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**ABSTRACT**

**Aims:** The purpose of this study was to compare the effect of enamel wetness on microshear bond strength using different adhesive systems.

**Objectives:** To evaluate microshear bond strength of three bonding agents on dry enamel; to evaluate microshear bond strength of three bonding agents on wet enamel; and to compare microshear bond strength of three different bonding agents on dry and wet enamel.

**Materials and methods:** Sixty extracted noncarious human premolars were selected for this study. Flat enamel surfaces of approximately 3 mm were obtained by grinding the buccal surfaces of premolars with water-cooled diamond disks. This study evaluated one etch-and-rinse adhesive system (Single Bond 2) and two self-etching adhesive systems (Clearfil SE Bond and Xeno-V). The specimens were divided into two groups (n = 30). Group I (dry) was air-dried for 30 seconds and in group II (wet) surfaces were blotted with absorbent paper to remove excess water. These groups were further divided into six subgroups (n = 10) according to the adhesives used. The resin composite, Filtek Z 250, was bonded to flat enamel surfaces that had been treated with one of the adhesives, following the manufacturer’s instructions. After being stored in water at 37°C for 24 hours, bonded specimens were stressed in universal testing machine (Fig. 3) at a crosshead speed of 1 mm/min. The data were evaluated with one-way and two-way analysis of variance (ANOVA), t-test, and Tukey’s Multiple Post hoc tests (α = 0.05).

**Results:** The two-way ANOVA and Tukey’s Multiple Post hoc tests showed significant differences among adhesive systems, but wetness did not influence microshear bond strength (p = 0.1762). The one-way ANOVA and t-test showed that the all-in-one adhesive (Xeno-V) was the only material influenced by the presence of water on the enamel surface. Xeno-V showed significantly higher microshear bond strength when the enamel was kept wet. Single Bond 2 adhesive showed significantly higher microshear bond strength as compared with Xeno-V adhesive but no significant difference when compared with Clearfil SE Bond adhesive in dry enamel. Single Bond 2 adhesive showed no significant difference in microshear bond strength as compared with self-etching adhesive systems (Clearfil SE Bond and Xeno-V), when the enamel was kept wet.

**Conclusion:** From the findings of the results, it was concluded that self-etching adhesives were not negatively affected by the presence of water on the enamel surface.

**Clinical significance:** The all-in-one adhesive showed different behavior depending on whether the enamel surface was dry or wet. So the enamel surface should not be desiccated, when self-etching adhesives are used.

**Keywords:** Enamel wetness, Microshear bond strength, One-step self-etch adhesive, Two-step self-etching adhesive.


**Source of support:** Nil

**Conflict of interest:** None

**INTRODUCTION**

Enamel bonding has been challenging in dentistry for many years. Bonding procedures to enamel play an important role in esthetic, preventive, and restorative procedures. Completely mature enamel consists of approximately 96% mineral, 4% organic material, and water. The fundamental units of enamel are the rods and interrod substance. These rods have a cross-sectional shape of keyhole, where the...
crystallites at the top of the keyhole shape are oriented parallel, while the crystallites at the bottom are oriented perpendicular to the axis of the rods. This difference in orientation is important, because etching of hydroxylapatite crystals occurs along the axis of the crystallites. The adhesive technology can be used to improve or repair the appearance of a tooth that has been stained, broken, or chipped. Generally, enamel is covered by organic pellicle. The procedure that leads to removal of this organic layer, making the enamel surface more reactive by increasing the wettability and increasing the surface area by exposing the inorganic crystallite component, is known as etching. Acid etching provides a porous layer where low-viscosity resin flows into the microporosities and polymerizes to form resin tags. Bonding to enamel depends primarily on resin tags becoming interlocked with the enamel microporosities or surface irregularities. Resin tags that form in the enamel rod peripheries between enamel prisms are known as macrotags and several finer small tags form across the end of each rod, called as microtags. Microtags are more important for micromechanical retention because of their large number, and also they offer greater surface area of contact.

