Effect of Double Coating of One-step Self-etching Adhesive on Micromorphology and Microtensile Bond Strength to Sound vs Demineralized Dentin

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ABSTRACT

Aim: The purpose of the present study was to evaluate (1) the one-step adhesive system application method (doubling the adhesive coatings) in regard to microtensile bond strength (MTB) and (2) the interfacial morphology of one-step adhesives to sound vs demineralized dentin.

Materials and methods: Forty dentin fragments were randomly allocated to 2 groups: D, demineralized dentin and S, sound dentin. Specimens were also subdivided into 2 groups (n = 10), according to the one-step adhesive [AEO (Adper Easy One), 3M ESPE] application method: M, according to the manufacturer’s instructions, and D, based on the application of two consecutive layers. After adhesive light polymerization, a resin composite block (Filtek Z250, 3M ESPE) was built on the dentin surface. Resin-tooth blocks were sectioned into 0.9 mm thick slabs, and one slab of each block was prepared for adhesive interface analysis by scanning electron microscopy (SEM). The remaining slabs were sectioned into 0.6 mm sticks that were subjected to tensile stress (0.5 mm/min). Data were subjected to two-way ANOVA and Tukey’s test (α = 0.05).

Results: The application of two consecutive layers of AEO adhesive system did not influence MTB values for sound dentin. When two consecutive layers of one-step adhesive system were applied, MTB was statistically greater in demineralized vs sound dentin. SEM analysis demonstrated that the application of two consecutive adhesive layers to sound and demineralized dentin produced longer resin tags.

Conclusion: It can be concluded that the application of two consecutive adhesive layers improved bond strength to demineralized dentin, but no such effect was observed for sound dentin.

Clinical significance: Application of double coats of one-step self-etching adhesive improved bond strength to demineralized dentin.

Keywords: Dentin, Demineralization, Adhesion.


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Conflict of interest: None declared

INTRODUCTION

Adhesion of resin-based materials to enamel has been well-established since the etch-and-rinse approach was developed. However, the challenge of ensuring adequate adhesion to dentin still remains. In general, the dentin substrate chemical composition consists of 50% minerals, 20% water and 30% organic matrix. However, it is known that this composition may change according to tooth depth, considering that superficial dentin has few tubules and is composed predominantly of intertubular dentin. Deep dentin (near the pulp) is composed mainly of larger funnel-shaped dentinal tubules with much less intertubular dentin, making adhesion to this region more critical.

Adhesion to dentin is based on the formation of a hybrid layer, first described by Nakabayashi and obtained by resin infiltration within the exposed collagen fibrils. However, the ideal situation in which the adhesive system completely penetrates demineralized dentin is rarely achieved. Incomplete resin impregnation and imperfect polymerization of adhesive resin may result in adhesive interface degradation and nanoleakage over time.

The self-etching adhesives (available as two-step or one-step adhesives) were proposed with the advantage that resin infiltration occurs simultaneously with the self-etching process. Accordingly, the risk of discrepancy between both processes (demineralization and resin infiltration) is low or non-existent. Moreover, the risk of making errors during application and manipulation are diminished. Despite these advantages, the clinical performance of these adhesives must be improved, especially regarding bond strength to dentin.

Another dental substrate commonly found in dental practice in addition to sound dentin is caries-affected dentin. Caries-affected dentin is defined as demineralized, low-contaminated or bacteria-free dentin capable of remine-
ralization and therefore requiring that it be maintained during caries removal. With this in mind, many studies have been performed on demineralized dentin, including those that evaluate bond strength of self-etching adhesives. However, lower bond strength is usually reported in demineralized dentin, compared to sound dentin.

In an attempt to increase clinical performance of one-step self-etching adhesives, the application of multiple consecutive layers of these adhesives has been recommended. This is especially important when mild-pH and ultra-mild-pH adhesives are considered, because they demineralize dentin only to a superficial depth. However, this application protocol has not been tested on demineralized dentin. Accordingly, it would be very interesting to verify if the application of two consecutive layers of a one-step self-etching adhesive would be able to enhance the bond strength to demineralized dentin, compared to sound dentin. So, the aim of this study was to evaluate the influence of the application method (one layer vs two consecutive layers) of a one-step self-etching adhesive system on micromorphology and microtensile bond strength to sound vs demineralized dentin.

MATERIALS AND METHODS

Ethical Aspects

The present study was approved by the Research Ethics Committee of São Leopoldo Mandic Institute and Research Center (#2011/0300).

Experimental Design

The factors studied were:
- Dentin condition, on two levels: demineralized and sound (control);
- Application method of an adhesive system, on two levels: one layer (as recommended by the manufacturer — group M) or two consecutive layers (group D).

