Influence of environmental conditions on dentin bond strengths of recently developed dentin bonding systems

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Abstract: A study was conducted to investigate the influence of temperature and relative humidity (RH) on the bond strengths of several recently developed dentin bonding systems. Six environmental conditions, (A) 25 ± 0.5°C, 50 ± 5% RH, (B) 25 ± 0.5°C, 80 ± 5% RH, (C) 25 ± 0.5°C, 95 ± 5% RH, (D) 37 ± 0.5°C, 50 ± 5% RH, (E) 37 ± 0.5°C, 80 ± 5% RH, (F) 37 ± 0.5°C, 95 ± 5% RH were used. Bovine mandibular incisors were mounted in self-curing resin and the facial surfaces were ground on wet #600 SiC paper to expose the dentin. After the tooth surface had been treated according to each manufacturer's instructions, adhesives were applied, followed by condensation of resin composites into a mold placed on the dentin surface. Fifteen specimens per group were stored in distilled water at 37°C for 24 h, and then shear-tested at a cross-head speed of 1.0 mm/min. Statistical analysis was carried out with two-way ANOVA followed by Tukey's test (P < 0.05). Dentin bond strengths decreased with increasing relative humidity but were not influenced by environmental temperature. Even though one-bottle adhesive systems require a wet dentin surface, their bond strengths are affected by an increase in environmental humidity. (J. Oral Sci. 43, 35-40, 2001)

Key words: dentin bonding; bovine tooth; temperature; relative humidity.

Introduction

Significant efforts have been made to develop restorative materials that bond to dentin in the oral environment without complicated clinical procedures. Several new adhesive systems, which employ simultaneous etching enamel and dentin with phosphoric acid (1, 2) or a self-etching primer (3-5), have been introduced. Screening of these materials is usually carried out by in vitro adhesion testing and microleakage evaluation under room temperature conditions of 23°C and 50% relative humidity (RH). However, environmental factors are known to have an important influence on bonding strength to dentin (6-9).

The influence of intrinsic dentinal fluid on bonding efficacy has been investigated with simulated pulp fluid (10-14). Variation in dentin depth and dentin permeability has a significant influence on dentin bonding strength. Environmental temperature and RH during the restorative procedure are other previously reported relevant clinical aspects (9). Resin composites have been considered to be susceptible to environmental moisture, which has been shown to decrease bond strength. Plasmons et al. (6) employed a 35°C, 95% RH environment as a high-humidity condition and concluded that the influence of extrinsic dentin wetness differed among the bonding systems tested in that some systems were more sensitive to wetness than others.

It has been suggested that bonding systems that use phosphoric acid with a one-bottle adhesive can form strong bonds with moist dentin (15-18). Water in the partially demineralized dentin may play an important role in preventing the collapse of collagen (19, 20). The ability to form strong bonds with moist dentin is a positive feature of these bonding systems. On the other hand, excess water may impair the mechanical properties of the adhesive and decrease bonding strength (21-23). Systems that use
hydrophilic dentin primers could minimize the effect of sensitivity to moisture on the dentin surface.

The null hypothesis tested in this study was that environmental humidity would not alter the dentin bonding strengths of recently developed two-step bonding systems. The purpose of the study was to investigate the influence of temperature and RH on the dentin bonding strengths of one-bottle adhesive and self-etching primer systems.

Materials and Methods

The bonding systems with a combination of resin composites employed in this study were three self-etching primer systems - Imperva Fluoro Bond/Lite-Fil II A (FB), Clearfil Liner Bond II Σ/Clearfil AP-X (LB) and Mac Bond II/Palifique Estelite (MB) - and three one-bottle adhesive systems - One Step/Elitefil (OS), Prime & Bond 2.0/Spectrum TPH (PB) and Single Bond/Z100 (SB) (Tables 1, 2).

A curing unit, Optilux 500 (sds Sybron, Danbury, CT, USA), was connected to a variable transformer in order to adjust the light intensity to 600 mW/cm² as measured with a dental radiometer (Model 100, sds Sybron, Danbury, CT, USA).