Unlike enamel, similar resin bonding is difficult to obtain with dentin due to persistent dentinal fluid perfusion and higher collagen component in dentin. For this reason, Nakabayashi et al. evolved “wet bonding technique.” According to the wet bonding technique, the demineralized dentin after acid etching should be kept moist to prevent the exposed collagen fibers from collapsing. It provides penetration of hydrophilic primers into collagen fibers for good dentinal bonding. Since dentin and enamel are a juxtaposed tooth structure, it is very difficult for a clinician to keep enamel dry and dentin moist at the same time. Therefore, the use of hydrophilic bonding adhesives in enamel has been associated with “wet bonding technique.” Decalcification of enamel is an ionic process that requires water. However, any remaining water and other organic solvents should be evaporated by air-drying because excess water and organic solvents interfere with polymerization in enamel microporosities and adhesive bond strength of monomer components of bonding agents. Therefore, water present on the enamel surface plays a significant role before the application of self-etching adhesives.

Current adhesive systems use one of the two available bonding approaches to interact with dental substrate: (1) The etch and rinse approach requires a separate phosphoric acid etching step to create microporosities on the enamel. (2) In self-etch approach, demineralization and infiltration occur simultaneously. Etch and rinse adhesives require either two or three steps, depending on whether the primer and bonding agents are separate or combined in a single bottle. Similarly, self-etch adhesives can be either one- or two-step systems, depending on whether the self-etching/primer solution is separated from the bonding agent or combined with it. The latter enables a single application procedure of an “all-in-one” adhesive.

Bond strength testing can be widely divided into macro- and microtests, depending on the size of the bonded area. The macrobond strength, i.e., with a bonded area of 3 mm² or larger, can be measured in shear or tensile mode. The microbond strength, that is, with a bonded area of 1 mm² or lesser, can be measured in microshear or microtensile mode.

Aims
The aim of the present study was to perform a comparative evaluation of enamel wetness effect on microshear bond strength using three different (one etch and rinse and two self-etch) adhesive systems.

MATERIALS AND METHODS

Armamentarium Used
• Satelec Ultrasonic Scalers (P5 Boosters, Mergnac, France)
• Universal testing machine (star testing system, STS 248, India)
• Straight handpiece (NSK Japan)
• Diamond discs (Suzhou, Japan)
• Stereomicroscope (Lawrence and Mayo).

Materials Used
• Adper Single Bond 2 adhesive (3M ESPE, St. Paul, MN, USA)
• Clearfil SE Bond adhesive (Kuraray Noritake Co Ltd. Osaka, Japan)
• Xeno V adhesive (Dentsply, Konstanz, Germany)
• Filtek Z 250 XT B2 shade (3M ESPE)
• Woodpecker LED Composite curing light
• Mylar strips
• Applicator tip
• BP blade No. 15 (Lister)
• Orthodontic wire 0.2 mm thick
• Absorbent paper
• Microbore Tygon tubing (R-3603, Norton Performance Plastic Co., Cleveland, USA).

Materials: Sixty human premolar teeth, extracted for orthodontic reasons from patients of both sexes ranging from 15 to 30 years of age, were collected and stored in saline at room temperature (Fig. 1).

Selection criteria: All the teeth that are free of caries, restorations, and cracks were selected for the present study. The teeth were stored in distilled water till further use.
Enamel Wetness Effects on Microshear Bond Strength of Different Bonding Agents (Adhesive Systems)

Preparation of specimens: The enamel surfaces of all specimens were cleaned of any debris and soft tissues with ultrasonic scaling and rinsed. Then each tooth was embedded in acrylic resin, exposing buccal surface. The buccal surfaces were polished with nonfluoridated pumice using rubber cups. The enamel surface of the specimens was flattened by using diamond discs with copious amount of water to obtain approximately 3 mm flat enamel surface. Then the sample teeth were randomly divided into two groups (n = 30).

Experimental groups: Group I (Dry, n = 30): Specimens were air-dried for 30 seconds to desiccate the enamel surface. Air-drying was performed at an approximate distance of 10 cm at an angle of 45° inclination.

Group II (Wet, n = 30): Surfaces of specimens were blotted with absorbent paper to remove excess water either after acid etching the enamel (for two-step etch and rinse adhesives) or before the application of self-etching adhesives while still leaving the enamel moist and visibly shiny.