Four experimental groups (n = 10) were formed according to variables of dentin condition and application method. After the application of the adhesive system, a block of resin composite was built on the dentin. The resin-dentin block was considered as the experimental unit. Resin-dentin blocks were sectioned into 0.9 mm thick slabs, and one slab of each block was prepared for adhesive interface analysis by scanning electron microscopy (SEM) (qualitative response variable). The remaining blocks were sectioned into 0.8 mm² sticks that were subjected to tensile stress (quantitative response variable). The bond strength average of sticks from the same tooth became the value for that particular experimental unit.

Dentin Fragments Preparation

Forty human third molars, extracted for reasons not related to those of the present research, were used in this experiment. They were stored in thymol (0.1%, pH 7.0) after extraction. The teeth were submitted to deburring with scalpel blades and periodontal curettes. They were then cross sectioned using the water-cooled diamond saw (15 HC series, Buehler Ltd, Lake Bluff, Illinois, EUA) of a sectioning machine (Isomet 1000 Precision Diamond Saw, Buehler Ltd, Lake Bluff, Illinois, EUA). The occlusal third of the crown was separated and a large dentin surface was obtained in the middle third, perpendicular to the long axis of the tooth. A second parallel section was made 4 mm from the first one to obtain 4 mm high fragments. These fragments were sectioned in the mesiodistal and buccolingual directions to obtain square slabs measuring 5 × 5 mm. Dentin slabs were flattened in a water-cooled polishing machine (Politriz Aropol 2V, Arotec, São Paulo, Brazil) with decreasing granulations (400, 600 and 1200) of water abrasive paper (Imperial Wetordry, 3M, USA). At this time, dentin specimens were randomly divided into 2 groups, according to the dentin condition: demineralized (D) or sound dentin (S).

Obtaining Demineralized Dentin

The demineralized group was submitted to a dynamic model of demineralization/remineralization proposed by Hara et al., which simulates high caries risk conditions. To this end, dentin slabs were covered with nail varnish, leaving only an exposed dentine surface area of 4 × 4 mm. The specimens were submitted individually to three 24 hour cycles that consisted of: 1 hour in demineralizing solution (2.0 mM of calcium, 2.0 mM of phosphate in a buffer solution of 74 mM of acetate at pH 4.3) and 23 hours in remineralizing solution (1.5 mM of calcium and 0.9 mM of phosphate in a buffer solution of 20.0 mM of tris (hydroxymethyl)-aminomethane at pH 7.0), similar to the procedure proposed by Featherstone and modified by Serra and Cury. Specimens were rinsed with distilled water between remineralizing cycles. At the end of the immersion time, the slabs were removed from the solutions, rinsed with deionized water and prepared for adhesive procedures.

Adhesive Procedures

Sound and demineralized specimens were randomly divided into two groups, according to the adhesive system application method: one layer (as recommended by the manufacturer — group M) or two consecutive layers (double layer — group D). The one-step adhesive system is shown in Table 1.

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For the group M, the adhesive was applied actively on the dentin surface with a disposable brush for 20 seconds, dried gently for 5 seconds for the solvent to evaporate and then light polymerized for 10 seconds.

For the group D, the adhesive was applied on the dentin surface with a disposable brush for 20 seconds, but without light polymerization. A second layer was applied over the first, dried for 5 seconds and then light polymerized for 10 seconds.

A composite resin block (Filtek Z 250, A3 color, 3M ESPE Irvine, CA, USA), measuring 5 × 5 mm (height × width) was built on the bonding surface according to the incremental technique. Each layer of composite (approximately 2 mm thick) was light polymerized individually for 40 seconds, with a visible light-curing unit (Ultralux EL, Dabi Atlante, Ribeirão Preto, SP, Brazil). Finally, the restoration was light polymerized for 20 seconds on each of its two sides. The output of the light-curing unit was a mean range of 620 mW/cm², as measured periodically by a radiometer (Newdent Equipamentos Ltda, Ribeirão Preto, SP, Brazil).

Micromorphological Evaluation

One slab of each resin-tooth block was flattened with decreasing granulations (400, 600 and 1200) of water abrasive paper under water-cooling, and polished on felt disks, with diamond pastes of sequentially decreasing granulation (6, 3, 1 e ½ μm) under mineral oil cooling. After thorough rinsing, samples were demineralized for 30 seconds with 6N HCl, rinsed again, deproteinized with 2.5% NaOCl for 10 minutes and serially dehydrated with 25, 50, 75, 95 and 100% ethanol, chemically dried in HMDS for 10 minutes, mounted on aluminum stubs, sputter-coated with gold, and examined under a scanning electron microscope (Jeol 5900LV, Jeol Ltd, Tokyo, Japan), at 500×, 1000× and 2000× magnifications. The operating voltage was 10 kv.

