Effects of One-year Storage in Water on Bond Strength of Self-etching Adhesives to Enamel and Dentin

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The aim of this study was to compare the bond strengths of three self-etching materials during one year of storage. Clearfil SE Bond (SE), Clearfil Protect Bond (PB), and Clearfil Tri-S Bond (TS) were used for bonding to dentin and enamel according to manufacturer’s instructions. Microshear bond strength values were measured after 24 hours, six months, and one year. Two-way ANOVA showed that the interaction of material type and storage time was significant for dentin. At baseline, SE had the highest bond strength to dentin. There were no significant changes in bond strength for each material during the storage period, except for PB which showed increased bond strength to dentin after one year. All materials performed reliably after one year. However, the antibacterial and fluoride-releasing effects of PB would further contribute to the long-term clinical benefits of this material.

Keywords: Microshear bond strength, Self-etching, Durability

INTRODUCTION

Achieving a strong and durable bond between adhesive resins and dental substrates is of critical importance for the longevity of bonded restorations. The introduction of dentin primers has remarkably improved the efficacy of dentin bonding systems. Self-etching primer systems combine the etching and priming steps into one agent, while all-in-one systems combine the self-etching primer and bonding agent into one application step. Most of the current adhesive materials are capable of achieving high bond strength values shortly after polymerization. However, the longevity of bonds is still an area of immense interest in adhesive dentistry. As reported by several researchers, a significant decrease in bond strength after long-term usage may reduce the success rate of restorations, thereby leading to failure. Against this background, it is undoubtedly important to investigate the durability of adhesive systems.

Clearfil SE Bond is received and perceived as one of the most reliable adhesive systems and has been chosen as the reference bonding system in numerous studies. Meanwhile, the addition of antibacterial and fluoride-releasing agents to adhesives has recently been considered beneficial for bonding durability by some researchers.

Therefore, the purpose of this study was to evaluate the microshear bond strength to enamel and dentin of three self-etching adhesive systems — a two-step system, an antibacterial and fluoride-releasing two-step system, and an all-in-one system — over a storage period of one year after bonding. The null hypothesis was that the bond strength of all materials did not change during the storage period.

MATERIALS AND METHODS

Adhesive materials

Three self-etching materials were evaluated: two-step adhesives Clearfil SE Bond (SE) and Clearfil Protect Bond (PB), and all-in-one adhesive Clearfil Tri-S Bond (TS). Table 1 lists the lot number and chemical composition of each adhesive material according to the information provided by the manufacturer (Kuraray Medical, Tokyo, Japan).

Tooth specimens

Forty caries-free, human third molar teeth were used in this study. Roots of the teeth were removed, and coronal slices — about 2-mm-thick each — were prepared by cutting parallel to the longitudinal axis and facial surface using a low-speed Isomet saw (Buehler, Lake Bluff, IL, USA) under running water as coolant. The convex enamel surfaces on the outermost buccal or palatal slices were reduced by gently polishing on a 600-grit silicone paper under running water to prepare a flat enamel surface. The outer surface of the
underlying slice was also polished to create a standard smear layer on the dentin. Two enamel and two dentin slices were obtained from each tooth and distributed randomly among the three groups of self-etching adhesive materials.

**Bonding procedures**

For SE and PB groups, the self-etching primer agent of each material was applied on the surface using a sponge supplied by the manufacturer and agitated for 20 seconds. After air-drying with oil-free compressed air, the bonding agent of the corresponding adhesive was applied using the sponge pieces. The adhesive was then air spread until a homogeneous layer was observed on the surface.

For TS, the adhesive was applied to the dentin surface for 20 seconds using a sponge, and then air-blown to remove water and solvent from the adhesive material.

Prior to irradiation of the bonding agent, tygon tubes (Tygon®, Norton Performance Plastic, Cleveland, OH, USA) with an internal diameter of 0.75 mm and 1 mm in height were placed on the enamel and dentin surfaces and photo-irradiated for 10 seconds. The lumens were then carefully filled with a hybrid restorative, Clearfil APX, shade A3 (Kuraray Medical, Tokyo, Japan). The composite was light-cured for 40 seconds. Specimens in each group were randomly divided into three subgroups and stored in deionized water at 37°C until the bond strength test. The tygon tubes around the composite cylinder were removed after one hour, by gently cutting the tube into two hemi-cylinders using a feather blade.

