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Reference


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Effect of 180-Day Water Storage on Bonding Effectiveness of Self-Adhesive Systems to Occlusal and Proximal Dentin

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Keywords
Dentin-bonding agent; dental bonding; bond strength; water storage; microshear; adhesive system.

Abstract
Purpose: This study aimed to evaluate the microshear bond strength (μSBS) values of adhesive systems to occlusal and proximal dentin submitted to water storage aging. Materials and Methods: Occlusal and proximal dentin surfaces were obtained from 90 molars, polished with 600-grit SiC and divided according to the type of adhesive system: one total-etch and two self-etch. Starch tubing was used to perform 1 mm² cylindrical composite resin restorations. The specimens were aged in distilled water during different storage times: 24 hours, 3 months, and 6 months (n = 10). The specimens were submitted to μSBS test. The μSBS values in MPa were subject to three-way ANOVA and post hoc Tukey test (p < 0.05). Results: There was no statistical difference in the μSBS values among the storage times (p = 0.72); however, the dentin region (p < 0.01) and the adhesive system (p < 0.01) significantly affected the μSBS. The proximal surface (14.7 ± 3.3 MPa) presented higher μSBS values than the occlusal dentin (10.9 ± 4.1 MPa). The all-in-one adhesive system (GB) achieved the highest μSBS mean (17.0 ± 1.7 MPa). Conclusion: Both material and dentin surface factors affected the composite-dentin bond strength; however, the water storage did not influence bonding effectiveness over time.

The bonding mechanism of self-etch adhesives has been intensely investigated and described.1-6 However, the durability of its adhesion to teeth is still questionable. The self-adhesive systems have been shown to lose bond strength over a long-term period, especially when compared with the simpler all-in-one system technique.2,6-8 Some studies have indicated controversies in the behavior of those self-etch systems, which are more hydrophilic and create a more permeable hybrid layer, a fact that could influence the bond stability.1,2,6 The water permeation within the hybridized area jeopardizes the mechanical properties,4 promoting alterations in the hybrid layer over time2 and a decrease in bond strength values of the adhesive systems.2,6 The decrease is attributed to a combined deterioration of both resin polymers and collagen fibrils.2,9 The stability of the bonding interface is also aggravated because after the dentin demineralization, the dentin permeability increases, and water from dentin and pulp permeates through the interface.10

As water is known to be a crucial factor in the adhesive interface degradation process, accelerated laboratory aging by aqueous storage is feasible because of the short diffusion distances required.2,4,11 Several approaches might be used to promote aging in the adhesive interface, and measuring the bond strength values of adhesive systems is a powerful tool to compare results with and without such aging effects. The microtensile test was introduced in 1994 to assess composite-dentin bond strength values,12 and later the microshear test was introduced.13 The method chosen to measure the bond strength is a controversial topic in dental adhesion as each test has its inherent benefits.

The microshear test presents advantages over other bond strength methods because of the easier specimen preparation and the smaller bonding surface area (1.0 mm²) used,14 which allows the storage medium to infiltrate the bonding area efficiently.15 When compared with the conventional shear bond test, the stress distribution of the microshear test is more concentrated at the adhesive interface, minimizing the chance of cohesive failures that do not represent the true interfacial bond strength.14,16 Nevertheless, some limitations of this test have been reported, such as the number of premature failures during the polyethylene tubing removal step.14 Thus, other types of tubing could be used to overcome that limitation.17

A correlation between aged bond strength and clinical data has been shown.18 In this context, the aim of this study was to evaluate the microshear bond strength (μSBS) values of...
different self-adhesive systems to occlusal and proximal dentin surfaces submitted to water storage aging times. The null hypotheses tested were: (1) the different adhesive systems and dentin surfaces promote no effect on μSBS; and (2) the water storage aging promotes a decrease in μSBS.

