests, and Figs. 2-5 show SEM micrographs of tensile fractured dentin-side surfaces.

Conventional specimens

After 1 day of water immersion, the bond strength of wet-bonded specimens was 13.4 MPa, while that of dry-bonded specimens was significantly lower at 3.7 MPa (p<0.01). The bond strength of wet-bonded specimens did not decrease significantly over the 2 year period. Through SEM observation, mixed failures — in terms of adhesive failure at bonded interface and cohesive failure within bonding resin — were seen in the period of 1 day to 1 year (Fig. 2a).

After 2 years of water immersion, numerous mixed failures of adhesive and cohesive failures in bonding resin and demineralized dentin were noted (Fig. 2b). Fracture of demineralized dentin occurred at and after 3 months of water immersion.

Dumbbell-shaped specimens

Bond strength of wet-bonded specimens was 29.7
MPa after 1 day of water immersion, but that of dry-bonded specimens was significantly lower at 7.4 MPa (p<0.01). SEM analysis of fractured surfaces of wet-bonded specimens showed numerous mixed failures of adhesive and cohesive failures in bonding resin and in demineralized dentin (Fig. 4). No tensile tests were performed at and after 2 weeks because cohesive failure in demineralized dentin was noted after 1 day. However, cohesive failure in demineralized dentin was not observed in dry-bonded specimens, and tensile testing and SEM observation were performed after 2 weeks and 1 month of water immersion. After 1 day and 2 weeks, it was chiefly adhesive failure in dry-bonded specimens (Fig. 5a). After 1 month, mixed failures of adhesive and cohesive failures in demineralized dentin with marked collagen fibrils were seen (Fig. 5b). There were no significant differences in bond strength for dry-
Fig. 5 SEM micrographs of fractured dentin-side surface of dry-bonded dumbbell-shaped specimen immersed in water for: a) 1 day; b) 1 month.
A=adhesive failure, DD=cohesive failure in demineralized dentin.

DISCUSSION

The adhesion technique of resin composite to dentin advanced rapidly since dentin primers were introduced, and resin systems that adhere to both enamel and dentin now play a very important role in daily dental practice. However, while adhesion to enamel can be achieved by simple mechanical retention\(^2^3\), adhesion to dentin is achieved via complex procedures, including smear layer treatment and hybrid layer formation\(^2^4\). In addition, whether bond strength can be maintained for a long period of time is another key issue to tackle. While long-term water immersion is a useful technique for determining bonding durability\(^9,16^\text{-}19\), results cannot be obtained rapidly. On the other hand, with tensile bond test using dumbbell-shaped specimens, regions with reduced bond strength (such as remaining demineralized dentin) can be detected early within a short period of time\(^2^0^\text{-}2^2,2^5\). These regions are prone to fractures, and are hence a useful indicator of bonding durability. In the present study, tensile bond tests were conducted using both conventional and dumbbell-shaped specimens in order to assess the bonding durability of a wet-bonding system (Prime & Bond\(^R\) NT). In an adhesive system where dentin is acid-etched, and if remaining demineralized dentin layer not impregnated by resin exists at the lower margin of hybrid layer, hydrolysis of this region can destroy adhesion in the long term\(^8,16^\text{-}19,3^0\). The main cause of this degradation is attributed to collagen fibrils that collapse due to air-drying after etching and rinsing\(^2^7,2^8\). As a result, these collapsed collagen fibrils prevent bonding monomer from penetrating into the demineralized dentin. The role of dentin primer, therefore, is to facilitate penetration of the bonding monomer by re-swelling the collapsed collagen fibrils\(^2^9^\text{-}3^2\). Wet-bonding is another technique in which the collapse of demineralized collagen fibrils is suppressed by minimizing air-drying after acid-etching and water-rinsing\(^3^3,3^4\). In addition, the permeability of bonding monomer can be improved by replacing water with an alcohol or acetone solvent in the bonding resin, ensuring that the bonding monomer penetrates into the deepest parts of the demineralized layer to suppress the formation of remaining demineralized dentin\(^3^5,3^6\).