Mandibular incisors extracted from 2- to 3-year-old cattle and stored frozen (-20°C) for up to 2 weeks were used as a substitute for human teeth. After the roots had been removed with a low-speed saw, the pulps were removed, and the pulp chamber of each tooth was filled with cotton to prevent penetration of the embedding media. The labial surfaces of bovine incisors were ground on wet 240-grit SiC paper to a flat surface. Each tooth was then mounted in cold-curing acrylic resin to expose the flattened area and placed in tap water to reduce the temperature rise from the exothermic polymerization reaction. The final finish was accomplished by grinding on wet 600-grit SiC paper until a sufficient area of dentin was exposed. After ultrasonic cleaning with distilled water for 3 min to remove the debris, these surfaces were washed and dried with oil-free compressed air. Double-sided adhesive tape with a 4-mm diameter hole was firmly attached to the flattened surface to restrict the adhesive area.

The embedded teeth were transferred to a chamber with controlled temperature and humidity (Type V-85, Atom Corp., Tokyo, Japan). Because the temperature of the samples was different from the temperature inside the chamber, samples were left for 10 min to equilibrate them to six environmental conditions: A) 25 ± 0.5°C, 50 ± 5% RH, B) 25 ± 0.5°C, 80 ± 5% RH, C) 25 ± 0.5°C, 95 ± 5% RH, D) 37 ± 0.5°C, 50 ± 5% RH, E) 37 ± 0.5°C, 80 ± 5% RH, F) 37 ± 0.5°C, 95 ± 5% RH.

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<tbody>
<tr>
<td>FB</td>
<td>Imperva Fluoro Bond</td>
<td>FB Primer (4-AET, HEMA, ethanol, water)</td>
<td>A: 109846</td>
<td>FB Bond (4-AET, HEMA, ethanol, water)</td>
<td>119853</td>
</tr>
<tr>
<td>LB</td>
<td>Clearfil Liner Bond II</td>
<td>LB Primer (3-MNSA, MDP, HEMA, ethanol, water)</td>
<td>A: 0002A2</td>
<td>LB Bond (MDP, HEMA, Bis-GMA, Filtek)</td>
<td>00050A</td>
</tr>
<tr>
<td>MB</td>
<td>Mac Bond II</td>
<td>Primer (MAC-10, HEMA, ethanol, water)</td>
<td>A: 0161</td>
<td>Bonding Agent (MAC-10, Bis-GMA)</td>
<td>013</td>
</tr>
<tr>
<td>OS</td>
<td>One Step</td>
<td>Uni-Etch (32% phosphoric acid)</td>
<td>009127</td>
<td>One Step (Bis-GMA, HEMA, acetone)</td>
<td>059236</td>
</tr>
<tr>
<td>PB</td>
<td>Prime Bond 2.0</td>
<td>Conditioner 36 (36% phosphoric acid)</td>
<td>00283</td>
<td>Prime Bond (PENTA, UDMA, R-14-2-1, acetone)</td>
<td>00908</td>
</tr>
<tr>
<td>SB</td>
<td>Single Bond</td>
<td>Etchant (35% phosphoric acid)</td>
<td>7EC</td>
<td>Single Bond (Bis-GMA, HEMA, Polyalkenoate copolymer)</td>
<td>6AB</td>
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The dentin surface was then treated according to each manufacturer’s instruction, followed by application of the bonding agent. A Duracon® mold (2 mm height, 4 mm internal diameter) was used to form and hold the resin pastes to the dentin surface. The resin composite was condensed into the mold, and then irradiated for 40 s. The finished specimens were transferred to 37°C distilled water for 24 h from the start of light exposure to the material. Fifteen specimens per group were shear-tested in an Instron testing machine (Type 4204, Instron Corp., Canton, MA, USA) at a cross-head speed of 1.0 mm/min. Shear bond strengths in MPa were calculated from the peak load at failure divided by the specimen surface area.

After testing, the specimens were examined in an optical microscope.

Table 2 Procedures for making bond strength specimens according to each manufacturer’s instructions

<table>
<thead>
<tr>
<th>Code</th>
<th>Etching/Primer</th>
<th>Adhesive</th>
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<tbody>
<tr>
<td>FB</td>
<td>10 s apply Air dry</td>
<td>10 s cure</td>
</tr>
<tr>
<td>MB</td>
<td>20 s apply Air dry</td>
<td>10 s cure</td>
</tr>
<tr>
<td>LB</td>
<td>30 s apply Air dry</td>
<td>20 s cure</td>
</tr>
<tr>
<td>OS</td>
<td>15 s etch 10 s rinse Blot dry</td>
<td>Apply two coats 10 s air dry 10 s cure</td>
</tr>
<tr>
<td>PB</td>
<td>20 s etch 15 s rinse Blot dry</td>
<td>30 s apply, 10 s cure Apply then 10 s cure</td>
</tr>
<tr>
<td>SB</td>
<td>15 s etch 10 s rinse Blot dry</td>
<td>Apply 5 s Air dry 10 s cure</td>
</tr>
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microscope at a magnification of ×10 to define the location of the bond failure. The types of failure were determined on the basis of the percentage of substrate-free material as: adhesive failure, cohesive failure in resin composite and in dentin, and mixed failure.