Bonding of specimens: The adhesive systems were applied according to manufacturer’s instructions (Table 1). The procedure was performed by applying the adhesive to the enamel surface. Before light curing of the adhesive resin, a small piece of Microbore Tygon tube with an internal diameter of 0.8 mm and a height of approximately 0.5 mm was placed on the uncured resin to restrict the bonding area (Fig. 2). Then the resin composite Filtek Z250 B2 shade was filled into the bonded tube by pressing it gently with the Mylar strip and light cured for 40 seconds. The obtained small cylinder of composite with the dimension approximately of the Tygon tube was stored at room temperature. After 1 hour, Tygon tubings were gently removed with a no 15 scalpel blade and specimens were stored in water at 37°C for 24 hours.

At room temperature, all the specimens were subjected to microshear bond strength testing using universal testing machine by the wire loop method. A shear force was applied to each specimen at 1 mm/min until debonding occurred (Fig. 3). All debonded enamel surfaces were examined at 20× magnification under a stereomicroscope. Debonding was assessed as adhesive enamel failure, that is, between enamel and adhesive, or as cohesive failure, that is, within composite resin.

Table 1: Composition and application protocol of adhesive systems

<table>
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<tr>
<th>Sl. no.</th>
<th>Adhesive</th>
<th>Composition</th>
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<tbody>
<tr>
<td>1</td>
<td>Adper Single Bond 2 (3M ESPE)</td>
<td>Bis-GMA, HEMA, dimethacrylates, polyalkenoic acid copolymer, photoinitiators, ethanol, and water</td>
</tr>
</tbody>
</table>
| 2      | Clearfil SE Bond (Kuraray) | Primer – MDP, HEMA, hydrophilic dimethacrylate, N, N-diethanol-p-toluidine, water, photoinitiator  
Bond – MDP, HEMA Bis-GMA, hydrophobic dimethacrylate, N, N-diethanol-p-toluidine, silanated colloidal silica, photoinitiator |
| 3      | Xeno V (Dentsply) | Bifunctional acrylic amides, acidic acrylates, functionalized phosphoric acid ester, acrylamido-2-methyl propenol-2-sulfonic acid, butylated benzenediol, camphorquinone, initiator, stabilizer, water, tertiary butanol |

Application protocol

<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>Adhesive</th>
<th>Application protocol</th>
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<tbody>
<tr>
<td>1</td>
<td>Adper Single Bond 2 (3M ESPE)</td>
<td>Phosphoric acid etching for 15 seconds. Rinse with water for 10 seconds. Dry gently, apply two coats, air-dry for 5 seconds, light cure for 10 seconds</td>
</tr>
<tr>
<td>2</td>
<td>Clearfil SE Bond (Kuraray)</td>
<td>Apply primer for 20 seconds, air-dry for 5 seconds, apply bond, air-dry for 5 seconds, light cure for 10 seconds</td>
</tr>
<tr>
<td>3</td>
<td>Xeno V (Dentsply)</td>
<td>Apply adhesive twice, gently agitate for 20 seconds, air-dry for 5 seconds followed by light cure for 20 seconds</td>
</tr>
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</table>
Groups and Subgroups of Specimens according to Specimens Used

60 Premolar teeth – Group I (Dry, n=30), Group II (Wet, n=30).

Group I (Dry):
Subgroup Ia – Two-step etch and rinse adhesive (n=10).
Subgroup Ib – Two-step self-etch adhesive (n=10).
Subgroup Ic – Single-step self-etch adhesive (n=10).

Group II (Wet):
Subgroup IIa – Two-step etch and rinse adhesive (n=10).
Subgroup IIb – Two-step self-etch adhesive (n=10).
Subgroup IIc – Single-step self-etch adhesive (n=10).

RESULTS

Statistical analysis of collected data was done.

Mean values and standard deviations obtained are shown in Table 2.

Tables 3 and 4 show comparison of microshear bond strength of six groups in dry and wet conditions respectively, by one-way analysis of variance (ANOVA) between the groups and within the groups. Microshear bond strength was statistically significant when compared among groups as well as within groups in both dry and wet conditions.

Table 5 shows comparison of microshear bond strength within the adhesive system in dry and wet conditions using student t-test. The one-step self-etching adhesive showed a significant increase in microshear bond strength when the enamel was kept wet before its application. The other two materials, two-step etch and rinse adhesive and two-step self-etch adhesive, showed an insignificant difference in microshear bond strength.

Table 6 shows comparison of microshear bond strength among six subgroups by t-test. The enamel wetness showed a nonsignificant effect on microshear bond strength (p=0.1762).

