Effect of Double-application or the Application of a Hydrophobic Layer for Improved Efficacy of One-step Self-etch Systems in Enamel and Dentin

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G Mattei • A Reis • AD Loguercio

Clinical Relevance
The double-application and placement of a hydrophobic resin coat can improve the performance (µTBS) of one-step self-etch systems to tooth substrates, mainly in dentin.

SUMMARY
Objectives: To evaluate the use of two alternative modes of application (double-application and placement of a hydrophobic resin coat) compared with the manufacturers’ directions on the microtensile bond strength (µTBS) of one-step, self-etch systems to enamel and dentin.

Materials and Methods: Resin composite buildups were bonded to the buccal and lingual ground enamel surfaces and occlusal dentin of third molars using the following adhesives: Xeno III (XE), GBond (GB), Adper Prompt L-Pop (AD) and Clearfil SE Bond (CSE) as the control. The adhesive systems were applied: 1) following the manufacturer’s directions (MD), 2) with double-application (DA) or with 3) additional placement of a hydrophobic resin layer (HR) after following the manufacturer’s directions. After storage in water (24 hours/37°C), the bonded specimens were sectioned into sticks (0.8 mm²) that were tested until failure at a crosshead speed of 0.5 mm/minute. Data from each substrate were subjected to two-way ANOVA and Tukey’s test (α=0.05).

Results: The interaction Adhesive vs Application mode was statistically significant.
(p<0.05) for both substrates. In dentin, XE and GB achieved high µTBS in the HR group (p<0.05). For AD, the highest µTBS were observed in the DA group (p<0.05). For enamel, similar µTBS was observed for XE and GB among the three modes of application, while the highest µTBS of AD was observed in the HR group.

Conclusion: The effects of using the double-application and placement of a hydrophobic resin coat on microtensile dentin-bond strength values seem to be effective; however, these techniques in enamel were adhesive-dependent.

INTRODUCTION

Today, bonding to tooth hard tissue can be accomplished by using one of two adhesion strategies: the etch-and-rinse or the self-etch approach. Contrary to the etch-and-rinse approach, the conditioning step in self-etch systems is not separated from the priming step and, therefore, demineralization and infiltration occurs simultaneously, at least from a theoretical viewpoint. Another difference from the etch-and-rinse approach is that the tooth is no longer rinsed, which not only lessens clinical application time, but also significantly reduces technique-sensitivity. Self-etch systems are also more user-friendly than the etch-and-rinse approach, as the separate etching step and the consequential management of dentin moisture was completely eliminated.

Initially, self-etch adhesives were introduced for use in a two-step procedure. After application of an acidic primer, a relatively hydrophobic bonding resin was to be applied on top of the primed surface. However, recently, the trend related to developing new dentin/enamel adhesives has focused on simplification of the clinical steps involved in the bonding protocol. One-step self-etch systems (also known as all-in-one) were introduced. The manufacturers of these products attempted to incorporate all of the components of an adhesive system (etchant, primer and bonding resin) into a single solution, resulting in a reduced-step, user-friendly adhesive. As these materials have become popular among clinicians, they are being introduced at a very fast rate, without comprehensive testing to substantiate their performance.

Different research centers have shown that some one-step self-etch adhesives exhibit relatively low bond strength values to both enamel and dentin, when compared to two-step self-etch or etch-and-rinse systems. This inferior performance has been attributed to certain factors. First, these products create very thin coatings, which may be oxygen inhibited, resulting in a poorly polymerized adhesive layer. Second, they are highly prone to phase separation as the solvent evaporates from the solution and, finally, they behave as permeable membranes after polymerization. The latter is due to the presence of water-attracting hydrophilic domains and interconnecting water-filled channels within the polymerized adhesives, permitting water to move from the underlying dentin through the adhesive.

