Influence of Application Methods of Self-etching Adhesive Systems on Adhesive Bond Strength to Dentin

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Purpose: To assess the influence of variations in the application technique of self-etching adhesive systems on the shear bond strength to dentin.

Materials and Methods: The buccal surface of 255 extracted bovine teeth was ground to expose a flat dentin surface; the teeth were divided into four experimental groups. The self-etching adhesive systems One Up Bond F Plus, Clearfil SE Bond, Xeno III, and FuturaBond NR were used, and the etch-and-rinse adhesive Adper Single Bond 2 was used as the control group. One or two layers of the self-etching systems were actively or passively applied. Cylinders of composite resin were bonded to dentin, and after 24 h, specimens were shear tested in a universal testing machine at a crosshead speed of 1 mm/min. The data were submitted to two-way ANOVA, Dunnett’s and Tukey’s test (5%).

Results: There were significant differences for the factors adhesive type, application method, and their interaction. All adhesive systems showed significant differences. The active application of two layers of self-etching bonding systems resulted in a significantly higher mean than the passive application method.

Conclusion: The active application of self-etching adhesive systems tends to increase the dentin shear bond strength, and the influence of the application method depends on the tested adhesive.

Keywords: self-etching adhesives, bond strength, dentin.

The quality of bonding produced by self-etching systems can also be modified according to the characteristics of the smear layer that is produced during cavity preparation and serves as a substrate for bonding.15,21 The thickness and density of the smear layer is a matter of concern when using self-etching systems, because these systems must have the ability to permeate dentin smear layer and impregnate the underlying dentin.11 Previous studies have shown that when thick smear layers were prepared, the bond strength values of self-etching adhesive systems decreased.5,14

In addition, due to the low availability of H+ ions, the self-etching systems can be easily neutralized by the mineral components dissolved in dental structure, before they allow an adequate etching.5,10 To increase the etching ability and reduce the possible difficulties of the self-etching systems applied on prepared dentin, the manufacturers have developed systems which have a lower pH and are, therefore, more aggressive.21

Changes in the application methods of bonding systems have also been proposed to increase bonding to dental substrate.10,23 Traditionally, self-etching adhesive systems are applied to dental substrate without agitation.
However, these systems can be applied actively by brushing an applicator soaked with self-etching primer or all-in-one adhesives on the dental substrate. This may improve bonding by enhancing the interaction of acid monomers with dental tissue and dispersing etching by-products into the hybrid layer.4,23

Another possibility is the application of an additional layer of acidic monomers on the cavity surface, increasing the application time of the self-etching primer or all-in-one adhesive in contact with the dental substrate, and providing a greater number of H+ ions to react with mineral composition. This additional supply of acidic monomers may improve their infiltration into the intertubular demineralized dentin.13

Based on these considerations, this study was performed to evaluate the influence of the self-etching adhesive system application technique on dentin bond strength. The null hypothesis tested was that the number of layers and agitation have no effect on dentin bond strength.

MATERIALS AND METHODS

Two hundred fifty-five bovine incisors were cleaned and stored in distilled water at a temperature of -18°C until they were used.3 The selected teeth were sectioned in the apical direction 3 mm from the cementoenamel junction with a carborundum disk at high speed (Fig 1A); the roots were discarded and the pulp tissue removed with endodontic files (Fig 1B). The pulp chambers were abundantly irrigated with distilled water and dried with a short blast of air.

By means of a round diamond bur in a high-speed handpiece with copious water spray, coronal access in the lingual face of the teeth was made, until the pulp chamber was exposed. The pulp chamber was filled with utility wax to avoid penetration of embedding media. The teeth were embedded in self-curing acrylic resin to allow the exposition of the buccal area parallel to the horizontal plane (Fig 1C).

The pulp chamber access was opened to allow measurement of the remaining dentin thickness with a thickness spring caliper (Fig 1D). The dentin surface was exposed using a wet 80-grit silicon carbide paper, and the dentin thickness was standardized at 2 mm. The dentin surface was polished with 600-grit sandpaper on a polishing machine (Politriz DP 10, Struers; São Paulo, SP, Brazil) for 30 s. To limit the area for the application of the adhesive system, a special Scotchtape mold with a standard central hole, 3 mm in diameter, was placed on each specimen (Fig 1E).

Specimens were randomly assigned into 4 experimental groups of 60 teeth each and a control group of 15 teeth. The tested materials are listed in Table 1.