The mean and standard deviation for each group were tested for homogeneity of variance using the Bartlett test, and then subjected to two-way ANOVA and the Tukey multiple comparison procedure at \( P < 0.05 \) using a computer statistics package (Sigma Stat® version 2.03, SPSS Inc., Chicago, IL, USA).

For ultrastructural observation of the resin-dentin interface, bonded specimens stored in distilled water at 37°C for 24 h were embedded in epoxy resin and then longitudinally sectioned with a diamond saw. The sectioned surfaces were polished to a high gloss with abrasive discs followed by diamond pastes down to 0.1 μm particle size. They were dehydrated in ascending grades of tert-butyl alcohol (50% for 20 min, 75% for 20 min, 95% for 20 min, and 100% for 2 h), and then transferred from the final 100% bath to a critical-point dryer (Model ID-3, Elionix, Tokyo, Japan) for 30 min. The polished surfaces were then subjected to argon-ion beam etching (EIS-200ER, Elionix, Tokyo, Japan) for 15 s with the ion beam (accelerating voltage 1.0 kV, ion current density 0.4 mA/cm²) directed perpendicular to the polished surface. The surfaces were coated in a vacuum evaporator with a thin film of gold. Observation was done under a scanning electron microscope (SEM, JSM-5400, JEOL, Tokyo, Japan) at an operating voltage of 10 kV.

**Results**

Mean shear bond strengths under various environmental conditions are presented in Table 3. Two-way ANOVA revealed differences in shear bond strength for the different levels of RH, but not for changes in temperature. The bond strength of each bonding system was lower under higher RH conditions. No interaction between temperature and RH was found.

The fracture modes after the bond strength test are listed in Table 4. The fracture mode of the debonded specimens showed an increased tendency for failure in the mixed and adhesive mode with higher RH. This tendency was more pronounced for the one-bottle adhesive systems than for the self-etching primer systems. For specimens made under the highest RH (95 ± 5%), the fracture of all the specimens tended to be in the adhesive failure mode.

The SEM observations of the resin-dentin interface of the specimens made under the condition 37°C, 95 ± 5% RH are shown in Figs. 1, 2. After argon ion beam etching, close adaptation between the resin and dentin, with low resistance to argon ion bombardment, was observed as a hybrid layer in the specimens made under conditions of lowest RH, and the width of this layer was ~0.5-1.0 μm for the self-etching primer systems, and ~3.0-4.0 μm for the one-bottle adhesive systems. Although the hybrid layer was seen, cracks inside the adhesive resin (Fig. 1) and gaps between resin and adhesive resin (Fig. 2) were observed for the highest RH conditions.

**Discussion**

The results of this study showed that the dentin bond

| Table 3 Influence of environmental conditions on bond strength (mean ± SD, MPa) of two-step bonding systems to bovine dentin (n = 15) |
|---|---|---|---|---|---|
| Code | A) 25°C, 50% RH | B) 37°C, 50% RH | C) 37°C, 95% RH | D) 25°C, 95% RH | E) 37°C, 95% RH |
| MB | 16.3 ± 3.5* | 12.6 ± 3.6* | 6.2 ± 1.9 | 16.1 ± 3.2* | 12.7 ± 4.1* |
| PB | 17.9 ± 3.6* | 16.2 ± 3.3* | 9.5 ± 2.4 | 17.7 ± 2.5* | 15.4 ± 3.5* |
| LB | 17.2 ± 3.0* | 16.2 ± 3.1* | 12.4 ± 3.1 | 15.6 ± 3.2* | 15.4 ± 3.5* |
| OS | 14.9 ± 1.9 | 9.9 ± 2.7* | 10.2 ± 2.1* | 14.9 ± 3.6 | 9.8 ± 2.3* |
| PB | 12.3 ± 2.5 | 9.0 ± 2.6* | 9.1 ± 2.1* | 13.2 ± 3.3 | 9.7 ± 3.7* |
| SB | 18.1 ± 3.5 | 11.3 ± 3.8* | 8.5 ± 3.8* | 16.4 ± 2.2 | 12.6 ± 2.7 |