Materials and methods

This in vitro study was approved by the Local Ethics Committee in Research (protocol 23081.010339/2008 – 22, UFSM). Ninety extracted human molars were selected, cleaned, and stored in 0.5% thymol aqueous solution (5°C). To obtain occlusal and proximal dentin surfaces, the specimens were prepared in two ways: (1) the proximal substrate was obtained from a longitudinal section of the distal or mesial third of the crown, exposing the superficial proximal dentin; and (2) the occlusal substrate was achieved by a complete cross-section at the middle third of the crown, exposing the superficial occlusal dentin. The cuts were performed with a low-speed diamond saw water-cooled cutting machine (Isomet 1000; Buehler, Lake Bluff, IL). The teeth were embedded with acrylic resin in Polyvinyl chloride (PVC) cylinders and polished with 600-SiC grit silicon carbide paper in a circular mechanical polishing machine (PL 4; AroTec, Sao Paulo, Brazil).

Figure 1 presents a schematic design of the study.

The specimens were randomly divided into three groups according to the adhesive systems tested: a one-bottle total-etch adhesive (Adper® Single Bond 2 – SB), a two-bottle self-etch adhesive (ClearfilTM SE Bond – SE), and a one-bottle self-etch adhesive (G BondTM – GB). The adhesive systems were applied on dentin following the manufacturer’s instructions (Table 1).

Starch tubing was used as tube to build up cylindrical composite resin restorations. The cylindrical tubes of starch (Spaghetti; Izabella Food Products S.A., Curitiba, Brazil) were prepared by cutting the tubes in parts (Fig 1) to obtain a composite resin restoration of 0.9 mm in diameter and 1.5 mm in height. Starch tubing was used as matrix because it is an inert material and does not promote any reaction with both the adhesive systems and composite resin. Moreover, it was shown to be an alternative to build up the microshear specimens.

Three starch matrixes were placed over each adhered surface, before light-cure of the adhesive systems. After light irradiation using a light emission diode (Olsen Ind. e Com. S/A, Palhoça, Brazil), with 800 mW/cm² intensity, the restorations were placed using a composite resin (Filtek Z250; 3M ESPE).

The irradiance was monitored using a powermeter (Fieldmaster; Coherent Commercial Products Division, Auburn, CA). The resin was carefully inserted into the tube matrix and light cured for 20-second increments. One operator previously trained made the adhesive procedures and restorations.

The specimens were randomized (taking into account each adhesive system) according to three water storage times (24 hours, 3 months, and 6 months), resulting in n = 10. The specimens remained immersed in distilled water, at 37°C, and the solution was replaced weekly. All specimens had the starch matrix carefully removed after immersion in water, for 24 hours, with a gentle air-water spray, without any cuts. The specimens were examined using a stereomicroscope (Discovery V20; Zeiss, Berlin, Germany) at a 10x magnification to detect any bonding defects, and specimens with interfacial gaps, bubble inclusion, or other defects were eliminated from the test and replaced.

After the water storage aging times, the specimens were tested to microshear bond failure using a bi-articulated and removable device, to hold it and fit it at the framework, at a 0.5 mm/min crosshead speed, with cell of 1 kN until fracture (EMIC DL 1000; Equipment and Systems Ltda., Sao Jose dos Pinhais, Brazil). A thin wire (diameter 0.2 mm) was looped around the resin composite cylinder, making contact through half of its circumference and adapted between the load cell and composite cylinder. The thin wire was gently held flush against the composite/dentin interface. The μSBS were expressed in MPa as derived from the division of the imposed force (N) at the time of fracture by the bonded area (mm²). The fracture pattern was determined at a magnification of 400× under a stereomicroscope and classified as adhesive/mixed failure, cohesive failure in the dentin, or cohesive failure in the composite resin.