![Fig. 1 Illustration of microshear bond strength test. A composite cylinder is bonded to a dentin disk from human molar, and the sample is tested in a universal testing machine.](image-url)

**Microshear bond strength test**

At 24 hours after bonding, tooth slices in one of the subgroups for each material-substrate set were attached to the test apparatus (Bencor-Multi-T, Danville Engineering, San Ramon, CA, USA) using a cyanoacrylate adhesive (Zapit, Dental Ventures of America, Corona, CA, USA) and tested in a universal testing machine (EZ-Test 500N, Shimadzu, Kyoto,
Japan). A thin steel wire (0.20 mm in diameter) was looped flush between the load cell projection and resin cylinder, making contact with the lower half-circle of the cylinder and touching the tooth surface.

Force was applied at a crosshead speed of 1 mm/min until failure occurred (Fig. 1). Maximum load at the time of failure was converted from kgf to MPa and recorded as the microshear bond strength of the baseline subgroup. The microshear bond test was repeated after six months for another subgroup, and after one year for the last subgroup of each material-substrate set.

A total of 180 bond strength values were obtained (n=10 for each subgroup). Results were statistically compared within and between material groups using two-way ANOVA and one-way ANOVA with Tukey’s and Dunnett’s post hoc tests.

RESULTS

Table 2 shows the means and standard deviations of shear bond strength to enamel or dentin for each adhesive at different storage periods. Two-way ANOVA indicated that material type had a significant effect on bond strength to both enamel and dentin (F=3.788, p<0.05 and F=3.723, p<0.05 respectively). When storage time was investigated as a factor, two-way ANOVA did not find any significant effect on the bond strength to any of the substrates (F=0.487, p=0.616 and F=0.135, p=0.847 respectively). A significant interaction between the two factors of material type and storage time suggested that the effect of material type on bond strength to dentin was influenced by storage time (F=4.356, p=0.005). The interaction was not significant for enamel (F=1.697, p=0.159).

Comparisons among the materials using one-way ANOVA with Tukey’s post hoc test at baseline (24 hours) showed that SE yielded a significantly higher bond strength to dentin than the other two materials. After one year of storage, the test revealed that PB had significantly higher bond strength to both enamel and dentin only when compared to TS.

Separately, two-sided Dunnett’s test among the subgroups within each material-substrate set indicated that the only significant change of bond strength over the storage period was found for PB to dentin, and which was an increase in microshear bond strength after one year of storage. There was a similar but not statistically significant trend for PB bonded to enamel. Moreover, changes noticed for the nominal mean values of bond strength of SE and TS to both substrates were not statistically significant.

DISCUSSION

This study measured the bond strengths of three adhesives to enamel and dentin during their one year of storage in water. In addition to common advantages of bond strength tests, the microshear bond strength was chosen because of the following advantages: (1) small bonding surface area; (2) easier sample preparation; (3) use of lesser amount of material and fewer teeth; and (4) suitable for testing of enamel bonding. As for the bonding substrate, similar regions on the axial surfaces of enamel and dentin slices were bonded for all specimens to eliminate interference from substrate variations.

Moreover, similar to some recent studies, this study focused only on bond strength as the indicator of bonding performance. Mode of fracture — due to concerns regarding stress distribution at the bonding interface — was not reported.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Material</th>
<th>Experiment Time</th>
<th>SE Bond</th>
<th>Protect Bond</th>
<th>Tri-S Bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dentin</td>
<td>Baseline (24h)</td>
<td>45.2±10.4a</td>
<td>34.5±6.0b</td>
<td>36.0±6.1b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>43.2±11.2c</td>
<td>38.3±7.0c</td>
<td>35.2±5.9c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>One year</td>
<td>33.9±11.6d</td>
<td>46.6±12.5e</td>
<td>32.5±10.7d</td>
<td></td>
</tr>
<tr>
<td>Enamel</td>
<td>Baseline (24h)</td>
<td>39.5±5.3f</td>
<td>35.8±6.9f</td>
<td>34.6±5.6f</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>35.9±7.1g</td>
<td>38.8±6.0g</td>
<td>35.2±9.2g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>One year</td>
<td>34.5±8.4h</td>
<td>40.6±8.9h</td>
<td>29.9±7.7h</td>
<td></td>
</tr>
</tbody>
</table>

For each experiment time in rows, subgroups indicated by similar letters are not significantly different (p>0.05, Tukey’s HSD test).