Table 7 shows pair-wise comparison of microshear bond strength of six groups in two conditions (dry and wet)
wet) by Tukey’s Multiple Posthoc procedures. The reading showed that the two-step etch and rinse adhesive showed statistically insignificant decrease in bond strength when the enamel was kept wet, whereas two-step self-etch adhesive showed a nonsignificant increase in bond strength when the enamel was wet. The one-step self-etch adhesive showed a significant increase in bond strength when the enamel was kept wet before its application.

Table 8 shows the failure patterns of adhesive systems. The failure modes were evaluated under a stereomicroscope at 20× magnification. It showed that specimens generally presented adhesive-enamel failure patterns (Figs 4A and B). That is why no relation between failure mode and microshear bond strength was found. It indicated that there was no relationship between failure mode and adhesive systems.

Graph 1 shows mean microshear bond strengths (MPa) and standard deviations of three adhesives in dry and wet conditions. It showed the highest mean microshear bond strength value of Single Bond 2 adhesive when the enamel was kept dry and the lowest mean microshear bond strength value of XENO-V adhesive in dry condition.

The results of Tukey’s Multiple Post hoc procedures are represented in Graph 2. The Single Bond 2 adhesive showed statistically insignificant decrease in the mean bond strength value when the enamel was kept wet and the self-etch adhesive showed opposite behavior. Xeno V self-etch adhesive showed an increase in the mean bond strength value when the enamel was wet, indicating high bond strength of Xeno V on wet enamel compared with dry enamel.

One-way ANOVA shows the following:
- Significant difference in microshear bond strength in between adhesives in dry condition, \( p = 0.00001 \); \( p < 0.05 \)
- Significant difference in microshear bond strength in between adhesives in wet condition, \( p = 0.0482 \); \( p < 0.05 \)
- Nonsignificant difference between group Ia and group IIa, \( p = 0.5684 \); \( p > 0.05 \).
Nonsignificant difference between group Ib and group IIb, \( p = 0.8765; p > 0.05 \)

Significant difference between group Ic and group IIc, \( p = 0.0023; p < 0.05 \)

It shows the following:

Significant difference among adhesive systems (\( p = 0.00001 \))

Nonsignificant difference in the two-way interaction effects between adhesives and wetness (\( p = 0.1762 \))

Table 7 also denotes pair-wise comparison of microshear bond strength among test groups Ia, Ila, Ib, IIb, Ic, IIc by Tukey’s Multiple Posthoc procedures and it shows the following information:

Nonsignificant difference between group Ia (mean = 24.50) and group IIa (mean = 23.24), \( p = 0.9831; p > 0.05 \), with group Ia having higher mean microshear bond strength values.

Nonsignificant difference between group Ib (mean = 20.87) and group IIb (mean = 21.13), \( p = 1.0000; p > 0.05 \), with group IIb having higher mean microshear bond strength values.

Significant difference between group Ic (mean = 12.29) and group IIc (mean = 18.35), \( p = 0.0218; p < 0.05 \), with group IIc having higher mean microshear bond strength values.

Significant difference between group Ia (mean = 24.50) and group Ib (mean = 20.87), \( p = 0.3773; p > 0.05 \), with group Ia having higher mean microshear bond strength values.

Significant difference between group Ia (mean = 24.50) and group Ic (mean = 12.29), \( p = 0.0001; p < 0.05 \), with group Ia having higher mean microshear bond strength values.

Significant difference between group Ib (mean = 20.87) and group Ic (mean = 12.29), \( p = 0.0004; p < 0.05 \), with group Ib having higher mean microshear bond strength values.

Significant difference between group Ila (mean = 23.24) and group IIb (mean = 21.13), \( p = 0.8620; p > 0.05 \), with group IIb having higher mean microshear bond strength values.
with group IIa having higher mean microshear bond strength values.

- Nonsignificant difference between group IIa (mean = 23.24) and group IIc (mean = 18.35), p = 0.1042; p > 0.05, with group IIa having higher mean microshear bond strength values.

- Nonsignificant difference between group IIb (mean = 21.13) and group IIc (mean = 18.35), p = 0.6635; p > 0.05, with group IIb having higher mean microshear bond strength values.