μTBS Testing

The remaining slabs were sectioned perpendicular to the bonding surface, into multiple beam-shaped sticks with a cross-sectional surface area of 0.8 mm². The cross-sectional area of each stick was measured with a digital caliper (Mitutoyo, Tokyo, Japan). The sticks were attached to an acrylic device using a cyanoacrylate adhesive (Super Bonder Gel, Henkel Ltda, SP, Brazil) and subjected to tensile stress in a universal testing machine (MEM-2.000 model, EMIC, São José dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm/minute and a 20N load cell until fracture. The bond strength values were reported in MPa dividing the imposed force (N) at the time of fracture by the bond area (mm²). The comparison was made using the mean of each tooth.

After bond strength testing, the failure pattern of each stick was analyzed under a stereomicroscope (EK3ST, CQA, São Paulo, Brazil) at 30× magnification to assess the failure modes, which were classified as adhesive (lack of adhesion), cohesive in dentin (failure of the dental substrate), cohesive in composite resin (failure of the resin composite) or mixed (adhesive and cohesive failures).

Statistical Analysis

The means and standard deviations were calculated and the data were subjected to two-way analysis of variance (ANOVA), followed by Tukey’s test (α = 0.05).

RESULTS

Two-way ANOVA revealed significant interaction between the factors studied (p = 0.05). Tukey’s test demonstrated that there was a significant increase in bond strength when demineralized dentin was submitted to the application of two consecutive layers of the one-step self-etching adhesive (Table 2). For sound dentin, the application of two consecutive layers of the one-step self-etching adhesive yielded bond strength values that were statistically similar to the group in which the adhesive was applied, as recommended by the manufacturer (one layer).

When adhesive application was performed as recommended by the manufacturer, it was observed that bond strength in demineralized dentin was statistically similar to bond strength in sound dentin. However, when two consecutive layers of the adhesive system were applied, bond strength in demineralized dentin was statistically superior to that of sound dentin.

Table 1: Description of the self-etching adhesive system

<table>
<thead>
<tr>
<th>Trade name/manufacturer/batch number</th>
<th>Classification solvent type</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adper Easy One</td>
<td>One-step adhesive system</td>
<td>HEMA, Bis-GMA, methacrylated phosphoric esters, 1,6-Hexanediol</td>
</tr>
<tr>
<td>3M ESPE, Germany</td>
<td>Water and alcohol</td>
<td>methacrylate, Vitrebond copolymer, finely dispersed bonded silica with 7nm filler particles, ethanol, water, initiators based on CQ, stabilizers</td>
</tr>
<tr>
<td># 431326</td>
<td>pH ~3.5</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Mean (standard deviation) in each experimental group

<table>
<thead>
<tr>
<th>Substrate condition</th>
<th>Application method</th>
<th>Two consecutive layers</th>
<th>Recommended by manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demineralized</td>
<td>29.42 (9.03)</td>
<td>18.25 (9.76)</td>
<td>19.38 (7.70)</td>
</tr>
<tr>
<td>Sound</td>
<td>19.75 (10.03)</td>
<td>19.75 (10.03)</td>
<td></td>
</tr>
</tbody>
</table>

Means followed by the same capital letters in column and small letters in row are not statistically different (p < 0.05).
Morphological evaluation demonstrated that when the adhesive system was applied to sound dentin, following the manufacturer’s instructions, numerous cone-shaped resin tags could be evidenced. Lateral branches were not observed (Figs 1A and B). The application of two consecutive layers of the adhesive system (Figs 1C and D) was incapable of increasing the number of resin tags, but these were longer than when the adhesive was applied as recommended by the manufacturer. When the adhesive system was applied to demineralized dentin, as recommended by the manufacturer (Figs 1E and F), morphological evaluation showed the formation of fewer resin tags than in the group in which the adhesive was applied in two consecutive layers (Figs 1G and H). The hybrid layer was recognized in all tested groups.

Fracture pattern evaluation (Graph 1) demonstrated that in the sound dentin group, in which the adhesive system was applied following the manufacturer’s instructions, 34% of the failures were adhesive, 28% were cohesive in resin com-
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Figs 1A to H: SEM micrograph of SM group under (A) 500× and (B) 1000× magnification. SEM micrograph of SD group under (C) 500× and (D) 1000× magnification. SEM micrograph of DM group under (E) 500× and (F) 1000× magnification. SEM micrograph of DD group under (C) 500× and (D) 1000× magnification.
D: Dentin; HL: Hybrid layer. Arrows indicate resin tags

Results of the present study are different from those of Arisu et al. which found that the application of three consecutive layers significantly increased bond strength of an one-step adhesive (Clearfil Tri S Bond, pH 2.7). Clearfil Tri S Bond adhesive has a lower pH (more acidic) than Adper Easy One adhesive (which has a pH of about 3.5). In fact, Adper Easy One has been classified as an ultramild pH adhesive. Accordingly, adhesive monomer penetration may be deeper with the application of a 2.7 pH adhesive (Clearfil Tri S Bond) as opposed to a 3.5 pH adhesive, such as that used in this study. This may explain the increase in bond strength to sound dentin reported by Arisu et al. Similarly, Bellis et al. found that the additional coats of ultramild pH adhesives (Clearfil Tri S Bond, pH 2.7 and Adper Easy Bond, pH 2.5) helped improve microtensile bond strength to sound dentin.