For each material-substrate set in columns, subgroup indicated by asterisk is significantly different from other groups (p<0.05, two-sided Dunnett’s test).
In terms of material choice, the materials selected in the present study were all produced by the same manufacturer and had similar basic compositions. In this manner, it was possible to better discern any effect that arose due to minor differences that existed between the materials. This approach stood in stark contrast to previous studies which reported on different performance characteristics for materials with different mechanisms of action and compositions\textsuperscript{13,14,16}.

According to the manufacturer, SE primer included a hydrophilic acidic monomer, MDP (10-methacryloyloxydecyl dihydrogen phosphate), as the functional monomer. Its other key ingredients included HEMA (2-hydroxyethyl methacrylate), DMA (dimethacrylate), and photoinitiator CQ (dil-campherquinone). For the bond agent, it contained filler and bis-GMA (bisphenol A diglycidylmethacrylate) in addition to primer ingredients — except water. The all-in-one system, TS, contained all the components named above mixed in a single bottle. As for PB, it differed from SE in that its primer incorporated an antibacterial monomer, MDPB (12-methacryloyloxy-dodecylpyridinium bromide), but lacked a photoinitiator. Further, PB’s bond agent also included a fluoride-releasing agent, sodium fluoride.

Judging from the results obtained after 24 hours of storage, SE showed higher bond strength to dentin compared to PB and TS. This was in line with other studies that suggested higher dentin bond strength with SE than with TS in short-term observation. This could be attributed to the lower mechanical properties of TS due to a lower polymerization efficacy, which was a result of incomplete removal of water and solvents\textsuperscript{7,11}.

Similarly for PB, it was suggested that the absence of photoinitiator in its primer led to a lower polymerization efficacy of the bonding layer\textsuperscript{22}. This then led to a lower bond strength compared to SE at just 24 hours after bonding, as also reported in another study\textsuperscript{13}.

Meanwhile, the bond strength to enamel at baseline did not reflect any differences among the materials. This might be due to the effect of bonding substrate not reflecting the potential differences among adhesives. Although bonding to enamel seems generally less problematic than to dentin, the inherent strength of enamel might be the weak link. Moreover, the brittle nature of enamel could limit the nominal strength values obtained for resin-enamel bonds\textsuperscript{14,15}.

Results of the current study showed a slight, but not statistically significant, decrease in bond strength for SE and TS to enamel during the storage period. Conversely, Wang \textit{et al.}\textsuperscript{16} reported on a significant decrease in bond strength with SE after one year of storage in sodium chloride, while others claimed that SE\textsuperscript{17} and TS\textsuperscript{18} showed durable bonding to enamel.

During the one-year storage in the present study, SE showed stable bonding to dentin. Studies conducted on the effects of long-term direct water exposure on bond strength of SE to dentin reported a reduction in bond strength to dentin, but concluded that SE still performed reliably after one-year direct water exposure\textsuperscript{19,20}.

Susceptibility of resin components to hydrolysis has been identified as a cause for decrease of bond strength. It has been suggested that the outstanding hydrolytic stability of MDP and its additional chemical interaction with enamel and dentin contributed to the long-term durability of dentin and enamel bonding with SE\textsuperscript{17,21} and TS\textsuperscript{18,22}. MDP has a special molecular structure that enables chemical interaction with residual hydroxyapatite after etching, and the calcium salts formed thereby also exhibit hydrolytic stability\textsuperscript{21}.

The materials used in this study were classified as mild self-etching systems with \textit{pH} values \textit{circa} 2.0 and higher: 2.0, 2.0, and 2.7 for SE\textsuperscript{8}, PB\textsuperscript{23}, and TS\textsuperscript{9} respectively. Long-term clinical evaluation of SE has suggested that more aggressive etching was not essential for the overall clinical performance of the restorations\textsuperscript{24}. In fact, mild acid-etching enables the bonding substrate to maintain a higher mineral content for chemical interactions. In addition, mild acid-etching of dentin bears the advantage of occlusion of the dentinal tubules to the effect of decreasing dentin permeability and fluid movement, which may otherwise lead to hydrolytic degradation and failure of the bond\textsuperscript{20}.