Statistical analysis

All the data were presented as mean and standard deviation values (n = 10). Nominal values for bond strength were tabulated and analyzed using descriptive statistics with SPSS Program (Statistical Product and Service Solutions, V18.0, Armonk, NY). The μSBS values, in MPa, were subjected to Levene test to evaluate homogeneity of variances, and then the data were analyzed with three-way ANOVA to assess the influence of surfaces (occlusal and proximal dentin), adhesive systems (SB, SE, and GB) and storage times (24 hours, 3 months, and
Table 1  Adhesive systems, manufacturers, pH, characteristics, compositions, and application techniques

<table>
<thead>
<tr>
<th>Adhesive system</th>
<th>Manufacturer</th>
<th>pH</th>
<th>Characteristics</th>
<th>Composition</th>
<th>Application technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adper Single Bond 2 (SB)</td>
<td>3M ESPE, St. Paul, MN</td>
<td>4.3</td>
<td>Total-etch adhesive system (one-bottle)</td>
<td>Components of the bottle: HEMA*, BisGMA**, dimethacrylates, polyalkenoic acid copolymer, initiators, water, and ethanol</td>
<td>Phosphoric acid-Apply gel for 15 seconds; clean with air/water spray for 20 seconds; apply two coats of primer/adhesive; gently apply compressed air jet; light-cure for 10 seconds. Phosphoric acid-Apply gel for 15 seconds; clean with air/water spray for 20 seconds; apply two coats of primer/adhesive; gently apply compressed air jet; light-cure for 10 seconds.</td>
</tr>
<tr>
<td>Clearfil SE Bond (SE)</td>
<td>Kuraray Medical Inc., Okayama, Japan</td>
<td>1.9</td>
<td>Self-etch adhesive system (two-bottles)</td>
<td>Primer: 10-MDP***, HEMA, hydrophilic dimethacrylate, initiators, and water. Bond: 10-MDP, BisGMA, HEMA, hydrophilic dimethacrylate, initiators, aromatic tertiary amine, and silanated colloidal silica</td>
<td>Apply primer; leave undisturbed for 20 seconds; dry lightly with dry air; apply adhesive; dry with compressed air; light-cure for 10 seconds.</td>
</tr>
<tr>
<td>G-Bond (GB) Lot: 09.2013</td>
<td>GC Corporation, Tokyo, Japan</td>
<td>2.0</td>
<td>Self-etch adhesive system (all-in-one)</td>
<td>4 MET****, UDMA***** phosphate ester monomer, acetone, water, silica filler, and initiators</td>
<td>Apply primer/adhesive for 10 seconds; apply a strong jet of air for 5 seconds; light-cure for 10 seconds.</td>
</tr>
</tbody>
</table>

*HEMA, 2-hydroxyethyl methacrylate; **Bis-GMA, bisphenol A diglycidylmethacrylate; ***MDP, 10-methacryloyloxydecyl dihydrogen phosphate; ****4-MET, 4-metacryloxyethyl trimethylatic acid; *****UDMA, urethane dimetacrylate.

6 months). Interactions were assessed through post hoc multiple comparisons using Tukey test ($\alpha = 0.05$).

Results

The $\mu$SBS, expressed in MPa, and standard deviations are shown in Table 2. Three-way ANOVA showed that the water storage aging times ($p = 0.724$) did not statistically influence the bond strength values; however, dentin regions ($p = 0.006$) and adhesive systems ($p < 0.001$) significantly affected the $\mu$SBS values. The interaction between adhesive system and dentin region was significant ($p < 0.001$); however, the other interactions were not ($p > 0.05$).

For the factor dentin surface, the proximal surface (14.7 ± 3.3 MPa) showed higher $\mu$SBS means than the occlusal surface (10.9 ± 4.1 MPa); however, the bonding effectiveness on the surfaces was dependent of the adhesive system used, being significant only for the ClearfilTM SE Bond (SE) adhesive ($p = 0.002$), which showed a superior performance on the proximal surface when compared with the occlusal dentin. The SE adhesive in the occlusal surface showed the lowest values. There was no effect of dentin surface for the other adhesive systems tested, which showed similar bonding performances in both proximal and occlusal surfaces.

For the adhesive system factor, the all-in-one adhesive (G-BondTM-GB) showed the highest $\mu$SBS values (17.0 ± 1.7 MPa). GB and AdperTM Single Bond 2 (SB) were statistically
stable for both dentin surfaces (proximal and occlusal) over time. SE was not stable after water storage aging times, showing differences in the $\mu$SBS values among the aging periods, mainly on the occlusal surface.

For all adhesive systems, regardless of the type of dentin region and aging, adhesive/mixed failures were predominant (93.4%). No cohesive failures in dentin occurred.