In this study, Prime & Bond\(^R\) NT system was used with wet-bonding technique according to manufacturer's instructions. For the conventional specimens, there were no significant decreases in bond strength after two years of water immersion. However, analysis of fractured dentin surfaces showed that while there were few fractures of demineralized dentin for the first year, numerous cohesive failures in demineralized dentin were seen after two years. A hybrid layer is formed when resin monomer penetrates and diffuses into demineralized collagen, and it has been reported that the deeper the hybrid layer, the lower the resin content\(^2^7\), and the more inferior the physical properties\(^3^8\). It has also been reported that there is a resin-poor, collagen-rich region in the hybrid layer, and nanoleakage occurs in this region when acid-etching system is used\(^3^9^\text{-}4^1\). Therefore, even if there is no demineralized dentin underneath a hybrid layer, regions of insufficient hybridization within the hybrid layer can be degraded by long-term water immersion. In the present tensile bond test using wet-bonded conventional specimens, cohesive failures that were seen in demineralized dentin after two years of water immersion could have represented regions with low resin content in the hybrid layer. As for wet-bonded dumbbell-shaped specimens, cohesive failure was observed in demineralized dentin only after one
day — as compared to wet-bonded conventional specimens after two years. It was found that the weakest region in resin-dentin bond was fractured first by tensile loading when dumbbell-shaped test specimens were used to examine tensile bond strength\(^\text{30-22}\). Therefore, while it took more than one year to detect the weak regions (i.e., regions with low resin content inside the hybrid layer or those of remaining demineralized dentin) of wet-bonded conventional specimens, such regions could be detected after only one day with wet-bonded dumbbell-shaped specimens. This finding supported the report by Nakabayashi et al. whereby regions with weak adhesion could be detected in a short period of time by tensile bond test using dumbbell-shaped specimens.

On the other hand, bond strength of dry-bonded conventional specimens (air-dried after etching and water-rinsing) was significantly lower than that of wet-bonded conventional specimens at every immersion period. Indeed, the bond strength of dry-bonded conventional specimens after one year was significantly lower than that of dry-bonded conventional specimens after one day to six months. In addition, cohesive failure in demineralized dentin was seen only after three months. Unlike the demineralized dentin observed in wet-bonded specimens after two years, this region represented hydrolysis of remaining demineralized dentin\(^\text{19-26}\). In dry-bonded specimens, the collapsed demineralized dentin permits little diffusion of monomers around the collagen fibrils, making it difficult to create good-quality hybrid layer — thus resulting in a region of non resin-impregnated demineralized dentin (i.e., remaining demineralized dentin). As compared to a region of low resin content inside the hybrid layer (as seen in wet-bonded specimens), this non-resin-impregnated demineralized dentin could be easy to hydrolyze. However, while the presence of remaining demineralized dentin with the dry technique was clear and distinct, tensile bond tests using dumbbell-shaped specimens showed that almost all adhesive failures occurred along a smooth intertubular zone, and there were very few instances of fracture in demineralized dentin after one day. This indicated that soon after adhesion (e.g., after one day), the weakest region of dry-bonded specimens was the adhesion interface, not demineralized dentin. In other words, while demineralized dentin is gradually weakened by hydrolysis over a long period of time, degradation is not advanced during the initial stages of adhesion and the tensile strength of remaining demineralized dentin is higher than that of the adhesion interface. On this note, the duration of water immersion for dry-bonded dumbbell-shaped specimens was extended in this study, and cohesive failure in demineralized dentin was seen after one month. The demineralized dentin of dry-bonded dumbbell-shaped specimens after one month had clear collagen fibrils when compared to that of dry-bonded conventional specimens after three months, thus suggesting that cohesive failure in demineralized dentin was detected earlier in dumbbell-shaped specimens than in conventional specimens. According to Hashimoto et al.\(^{42}\), Yoshida et al.\(^{43}\), and Nakajima et al.\(^{44}\), when specimens are made into a dumbbell shape, the resin-dentin interface comes into direct contact with water. As a result, it helps to accelerate the degradation of demineralized dentin and the extraction of resin components from the hybrid layer — hence leading to earlier detection. Nevertheless, in some tensile bond tests using dumbbell-shaped specimens, such demineralized dentin regions may not be detected after only one day of water immersion. This is because degradation of demineralized dentin advances gradually with time. Therefore, when assessing the bond strength of resin-dentin bonds by tensile bond test using dumbbell-shaped specimens, it should be noted that demineralized dentin may not be detected soon after immersion. Hence, if bond strength is relatively low, it is necessary to further examine the specimens by extending the duration of water immersion.

**CONCLUSION**

Using Prime & Bond\(^9\) NT, which was a wet-bonding system, the bond strength of dry-bonded restorations (air-drying after etching and water-rinsing) was markedly lower than that of wet-bonded restorations (blotting after etching and water rinsing). Based on the results of this study, we concluded that tensile bond test using dumbbell-shaped specimens was capable of detecting demineralized dentin more rapidly, as compared to one using conventional specimens.

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