For adhesive materials with aggressive etching effects, the dentin collagen network would be deprived of the hydroxyapatite coating. This would mean the absence of an effective chemical interaction and hence inadequate hybridization with dentin. Consequently, the bond strength to dentin would undergo a significant loss after storage for a long time due to the hydrolysis of collagen fibrils\textsuperscript{26}. However, two-step self-etching materials such as SE and PB are unlike bonding systems that have a separate, aggressive acid-etching step. With two-step self-etching systems, etching and penetration of the primer monomers occur simultaneously\textsuperscript{19}. TS, on the other hand, was an all-in-one material that required only one application step. Some researchers have even highly lauded such single-step self-etching systems for their simultaneous monomer penetration and complete impregnation of the collagen network, which then prevents the hydrolysis of collagen fibrils\textsuperscript{22}. This is because the formation of a homogenous and void-free interfacial zone improves the quality of the hybrid layer and contributes to long-term sealing of the dentin surface\textsuperscript{27}. Studies on long-term nanoleakage have reported superior leak-
age resistance for both SE⁹ and TS⁹. In the current study, similar durability results were obtained for SE and TS. Therefore, it was suggested that despite the differences that existed between all-in-one and two-step self-etching adhesives, their performance might be comparable in the long run.

As for comparison between the two-step self-etching systems used in this study, Nakajima et al. reported that the performance of PB was superior to SE in that it exhibited no changes in dentin bond strength over six months of water storage⁹. Their finding was later supported by a TEM investigation on nanoleakage over six months of water storage⁹. In addition, PB has shown promising results with respect to long-term bonding stability in vivo⁹.

In the present study, the only statistically significant change in bond strength over time was observed for PB to dentin after one year. This was a rather unusual result for a bonding system which already exhibited good initial bond strength. In previous studies, at best only a slight increase in bond strength was reported after long-term storage¹⁰–²². It has been suggested that loss of minerals from enamel and dentin during *in vitro* water storage would lead to deterioration of mechanical properties of teeth³¹. Such deterioration of the substrate over time may affect the bond strength. However, fluoride release by adhesive materials might be effective in protection against mineral loss from enamel and dentin surfaces²².

It has been reported that fluoride release from PB resulted in increased resistance of sound dentin to acid challenge⁹ and remineralization of the demineralized dentin³³, as compared to other materials that did not release fluoride. It should also be pointed that another study has challenged the remineralizing effect of PB on dentin, but suggested that PB might have released sufficient fluoride to inhibit any salivary or dentin matrix-bound esterases that could have been responsible for the decreased bond strength seen in fluoride-free adhesives³³¹. One of the mechanisms suggested for bond strength degradation is the enzyme-catalyzed hydrolysis of the methacrylate derivatives used in dental adhesives by salivary esterases³³².

Nevertheless, according to the present results, significant increase in bond strength to dentin was not observed earlier than a year after bonding. This indicated that the beneficial effects of additional chemical interactions from fluoride might not be significant — except with a prolonged duration.

As for the effect of antibacterial agents, the MDPB-containing primer has been shown to be promising for inactivating residual bacteria in cavities in *in vitro*²⁰ and *in vivo*³⁵ studies. Although the antibacterial effect might seem to be of little relevance to the current study since it was conducted on caries-free teeth *in vitro*, it is noteworthy that MDPB contained C=C bonds which are capable of undergoing a free radical polymerization. This stood in sharp contrast to the soluble antibacterial agents in some other dentin bonding systems. Thus, MDPB would be immobilized within the polymer network and would not induce weakness or degradation in the bonding layer through dissolution and substitution by water²⁰. On this score, PB exhibited significantly lower permeability compared to TS and other adhesives under simulated pulpal pressure³⁶.

With enamel, PB exhibited a bonding behavior different from that with dentin. Increase in bond strength was not statistically significant after one year of storage and there was no interaction between factors of material type and storage time. Pertaining to the similarity in baseline results among the materials, it might be due to the characteristics of enamel limiting the bond strength values, as mentioned earlier during the discussion. In view of the results obtained for enamel and dentin with PB, further research is indeed warranted for PB to thoroughly investigate and confirm its effects on dental substrates.

The null hypothesis of the present study was rejected. The performance of materials in terms of bond strength changed along with time, indicating that short-term bonding test results might not always soundly reflect the advantages of long-term use of different adhesives. In particular for dentin, the choice of adhesive material had a significant effect on long-term bond durability. On PB being the adhesive material of choice, it was the only material that did not show any nominal decrease over time. Therefore, use of this material is recommended for adhesive restorations, particularly for patients with high risk of developing recurrent or secondary caries.

**CONCLUSION**

Within the limitations of this *in vitro* study, it could be concluded that all tested adhesives showed good performance after one year of storage. In particular, the combination of an antibacterial, MDP-containing self-etching primer with a fluoride-releasing bonding agent might offer additional clinical benefits besides bond strength durability.

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