All groups except the control were divided into 4 subgroups of 15 specimens each, according to the application techniques:

• One passive layer (1P) – One layer of the respective adhesive systems was applied passively, followed by a waiting time of 20 s for it to act, as recommended by the manufacturers. Then, gentle air blowing (35 psi) was performed to help solvent evaporation for 5 s at a distance of 10 cm, followed by light curing for 20 s.

• Two passive layers (2P) – The first layer was applied in a manner similar to that for subgroup 1P, without light polymerization. A second layer was applied in the same way as the first, and light cured for 20 s.

• One active layer (1A) – A layer similar to that for subgroup 1P was applied actively by rubbing a microbrush-type applicator (Microbrush International; Orlando, FL, USA) in circular movements for the action time recommended. The solvent was evaporated with gentle air blowing (35 psi) for 5 s at a distance of 10 cm, and light cured for 20 s.

• Two active layers (2A) – Application was done in a manner similar to that for subgroup 1A, without light polymerization. Next, a second layer was applied, similar to the first, followed by air blowing and light curing.
In the control group, the etch-and-rinse adhesive system Adper Single Bond 2 (3M ESPE; St Paul, MN, USA) was applied according to the manufacturer’s instructions.

After the described treatments, the specimens were mounted in an apparatus containing a split Teflon mold with a circular hole 3 mm in diameter and 4 mm high (Fig 1F). Two increments of a composite resin (Filtek Z250, 3M ESPE) were inserted into the opening of the split mold and each one was light cured for 40 s. After curing, the split mold was removed and the composite cylinder was additionally light cured for 40 s from the two opposing sides (Fig 1G).

After storage in distilled water at 37°C for 24 h, the teeth were locked in a special device that was seated on the universal testing machine (EMIC; São José dos Pinhais, PR, Brazil). A shear load was applied to the base of the cylinder with a 1-mm-thick knife-edge rod at a crosshead speed of 1 mm/min (Fig 1H).

The results were expressed in MPa and were subjected to Dunnett’s test, two-way analysis of variance (ANOVA), and Tukey’s test at the 5% level of significance.

### Failure Analysis

After the shear test, the fractured dentin surfaces were analyzed with a stereomicroscope at 20X magnification (Stemi 2000C, Carl Zeiss; Jena, Germany) to determine failure pattern. Four fracture modes were distinguished: adhesive failure, cohesive failure in resin, cohesive failure in dentin, mixed failure. The failure was defined as adhesive when it occurred between bonding resin and dentin or composite resin over more than 75% of the analyzed area. Cohesive failure occurred when more than 75% of the bonding area appeared to have failed within the composite or within the dentin. When 25% to 75% of the failure was both adhesive and cohesive, the failure was considered mixed.

### Scanning Electron Microscopy (SEM) analysis

Four specimens of each group were additionally prepared for SEM analysis. The adhesive systems were used according to the application methods described above and light curing was not performed. The specimens were rinsed with acetone for 5 s to remove monomers. They were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 12 h at 4°C, rinsed with 20 ml of 0.2 M sodium cacodylate buffer for 1 h in three different baths, and rinsed with distilled water for 1 min. The teeth were dehydrated in ascending grades of ethanol (25% for 20 min, 50% for 20 min, 75% for 20 min, 95% for 30 min, 100% for 60 min), transferred to HMDS for 10 min to complete dehydration and stabilization of the collagen fibers, then placed on a filter paper inside a covered glass vial, and dried at room temperature for 30 min. Finally, the specimens were mounted on aluminum stubs, sputter coated with gold using a sputtering device (DeskII, Denton Vacuum; Moorestown, NJ, USA) for 2 min.