### Discussion

In the present study, the dentin regions and the type of adhesive system significantly affected the bond strength, thus the first null hypothesis should be rejected. The proximal surface demonstrated higher $\mu$SBS than the occlusal surface; however, it was dependent from the adhesive system, being significant only for SE. The difference between the dentin surfaces is consistent with previous studies,$^{19,20}$ indicating that the direction of dentinal tubules can be an important variable in determining the strength of the composite-dentin bonding; however, there are contradictory results for some adhesive systems,$^{5,21}$ where the bond strength was not dependent of dentin location and consequently by tubule orientation.

Dentin structural arrangement is varied,$^6$ which may be one reason that the bond strength is not uniform inside the cavity walls. The bond strength varies because of dentin morphological differences, indicating that dentin permeability may be an important factor to be considered, as it influences the quality of the adhesive interface and affects the bond strength.$^{19,20}$ A scanning electronic microscopy analysis demonstrated that the orientation of the dentinal tubules could impact the formation of the hybrid layer,$^{19,22}$ and consequently influence the bond strength of the adhesives. Dentin depth is another factor that could influence it. In addition, studies have shown that the adhesive line is associated with the bond strength because of stress distribution, though there is no relationship between bond strength and thickness of the hybrid layer.$^23$

Self-etch adhesive systems with a low pH have an interaction with the tooth structure similar to the total-etch by forming a micromechanical bond to dentin.$^7$ Alternatively, systems with a higher pH, such as SE and GB, are supposed to present a chemical interaction in addition to the micromechanical bond.$^{24}$ Despite these two adhesive systems presenting a chemical interaction, GB also has nanointeraction ability, providing an intimate contact between the adhesive resin and the dental substrate, forming a hybrid nanolayer.

SB, a two-step total-etch system, was included in this present study for comparison with the behavior of self-etch adhesive, although no significant difference was found among them. SB showed an improvement of $\mu$SBS on the occlusal surface and a worsening on the proximal surface over time. The compromising values of the proximal surface can be explained by the difficulty of this type of adhesive to fully infiltrate the demineralized collagen fibers and to remove all residual ethanol solvent with lower vapor pressure. The phosphoric acid causes the denaturation of the top collagen layer, as the SB needs a conditioning step prior to its application. Dentin permeability increases after the surface modification by phosphoric acid.$^{16}$ This fact may help to understand the values of adhesive bond strength for full-etch systems.

SE showed average values of $\mu$SBS. It showed a statistically significant deterioration over time with lower bond strength values than control (no aging) groups after aging, more discreet and with higher $\mu$SBS being at the proximal surface than the occlusal surface. The 10-MDP monomer contained in the composition of SE is mainly used as an etching monomer and seems to be relatively hydrolysis-stable by suffering less influence of water.$^{24}$ This monomer has been reported to be promising for chemical bonding to hydroxyapatite; however, in this present study, SE was not stable over the water storage time, diverging from other studies.$^{25,26}$

The 4-MET monomer is also able to establish an ionic bond with calcium in hydroxyapatite, but this link has been reported to be less intense than that found with 10-MDP.$^{27}$ Nevertheless, the 4-MET adhesive system (GB) showed statistically significantly higher $\mu$SBS than the 10-MDP adhesive system (SE). Furthermore, GB was stable over time, even after longer aging periods, showing the major $\mu$SBS means, which did not decrease over time. The GB achieved the highest value of bond strength for the 6-month storage time. GB also has shown suitable performance in other studies.$^{28,29}$

Degradation of the composite-dentin bond has increased with the use of more hydrophilic monomers as the water sorption affects the hydrolytic stability. Thus, an increase in the amount of hydrolytic components, like HEMA, could jeopardize the quality and durability of the adhesive interface.$^{2,11}$ GB is an all-in-one system, which might affect its hydrolytic stability.$^{14,8}$ However, the results for GB (an HEMA-free adhesive), which is able to chemically link and nano-interacts with dental structure, might be due to the fact that the $\mu$SBS increased over time.