Some authors have indicated that treating one-step self-etch systems as a primer and covering them with a less hydrophilic resin coating (converting them into two-step materials) can be an option for resolving their drawbacks. Other authors, however, have suggested placing multiple layers in a clinical attempt to improve their clinical efficacy. Despite these positive findings, the performance of the above mentioned alternative techniques seem to be dependent on the brand of adhesive tested. In addition, hardly any attention has been given to the enamel substrate, as most of the previously cited studies were conducted in dentin.

Concerns regarding the bonding efficacy of one-step self-etch systems to enamel have recently been raised. It was demonstrated that employing simplified self-etch adhesives in enamel can result in osmotic blistering and, consequently, bond failure when they are not covered by a hydrophobic resin layer or a resin composite.

Thus, this study evaluated two alternative modes of application for one-step self-etch adhesive systems (multiple coats and placement of a hydrophobic resin coat) and compared them with the manufacturers’ directions on ground enamel and dentin.

METHODS AND MATERIALS

Three one-step self-etch adhesive systems were tested: Adper Prompt L-Pop (AD, 3M ESPE, St Paul, MN, USA), Xeno III (XE, Dentsply De Trey, Konstanz, Germany) and G-Bond (GB, GC, Tokyo, Japan). Clearfil SE Bond (CSE, Kuraray, Osaka, Japan), a two-step self-etch adhesive system, was used as the control. The composition, application mode and batch number are described in Table 1.

Ninety extracted, caries-free human third molars were used. The teeth were collected after obtaining the patients’ informed consent under a protocol approved by the University of Oeste of Santa Catarina Institutional Review Board. The teeth were disinfected in 1% thymol stored in distilled water and used within six months of extraction. For dentin, 60 teeth were used. A flat dentin surface was exposed after wet grinding the occlusal enamel on #180 grit SiC paper. The exposed dentin surfaces were further polished on wet #600-grit silicon-carbide paper for 60 seconds to standardize the smear layer.

For enamel, 30 teeth were employed. They were sectioned in a mesio-to-distal direction in order to obtain buccal and lingual tooth halves. These surfaces were
cleaned with slurry of pumice and water and examined under a 40x stereomicroscope (HMV-2, Shimadzu, Tokyo, Japan) to ensure that the teeth were free of surface cracks, decalcification or any sign of previous grinding. Upon completion of the examination, the enamel was demarcated to outline the flattest area for bonding. The mid-coronal third of the buccal and lingual surfaces were usually outside the bonding area due to their inclination. The demarcated enamel surfaces were then flattened with a fine-grit diamond bur (#2135F, KG Sorensen, Barueri, São Paulo, Brazil) attached to a high-speed handpiece under water irrigation to remove the superficial 0.5 mm enamel layer. The abraded surfaces were then polished with wet 600-grit SiC paper to produce a standard smear layer.

For each substrate, the adhesives listed in Table 1 were applied to enamel according to three different modes. In the MD group, the adhesive systems were applied according to the manufacturers’ directions (Table 1). In Group DA, each adhesive system was applied following the manufacturer’s directions; however, the number of recommended coats was duplicated (double-application). In the HR group, each adhesive system was applied according to each manufacturer’s directions, however, each tooth received an additional coat of a hydrophobic resin layer (Bond bottle, Clearfil SE Bond).

The adhesives were light cured for the recommended time (Table 1) using a VIP light unit set at 600 mW/cm² (BISCO, Schaumburg, IL, USA). Resin composite build-ups (Z250, 3M ESPE, St Paul, MN, USA) were constructed on the bonded surfaces in three increments of 1 mm each, which were individually light cured for 30 seconds with the same light intensity. All the bonding procedures were carried out by a single operator at a room temperature of 24°C and constant relative humidity.