Adhesive was actively applied to dentin, i.e. by rubbing the dentin surface with a disposable brush. Zhang and Wang reported that this procedure (called ‘agitation’) could benefit polymerization (degree of conversion) of a strong self-etching adhesive (Adper Prompt L-Pop, pH~0.8) by enhancing chemical interaction with dentin. However, in their study, the degree of dentin demineralization and the degree of conversion to a mild-pH adhesive (Adper Easy Bond, pH~2.5) was not as dependent on the application method (active vs. inactive). Again, a likely observation is that the more acidic the adhesive pH, the greater the interaction/infiltration of resin monomers into dentin.

DISCUSSION

The application of two consecutive layers of one step adhesive system was incapable of increase significantly the bond strength in sound dentin. Morphologically, it appears that resin tag lengths were longer in the case of application of two consecutive layers (Figs 1C and D) as opposed to application of a single layer (Figs 1A and B). Nevertheless, bond strength should not be associated only to the formation of resin tags, but also to the penetration of resin monomers into intertubular dentin. Results of the present study are different from those of Arisu et al. which found that the application of three consecutive layers significantly increased bond strength of an one-step adhesive (Clearfil Tri S Bond, pH 2.7). Clearfil Tri S Bond adhesive has a lower pH (more acidic) than Adper Easy One adhesive (which has a pH of about 3.5).

Analysis of fractured sticks in demineralized dentin showed that when the adhesive was applied following the manufacturer’s instructions, 50% of the fractures were classified as adhesive, 10.9% as cohesive in resin composite, 6.5% as cohesive in dentin, and 32.6% as mixed. When the adhesive was applied with two consecutive layers, demineralized dentin failures exhibited the following modes: 60.9% were adhesive, 4.3% were cohesive in dentin, 2.2% were cohesive in resin composite and 32.6% were mixed.
The present study demonstrated a significant increase in bond strength when a one-step self-etching adhesive was applied to demineralized dentin with two consecutive layers. Morphologically, the DD group showed abundant resin tag formation (Figs 1G and 1H). This result may be attributed to the demineralizing/remineralizing pH-cycling model applied in this study. This model may have caused dentin demineralization to such a depth that penetration of acidic monomers of the adhesive system may have been boosted when applied in two consecutive layers.

When the one-step self-etching adhesive system was applied according to the manufacturer’s instructions, bond strength to sound dentin and demineralized dentin was statistically similar. These results corroborate those of Mobarak and El-Badrawy which did not find significant differences in microshear bond strength of self-etching adhesives to caries-affected versus sound dentin. However, the findings of the present study are different from those of the study by Xie et al. which used an acidified gel technique to induce artificial carious dentin formation for 3 weeks. In their study, adhesion of an etch-and-rinse adhesive to demineralized dentin was 55% inferior to that of sound dentin.

Studies that use natural carious teeth commonly report that bond strength of etch-and-rinse and self-etching adhesives is lower to caries-affected dentin than to sound dentin. These studies commonly use teeth with pre-existing caries lesions, submitted to natural caries evolution under clinical conditions. In vivo, dentin is a tissue that reacts to transmission of stimuli from the oral cavity through its microporous enamel. The most common defense reaction by the pulp-dentin complex against the caries process is the deposition of minerals within the dentinal tubules. This is called ‘tubular sclerosis’, a process that requires a vital odontoblast, and that may influence dentin permeability and negatively affect bond strength to dentin, among other factors. The dynamic pH-cycling model, such as that used in the present study, is unable to induce tubular sclerosis and other dentin reactions. In a clinical situation, if tubules are empty (not occluded by mineralized deposits), voids and water channels may be formed with the use of one-step adhesives. This may be a sign of the high permeability of the dentin sub-surface when such adhesives are used. Considering this, long-term bond efficacy of one-step adhesives should be evaluated in further studies.

Considering that demineralized dentin remains in cavity preparation after caries removal and that it is very difficult to differentiate demineralized from sound dentin, clinically, the results of the present study recommend the application of two layers of ultramild self-etching adhesive to dentin.

CONCLUSION

It can be concluded that the application of two consecutive layers of ultramild one-step adhesive increased bond strength to demineralized dentin.

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REFERENCES

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