The effect of different levels of temperature does not depend on what level of RH is present. Values with * are not significantly different compared within the same temperature, p<0.05.
strengths of the recently developed bonding systems were not affected by changes in the environmental temperature, but were influenced by the changes in RH. When the bonding specimens were made under higher RH conditions, lower bond strengths were observed. Burrow et al. (8) employed a 30°C, 80 % RH environment for the higher humidity condition and concluded that the simulated oral environment had no effect on dentin bond strength. In this study, the same tendency for 80 % RH was seen for the self-etching primer systems but not for the one-bottle adhesive systems. When the specimens were made under the 95 % RH conditions, the bond strength of all the bonding systems decreased dramatically. Extrapolating these results to the influence of environmental RH on dentin bond strength, it might be independent of RH as long as the moisture level is below a critical level.

The wetting ability of the restorative material is important for dentin bonding (24). Resin composites with one-bottle adhesive systems rely on phosphoric acid and adhesives for good wetting in order to create adhesion (1, 2). One-bottle adhesive systems, which employ a wet bonding technique, produce a relatively higher bond strength to moist dentin (15-18). The presence of water in and on the dentin plays an important role in preventing collapse of the collagen fiber matrix after acid etching, maintaining open diffusion channels for adhesive resin infiltration (19, 20). The bonding agent of the one-bottle adhesive system is a hydrophilic solution that is extremely effective in wetting the etched dentin. The solvent of the bonding agent dynamically replaces the water until equilibrium is established; thus the diffusion of the adhesive resin through the entire thickness of the etched dentin might be expected. On the other hand, if the dentin surface is "too wet", the so called "over-wet phenomenon" might take place, and hybrid layer formation might be prevented by phase separation of the hydrophobic components of the bonding agent (21-23). It has been emphasized that the wet bonding technique has material-related, technique-sensitive factors that have a significant effect on dentin bond strength (25-28). When the specimens are made under conditions of higher RH, the over-wet phenomenon might occur so that the bond strength is diminished. Although the one-bottle adhesive systems use a total-etch technique in order to simplify the clinical application steps, it is difficult to determine the appropriate extent of wetness in the more-complex clinical situation.

Self-etching primers are applied to the tooth surface before application of the bonding agent to ensure maximum adhesion by improving monomer penetration into hydrophilic dentin substrate and improve wettability of the tooth surface by the bonding agent. After application of the self-etching primer, the primed dentin surface should be air-dried because the primer contains solvents like water, ethanol and acetone (29). Beyond a critical level, excess water may dilute the self-etching primer and weaken the etching effect. Water remaining on the dentin surface might have an adverse effect on polymerization of the bonding agent applied after the dentin priming step (19). Under high-RH conditions, it would be difficult to evaporate all of the water from the dentin surface. The primed dentin

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**Fig. 1** Scanning electron microscopy observation of the dentin (D)-resin interface of Mac Bond II (original magnification ×1,500). A thin layer, hybrid layer, and close adaptation of the adhesive layer were detected. Small cracks were observed inside the adhesive resin just above the hybrid layer (H).

**Fig. 2** Scanning electron microscopy observation of the dentin (D)-resin interface of a Single Bond (original magnification ×3,500). Small cracks were observed inside the adhesive resin just above the hybrid layer (H).
would be expected to contain intrinsic as well as extrinsic water, even after primer application and further air drying. Therefore, a wet bonding technique is not recommended for self-etching primer systems. Another reason for decreased bond strength under higher RH conditions might be the presence of moisture on the hardened adhesives. If the adhesive surface is wetted by oral humidity before placement of the resin composite, close adaptation between the resin composite and adhesive might be prevented. The presence of water on the hardened adhesive resin might interfere with the polymerization of resin composites so that a poorly polymerized resin layer remains at the adhesive interface, leading to lower bond strength values.

The results of this study suggest that the extrinsic water derived from oral humidity above a critical level may adversely affect the dentin bonding of two-step bonding systems. Even with one-bottle adhesive systems, the presence of moisture after the adhesive hardens might negate its effectiveness. Care must be taken when using two-step bonding systems in the oral environment, even though the systems employ hydrophilic components in the adhesive or the primer.

Acknowledgments
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References