### Table 1: Adhesive Systems: Composition, Batch Number and Application Mode of Different Groups

<table>
<thead>
<tr>
<th>Adhesive Systems (manufacturer)</th>
<th>Composition (batch #)</th>
<th>Manufacturers’ Directions (MD)</th>
<th>Double-Application (DA)</th>
<th>Hydrophobic Resin Layer (HR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearfil SE Bond (Kuraray)</td>
<td>Primer—water, MDP, HEMA, camphoroquinone, hydrophilic dimethacrylate (00176A); Adhesive—MDP, Bis-GMA, HEMA, camphoroquinone, hydrophobic dimethacrylate, N,N-dethanol p-toluidine bond, colloidal silica (001185A)</td>
<td>1. Apply two coats of the primer under pressure (20 seconds); 2. Air stream (10 seconds at 20 cm) after application of each coat; 3. Apply one coat of the adhesive (15 seconds); 4. Air stream to make the bond film uniform (3 seconds at 20 cm); 5. Light-activation (10 seconds—600 mW/cm²).</td>
<td>1. Steps 1 through 4 from MD; 2. Repeat steps 1 through 4; 3. Step 5</td>
<td>1. Steps 1 through 5 from MD; 2. Repeat steps 4 through 5.</td>
</tr>
<tr>
<td>Adper Prompt L-Pop (3M ESPE)</td>
<td>Liquid A—water, HEMA, camphoroquinone and hydrophilic dimethacrylate (225666); Liquid B—water, HEMA, polyalkenoic acid, stabilizers (250246)</td>
<td>1. Mix liquids A and B for 5 seconds; 2. Apply one coat of the adhesive under pressure (15 seconds); 3. Gentle air stream (10 seconds at 20 cm); 4. Apply a second coat of the adhesive under pressure (15 seconds); 5. Gentle air stream (10 seconds at 20 cm); 6. Light-activation (10 seconds—600 mW/cm²).</td>
<td>1. Steps 1 through 5 from MD; 2. Repeat steps 2 through 5; 3. Step 6.</td>
<td>1. Steps 1 through 6 from MD; 2. Apply one coat of adhesive from CSE; 3. Air stream to make the bond film uniform (3 seconds at 20 cm); 4. Light-activation (10 seconds—600 mW/cm²).</td>
</tr>
<tr>
<td>Xeno III (Dentsply)</td>
<td>Liquid A: HEMA, ethanol, water, aerosil, stabilizers (0601002833) Liquid B: Pyro-EMA, PEF, UDMA, canforquinone, stabilizers, ethyl-4-dimethylaminobenzoxazote (cointiator) (0601002832)</td>
<td>1. Mix liquids A and B for 5 seconds 2. Apply one thick coat of the adhesive under pressure (30 seconds); 3. Gentle air stream (10 seconds at 20 cm); 4. Light-activation (10 seconds—600 mW/cm²).</td>
<td>1. Steps 1 through 3 from MD; 2. Repeat steps 2 and 3; 3. Step 4.</td>
<td>1. Steps 1 through 4 from MD; 2. Apply one coat of adhesive from CSE; 3. Air stream to make the bond film uniform (3 seconds at 20 cm); 4. Light-activation (10 seconds—600 mW/cm²).</td>
</tr>
<tr>
<td>G Bond (GC Corp)</td>
<td>4-MET, UDMA, phosphate monomer, DMA component, fumed silica filler, acetone, water and photoinitiator (0606231)</td>
<td>1. Apply one coat of adhesive, scrub for 5 seconds; 2. The adhesive was left undisturbed for 5-10 seconds; 3. Air dry under maximum pressure for 5 seconds; 4. Light-curing for 10 seconds at 600 mW/cm².</td>
<td>1. Steps 1 through 3 from MD; 2. Repeat steps 1 through 3; 3. Step 4.</td>
<td>1. Steps 1 through 4 from MD; 2. Apply one coat of adhesive from CSE; 3. Air stream to make the bond film uniform (3 seconds at 20 cm); 4. Light-activation (10 seconds—600 mW/cm²).</td>
</tr>
</tbody>
</table>

MDP: methacryloyloxydodecylpyridinium; HEMA: 2-hydroxyethyl methacrylate; Bis-GMA: bisphenol A diglycidyl methacrylate; 4-MET: 4-methacryloyloxyethyl trimellitic acid; Pyro-EMA: tetramethacryloyloxyethyl pyrophosphate; PEF: pentamethacryloyloxyethylcyclohexaphosphazene monofluoride; UDMA: urethane dimethacrylate; DMA: dimethacrylate
humidity. Five teeth (dentin) and five hemi-teeth (enamel) were used for each combination of the factors Adhesive vs Application mode.