### Table 1 Composition and manufacturer of materials used

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<th>Product</th>
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| One Up Bond F Plus           | Agent A: MAC-10 (malonic acid 10-methacryl oxide camethylene); methacryloyloxyalkyl acid phosphate, bis-GMA, TEG-DMA, photoinitiators  
Agent B: HEMA, water, aluminum glass powder silica; photoinitiators | Tokuyama Dental; Tokyo, Japan          |
| Clearfil SE Bond             | Primer: 10-MDP, HEMA, hydrophilic dimethacrylate, di-camphorquinone, N,N-diethanol-p-toluidine, water  
Bond: 10-MDP bis-GMA, HEMA, hydrophobic dimethacrylate, N, N-diethanol-p-toluidine, di-camphorquinone, silanated colloidal silica | Kuraray; Tokyo, Japan                  |
| Xeno III                     | Liquid A: HEMA, purified water, ethanol, butylated hydroxy toluene, highly dispersed silicon dioxide  
Liquid B: phosphoric acid functionalized polymethacrylate resins, di- and polyfunctionalized methacrylate resins, butylated hydroxy toluene, camphorquinone, 4-dimethylaminoethyl benzoate. | Dentsply DeTrey; Konstanz, Germany     |
| FuturaBond NR                | Bis-GMA, dimethacrylates, hydroxyethyl methacrylate, BHT, ethanol, organic acids, fluorides, silicate filler  
Liquid A: methacryloyloxyalkyl acid phosphate (phosphoric acid monomer) | Dentsply DeTrey; Konstanz, Germany     |
| Adper Single Bond Plus       | Bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator methacrylate functional copolymer of polyacrylic, polyitaconic acids and 10% by weight of 5-nm-diameter spherical silica particles | 3M ESPE; St Paul, MN, USA              |
| Scotchbond Etchant           | 35% phosphoric acid, silica                                                                                                                                                                                  | 3M ESPE                                |
and observed under a scanning electron microscope (JSM 5310, JEOL; Tokyo, Japan).

**RESULTS**

Results of the two-way ANOVA are presented in Table 2. When analyzing the factors “type of adhesive” and “application technique”, significant differences and a significant interaction between them were found ($p = 0.0001$).

Table 3 shows the results of Tukey’s test for the adhesive factor. There were significant differences for all adhesive systems tested, and Clearfil SE Bond presented the highest mean values. The results of Tukey’s test for the factor “application technique” are shown in Table 4. It can be observed that the active application of two layers resulted in mean values significantly higher than the passive application.

Table 5 exhibits the results of Dunnett’s test for the comparison of all groups tested with one control group. It shows that One Up Bond F Plus and Futurabond NR presented significantly lower bond strengths compared to the etch-and-rinse adhesive system tested. The results of passive application of one and two layers of Xeno III also showed significantly lower values than the control group.
Figure 2 compares the means obtained for the different types of adhesive and application techniques tested. For One Up Bond F Plus and FuturaBond NR groups, there were no significant differences for the different application techniques. The active application of two layers of Xeno III resulted in significantly higher bond strength means than the other application techniques tested for the same adhesive. For Clearfil SE Bond groups, the passive application of one layer produced significantly lower means than the other application techniques tested.

**Failure Analysis**

The control group and the group OU showed predominantly adhesive failures. The group XE presented predominantly mixed failures and the FB group showed predominantly adhesive failures when one passive or active layer was applied, and mixed failures when two layers were applied. For the group CL, the application of one layer resulted predominantly in mixed failures; when actively or passively two layers were applied, the failures were predominantly adhesive (Table 6).
SEM Analysis

Figure 3 depicts the SEM analysis of the control group’s dentin surfaces etched with phosphoric acid with exposed collagen fibrils on the surface, showing a porous appearance. Figures 4 to 7 show the dentin surfaces after the application of adhesive systems with different techniques. With passive application, all self-etching systems tested showed occluded tubules and smear layer clearly visible on the dentin surface when one layer was applied (Figs 4A, 5A, 6A, and 7A).
With One Up Bond F Plus and Clearfil SE Bond, the application of two layers without agitation did not result in clearly visible alterations in dentin conditioning pattern (Figs 4C and 7C), compared to the application of one layer. When one layer was applied actively, some tubules were partially opened, but the smear layer was present on most parts of the dentin surface when One Up Bond F Plus was used (Fig 4B) and on some parts of the dentin surface using Clearfil SE Bond (Fig 7B).

The active application of one layer and the passive application of two layers resulted in similar conditioning patterns for Xeno III and FuturaBond NR, showing tubules partially occluded and smear layer present over most parts of the dentin surface (Figs 5B, 5C, 6B, and 6C). The application of two layers with agitation resulted in a more pronounced conditioning pattern, with tubules almost completely opened for all adhesive systems tested (Figs 4D, 5D, 6D and 7D).