Dentin structure consists of dentin tubules surrounded by a peritubular dentin, which is a highly mineralized structure, and the intertubular dentin that consists of collagen embedded with apatite crystals. The GB and SE are self-etch adhesive systems

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### Table 2 $\mu$SBS means (MPa) and standard deviations (SD), n = 10

<table>
<thead>
<tr>
<th>Material</th>
<th>24 hours</th>
<th>3 months</th>
<th>6 months</th>
<th>24 hours</th>
<th>3 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB</td>
<td>10.9 (4.2)$^{Aa}$</td>
<td>8.8 (2.3)$^{Ab}$</td>
<td>13.9 (1.7)$^{Aa}$</td>
<td>14.7 (2.5)$^{Aa}$</td>
<td>11.2 (4.9)$^{Aa}$</td>
<td>9.1 (6.9)$^{Aa}$</td>
</tr>
<tr>
<td>SE</td>
<td>8.9 (3.5)$^{AAb}$</td>
<td>7.8 (4.1)$^{AAb}$</td>
<td>5.7 (1.7)$^{Ab}$</td>
<td>14.7 (4.8)$^{Aa}$</td>
<td>11.5 (6.5)$^{AAb}$</td>
<td>13.2 (5.6)$^{AAb}$</td>
</tr>
<tr>
<td>GB</td>
<td>13.9 (6.1)$^{Aa}$</td>
<td>16.9 (2.9)$^{Aa}$</td>
<td>17.1 (3.6)$^{Aa}$</td>
<td>16.9 (1.8)$^{Aa}$</td>
<td>17.6 (7.4)$^{Aa}$</td>
<td>18.9 (6.6)$^{Aa}$</td>
</tr>
</tbody>
</table>

For each line, values with different capital letters indicate a statistically significant difference ($p < 0.05$), keeping the same type of adhesive, located at proximal and occlusal surfaces. For each column, values with different lowercase letters indicate significant differences between the adhesives when kept the same area and age ($p < 0.05$).
that are able to establish a chemical bond to tooth, independent of where it is located (proximal or occlusal), since all dental tissues have hydroxyapatite. Based on this, both systems might present similar performances in all dentin regions; however, in the present study it only occurred with GB, while SE showed higher bond strength values for proximal than for occlusal dentin. The peritubular dentin (more mineralized) is more current in proximal dentin, which could explain the reason for major $\mu$SBS of SE adhesive in this structure. Thus, the $\mu$SBS values in dentin are dependent on the adhesive used and the dentin surface; however, this interaction should be further investigated.

There is a correlation between aged bond strength and clinical data. Adhesive systems that present bond strength values around 17 to 20 MPa have been reported as satisfactory to promote up to 40% of Class V retention rate after 5 years. The adhesive systems tested in the present study did not present high $\mu$SBS values; however, they were stable after aging. Studies have shown a reduction in bond strength over time, mainly because of the degradation of the adhesive interface. Those results diverge with the present research, where the aging factor did not statistically promote a decrease in bond strength; however, water storage aging may have exerted some influence on the adhesive interface. The lack of significant changes in the $\mu$SBS values after 6-month water storage aging suggests that the adhesive systems were stable up to this time and did not suffer long-term negative effect under the conditions of this study.

The evaluation of failure modes revealed mainly adhesive/mixed failures for all experimental groups, which is a common occurrence on microshear tests as “micro” bonded areas present few voids leading to fractures restricted to the interface adhesive. The smaller area used for the preparation of the specimens results in a more uniform stress distribution throughout the adhesive interface during the mechanical test, preventing fractures in the substrate.

Conclusions

Within the limitations of this in vitro study, it can be concluded that:

1. $\mu$SBS values were affected by the type of adhesive systems ($p < 0.001$), with the all-in-one adhesive showing the highest bond strength values ($p < 0.001$).
2. $\mu$SBS values were affected by the dentin surfaces, showing that the bonding effectiveness depends not only on the material, but also on other factors, such as the dentin surface location.
3. The proximal surface showed higher $\mu$SBS values than the occlusal surface ($p = 0.006$).
4. The adhesive systems were stable over time ($p = 0.724$).

References