After the restored teeth were stored in distilled water at 37°C for 24 hours, they were longitudinally sectioned in both the “x” and “y” directions across the bonded interface using a diamond saw in a Labcut 1010 machine (Extec Corp, Enfield, CT, USA) to obtain sticks, each with a cross-sectional area approximately 0.8 mm². The number of premature debonded sticks (D) per tooth during specimen preparation was recorded. The cross-sectional area of each stick was measured with the digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) to the nearest 0.01 mm for calculation of the actual microtensile bond strength values (µTBS).

Each bonded stick was attached to a modified device for microtensile testing with cyanoacrylate resin (Zapit, Dental Ventures of North America, Corona, CA, USA) and subjected to a tensile force in a universal testing machine (EMIC, São José dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm/minute. The failure modes were evaluated at 400x (HMV-2, Shimadzu, Tokyo, Japan) and classified as cohesive ([C] failure exclusive within dentin or resin composite), adhesive ([A], failure at the resin/dentin interface) or adhesive/mixed ([A/M], failure at the resin/dentin interface), which included cohesive failure of the neighboring substrates).

The resin-dentin and resin-enamel bond strength of all sticks with adhesive or mixed fracture mode from the same tooth (dentin) or hemi-tooth (enamel) were averaged for statistical purposes. The premature debonded specimens were also included in the mean. The value attributed to these specimens was half of the minimum bond strength value measured in the current study for each substrate.

The data from each substrate were subjected to two-way ANOVA (Adhesive vs Application Mode) and Tukey’s test for pairwise comparisons at a pre-significant level of α=0.05. For enamel, an additional random factor was added to the statistical model as correction for the two half samples gathered from the same tooth.19

### RESULTS

#### Dentin

The mean cross-sectional area ranged from 0.80 to 0.92 mm² and no difference among the groups was detected (p>0.05). The mean and standard deviations of the resin-dentin µTBS means (MPa) are depicted in Table 2, as well as the number of tested vs premature debonded sticks. The interaction of the Adhesive vs Application Mode was statistically significant (p=0.0001). As seen in Table 2, CSE, AD and XE applied according to the manufacturers’ directions showed resin-dentin µTBS means inferior to those obtained in the DA (double-application) and HB (hydrophobic bonding layer) groups. For XE and GB, the highest resin-dentin µTBS were obtained under the HB group (p<0.05), while for AD, the highest µTBS were observed under the DA group (p<0.05). The CSE adhesive, used as the control, showed similar resin-dentin µTBS under all experimental conditions (p>0.05).

#### Enamel

The mean cross-sectional area ranged from 0.73 to 0.90 mm² and no difference among the treatment groups was detected (p>0.05). The mean and standard deviations of the resin-enamel µTBS means (MPa) are depicted in Table 3, as well as the number of tested vs premature debonded sticks. The interaction Adhesive vs Application mode was statistically significant (p<0.03). As seen in Table 3, XE and GB applied according to the manufacturers’ directions showed resin-enamel µTBS means similar to or higher than the DA or HB groups (p>0.05); however, GB showed a tendency towards superior performance under the HB group. For AD, the higher µTBS values were found in the HB group (p<0.05). The CSE system, used as the control, showed

### Table 2: Microtensile Resin-dentin Bond Strength Values (MPa): Means and Standard Deviations (number tested/premature debonded sticks)