**DISCUSSION**

Bovine dentin was used because of the large number of teeth needed for this experiment, and teeth of similar age and size were necessary to standardize the specimens. Bovine dentin has been reported to be a viable alternative for human dentin in bond strength testing. Previous studies have shown that adhesion to superficial dentin is not markedly different from that to human dentin, although the mean values were slightly lower in bovine specimens.12,19 In addition, bovine dentin is similar to the human substrate in respect to radiodensity, which reflects similarity in atomic composition and physical structure.6

Changes in application methods and the use of multiple layers of self-etching adhesive systems have been proposed to improve the bond performance to dentin.10,13,20 One alternative is the application of a second layer of self-etching adhesive systems to dentin. This procedure aimed to increase the number of H⁺ ions supplied to the substrate, improve the infiltration of resin monomers into the intertubular demineralized dentin, and increase the action time of the acidic solution.13

The application of multiple layers of self-etching bonding systems can significantly increase the application time, increasing the bond strength.8 In the present study, there was no significant increase of bond strengths when two layers of the adhesives were applied without agitation, when compared to one layer (Table 4), although a slight variation of the conditioning pattern was observed for Xeno II and FuturaBond NR (Figs 5B and 6B, respectively). Nakaoki et al13 also found no significant differences in bond strength between the single and double application of self-etching adhesive systems. This may be due to the deposition of insoluble products from hydroxyapatite dissolution on the surface of the substrate, preventing an additional conditioning effect. Furthermore, the application of an air blast between the first and the second layer may have increased the viscosity of the adhesive monomers, creating a film that could impede good contact of the additional layer of monomer with the dentin.

The increased thickness of the adhesive could also have influenced the shear bond strength. According to Zhen et al,25 the thickness of the adhesive layer can interfere with the bond strength. Nevertheless, previous studies have shown that bonding can be improved with the application of an additional adhesive layer after light curing the first layer.8,16

When active application was performed, an increase of the shear bond strength for the tested adhesive systems was observed (Table 4). Previous studies also found a significant increase in bond strengths when self-etching adhesive systems were applied on dentin under agitation.4,23 This result can be justified by the better conditioning of the substrate compared to the passive application method.

Figures 4C, 5C, 6C, and 7C show partial opening of dentinal tubules, compared to the passive application of one layer (Figs 4A, 5A, 6A, and 7A). This may be caused by the better contact of the hydrogen ions of the monomer solution with substrate. This way, the reaction would not depend only on ion diffusion of the solution, which can be higher or lower depending on its viscosity and water content. With active application, the entire volume of adhesive applied will contact the bonding area, promoting the reaction of free H⁺ ions with the dental mineral composition, and drawing fresh, unbuffered acidic resin monomers to the demineralization front.4

The active application of two layers of the adhesive system significantly increased the shear bond strength for Xeno III, while Clearfil SE Bond showed a discrete
increase (Fig 2). These results may be explained by the additional conditioning effect of the second layer application added to the greater agitation time. These factors would increase the number of dentin tubules opened, and consequently produce higher bond strength values. In a previous study, it was observed that demineralization of dentin surface was more pronounced with longer application duration of single-application bonding systems, increasing the bond strength.9

The SEM analysis shows a more aggressive conditioning pattern when two layers of adhesive systems were applied actively, with an increase of the opening of dentin tubules for all adhesive systems tested (Figs 4D, 5D, 6D, and 7D). Chan et al4 also observed that patent dentin tubules were rendered by partial or complete removal of smear plugs when self-etching systems were applied on dentin with continuous agitation.

Table 5 shows the comparison of the tested adhesives with control group (etching and rinse system). Only Clearfil SE Bond presented higher bond strength values than the control group, while the application of Xeno III with agitation presented a statistically similar performance. It is important to emphasize that this result was obtained without acid etching, showing that the bond strength is influenced by other factors, such as solvent, primer, and bond composition, and wetness of substrate.17,20,23

Furthermore, the results of this study showed that alterations in the application method caused different effects on bond strengths, depending on the tested adhesives. The null hypothesis was rejected for the application technique factor. Therefore, additional studies should be accomplished to clarify the interaction between self-etching systems and dental substrate, as well as the influence of the application methods in dental bonding. This would probably result in more effective and less aggressive adhesive systems, and in more reliable bonding.

**CONCLUSION**

Within limits of the present study, it can be concluded that:

1. The active application of self-etching adhesive systems tends to increase the dentin shear bond strength.
2. The influence of the application method depends on the tested adhesive.
3. The active application of one and two layers of Xeno III and Clearfil SE Bond produced bond strength means similar to the control group.

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REFERENCES


Clinical relevance: The variation in the technique proposed by this study can improve the effectiveness of some self-etching adhesive systems, eg, Clearfil SE Bond and Xeno III, resulting in increased bond strength values.