**DISCUSSION**

Adhesive technology has become increasingly important for two reasons. Firstly, adhesive techniques combined with the use of tooth-colored restorative materials allow clinician to restore teeth not only anatomically and functionally but also esthetically. Secondly, today’s operative dentistry should primarily involve procedures that are minimally invasive.2 For a restorative material, adhesion is the primary requirement so that the restorative material can be bonded to enamel or dentin without the need for extensive tooth preparation. After the introduction of the acid etch technique in clinical practice, there has been continuous progress in developing more refined and diversified restorative resin composites, along with steady improvement in bonding agents. The major problems associated with bonding to tooth surface are the inadequate removal of etching debris, smear layer, contamination by saliva, and water. Since dentin and enamel are a juxtaposed structure, it is very difficult for a clinician to keep the enamel dry and the dentin moist at the same time. Silverstone et al10 reported that dried etched enamel should not be contaminated with water or saliva. Otherwise it would compromise the bonding between the enamel and the resin material. The water is required to decalcify the enamel because decalcification is an ionic process. According to Spreataco et al,11 it must be considered that any remaining water and any organic solvents should be evaporated by air-drying because excess water and organic solvents could decrease the adhesive monomeric components within the microporosities of enamel and probably interfere with polymerization. So the dental adhesives must displace the water, react with it, or wet the surface more effectively than the water already present on the surface.

To enhance adhesive bonding, manufacturers and dentists are developing and using more hydrophilic resins that are not as sensitive to the presence of moisture. Recent literature2 divides the adhesive systems into major categories, i.e., etch and rinse or self-etch categories. Etch and rinse adhesives require either two or three steps, depending on whether the primer and bonding agent are separate or combined in a single bottle. Similarly, self-etch adhesives can be either one- or two-step systems depending on whether the self-etching primer solution is separated from the bonding agent or combined with it. The latter enables a single application procedure of a so-called “all-in-one adhesive.”12 So the adhesive used in this study can be categorized based on recent literature classification.9 Single Bond 2 (5th generation) two-step etch and rinse single bottle adhesive system, Clearfil SE Bond (6th generation), two-step self-etch adhesive, Xeno V (7th generation), one-step all-in-one adhesive.

Fifth-generation dentin bonding agents have been successfully used over a significant period of time and with consistent clinical success because of fewer steps involved.13 They are considered to be a benchmark against which newer systems should be compared. So Single Bond 2 (5th generation) adhesive has been chosen.14

Attempting to provide faster and more user-friendly adhesives, manufacturers have introduced one-step self-etch adhesives (7th generation), which etch, prime, and bond the dental substrate simultaneously. Xeno V has been chosen because it has been propagated by the manufacturer as a unique all-in-one adhesive system, containing tertiary butanol as a solvent rather than ethanol or acetone. A common restorative material, Filtek Z 250, was used for all the specimens. The rationale behind the bond strength testing is that the higher the actual bonding capacity of an adhesive, the better it will withstand stresses and the longer the restoration will survive in vivo. The microbond strength with a bonded area of 1 mm² or lesser can be measured in microshear mode. A better stress distribution can be accomplished in smaller specimens, since the number of voids and stress raising factors are lower than the ones that possibly occur in larger areas. In this in vitro study, the microshear bond strength testing was used because the microshear bond strength test is considered to be more suitable than the microtensile bond test, when enamel bonding is evaluated. In microshear bond test, the shear force can be applied by the blade or wire loop method. The wire loop method is preferred because it is able to apply a more even distribution of shear force on the specimen by wrapping around half the circumference of the composite cylinder. Positioning the wire is also much simpler and can be consistently placed flush at the enamel adhesive interface.15,16

In this study, human premolar teeth were selected because these teeth are extracted more frequently for orthodontic purpose and are easily available. In order to standardize the adhesion area, enamel surfaces were mechanically flattened. This procedure could substantially affect the microshear bond strength because many studies17,18 have shown different bonding behaviors of adhesives on ground and intact enamel surfaces. The
The following conclusions can be drawn from this study.

- The two-step etch and rinse showed higher bond strength when the enamel was kept dry. So we can desiccate the enamel, while using the two-step etch and rinse approach.
The self-etching adhesive systems showed higher bond strength, when the enamel was kept wet. So these adhesives are not negatively affected by moisture on enamel surfaces.

When a self-etching adhesive is used, the enamel surface should not be desiccated.

Further studies need to be undertaken to better understand the efficiency of the other all-in-one adhesives on the wet enamel surface.

REFERENCES