<table>
<thead>
<tr>
<th>Adhesive</th>
<th>Manufacturer’s Directions</th>
<th>Double Application</th>
<th>Hydrophobic Resin Layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSE</td>
<td>18.0 ± 4.7 (30/05) f</td>
<td>25.2 ± 1.6 (29/04) e,f</td>
<td>24.5 ± 4.6 (35/04) e,f</td>
</tr>
<tr>
<td>AD</td>
<td>24.3 ± 3.1 (28/09) e,f</td>
<td>45.3 ± 6.4 (28/01) a,b</td>
<td>29.5 ± 4.9 (36/14) d,e</td>
</tr>
<tr>
<td>XE</td>
<td>30.0 ± 1.2 (26/07) d,e</td>
<td>33.8 ± 3.4 (24/07) c,d</td>
<td>49.8 ± 3.7 (29/02) a</td>
</tr>
<tr>
<td>GB</td>
<td>37.1 ± 2.4 (38/06) b,c,d</td>
<td>20.6 ± 3.9 (31/04) f</td>
<td>44.5 ± 3.7 (27/03) a,b</td>
</tr>
</tbody>
</table>

*Groups identified with the same letters are not statistically different (p<0.05).

### Table 3: Microtensile Resin-enamel Bond Strength Values (MPa): Means and Standard Deviations (number tested/premature debonded sticks)

<table>
<thead>
<tr>
<th>Adhesive</th>
<th>Manufacturer’s Directions</th>
<th>Double Application</th>
<th>Hydrophobic Resin Layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSE</td>
<td>33.1 ± 7.9 (18/03) a</td>
<td>22.7 ± 5.0 (20/04) b</td>
<td>25.8 ± 7.1 (17/04) a,b</td>
</tr>
<tr>
<td>AD</td>
<td>13.5 ± 5.4 (14/02) c</td>
<td>17.9 ± 4.7 (24/05) b,c</td>
<td>23.1 ± 5.9 (21/04) b</td>
</tr>
<tr>
<td>XE</td>
<td>20.1 ± 4.3 (15/06) b</td>
<td>21.2 ± 7.4 (18/07) b</td>
<td>15.3 ± 4.4 (17/05) b,c</td>
</tr>
<tr>
<td>GB</td>
<td>25.3 ± 5.6 (16/03) a,b</td>
<td>28.9 ± 6.8 (22/04) a,b</td>
<td>32.0 ± 8.2 (18/04) a</td>
</tr>
</tbody>
</table>

*Groups identified with the same letters are not statistically different (p<0.05).
lower resin-enamel \( \mu \)TBS values in the DA group (\( p<0.05 \)).

**DISCUSSION**

The double application of Adper Prompt L-Pop enhanced the performance of this material to dentin. This finding was already demonstrated by previous literature findings, which reported that the application of only one coat of Adper Prompt L-Pop is not enough to adequately create a sufficiently thick hybrid and adhesive layer that will couple to subsequently applied resin composite.6-8 The increased resin-dentin \( \mu \)TBS under double application is due to several mechanisms operating simultaneously. As the solvent is evaporated between each coat, the concentration of co-monomers that exist after each coating increases,\(^8\) thereby improving the quality of the hybrid layer\(^20\) and the ratio of the polymerized vs unpolymerized adhesive layer due to oxygen inhibition.\(^21\) According to Hashimoto and others,\(^20\) the improved bonding performance when multiple coats are applied but not cured cannot be attributed to the increase in adhesive thickness but to the improved quality of the adhesive layer. Only when each coat is light-cured, is the thickness of the adhesive layer increased.\(^6\)

On the other hand, no improvement in resin-dentin \( \mu \)TBS values was observed for Xeno III and G-Bond with double application. One important difference between XE and GB in relation to AD is their filler content. Filled adhesives, such as XE and GB, produce a thicker adhesive layer with only one coat,\(^16,22\) resulting in this layer being less susceptible to oxygen inhibition.\(^21\) That is why the manufacturers of unfilled adhesives often recommend the application of at least two coats of adhesives. This ensures that the etched dentin will be adequately covered, reducing the deleterious effects of oxygen inhibition, such as defective bond formation both to self-etch\(^6,8\) and etch-and-rinse systems.\(^21\)

For G-Bond, the resin-dentin \( \mu \)TBS was statistically decreased after double application. The rationale behind this finding was not clearly identified by the authors of the current investigation. The most plausible explanation is likely to rely on the solvent differences between G-Bond and Xeno III. While Xeno III is composed of a mixture of water and ethanol, G-Bond contains water and acetone as solvents. As the evaporation of acetone is much faster than water, after acetone evaporation, the water content and monomer concentration is increased proportionally, reducing further water evaporation. This situation can be even worse under double-application, permitting water-accumulation in the adhesive layer. The water molecules compete with the adhesive monomers for binding sites on collagen, and this might have caused reductions in resin-dentin \( \mu \)TBS.\(^24,25\)

Contrary to G-Bond, Xeno III contains a mixture of water-ethanol as the solvent. This mixture at a specific ratio (95.6% ethanol and 4.4% water) creates azeotrope, which is a mixture of two or more miscible liquids that retain the same composition in vapor as in liquid state when distilled or partially evaporated under certain pressure.\(^26\) This implies the formation of hydrogen bonds between water and ethanol molecules, resulting in better evaporation of these water-ethanol aggregates than pure water. In addition, the formation of hydrogen bonds is much lower with ketones (acetone) than with alcohol.\(^27\)

The additional application of a hydrophobic solvent-free resin layer improved the performance of Xeno III and G-Bond in dentin. When one-step self-etch systems were applied according to a two-step protocol, the additional application of the hydrophobic bonding layer must have increased concentration of the hydrophobic monomers, reducing the relative concentration of solvents and hydrophilic monomers within the adhesive interface, which explains increased \( \mu \)TBS. This finding was already demonstrated by previous literature findings.\(^8,14-15\) The application of a hydrophobic coat also seems to limit the diffusion of water through the hybrid layer to the interface between the adhesive and resin composite, otherwise, this diffusion might have occurred rapidly.\(^11,28\) The additional layer of hydrophobic adhesive increased the thickness of the adhesive layer, which is known to reduce polymerization stresses\(^29\) and improve stress distribution during testing.

In regard to enamel substrate, a different scenario was detected. In fact, no significant improvement was shown with double-application. One could expect that this technique would improve the quality of the adhesive layer, leading to better bonding to both substrates.\(^8,31,30\) Obviously, one cannot rule out the fact that the improvement of the adhesive layer properties might have occurred; however, it seems that this fact was of less importance for resin-enamel strength. These results are similar to those reported by Perdigão and others.\(^11\) They showed that the double-application of Adper Prompt L-Pop did not improve bond strength to ground enamel.

In regard to the additional application of a hydrophobic solvent-free resin layer, no significant improvement was observed, except for the AD system. Previous literature findings have demonstrated that low-pH self-etch systems, such as AD, do not present good performance on tooth substrates\(^31,32\) despite their creating a deeper etching pattern than moderate and less acidic self-etch systems.\(^32\) These findings can be attributed to the low cohesive strength of the adhesive layer formed by the AD system. It was already demonstrated that the cohesive strength of the adhesive layer may be more important than the etching potential of an enam-
el adhesive. By increasing the mechanical properties of the adhesive layer through the inclusion of hydrophobic monomers, the application of an extra coat of a hydrophobic layer improved the resin-enamel bond strength values of the AD system.

CONCLUSIONS

The results of the current investigation showed that the alternative modes of application can lead to different results, depending on the material and substrate that prevents researchers from suggesting the use of these alternative modes of application for all one-step systems.

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References


