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Bond Strength of Self-etch Adhesives After Saliva Contamination at Different Application Steps

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Clinical Relevance

The effects of oral fluids on the bond strength of bonding systems to enamel/dentin must be considered during clinical application of these materials.

SUMMARY

This study evaluated and compared the effect of saliva contamination and possible decontamination methods on bond strengths of two self-etching adhesive systems (Clearfil SE Bond [CSE], Optibond Solo Plus SE [OSE]). Flat occlusal dentin surfaces were created on 180 extracted human molar teeth. The two bonding systems and corresponding composite resins (Clearfil AP-X, Kerr Point 4) were bonded to the dentin under six surface conditions (n=15/group): group 1 (control): primer/bonding/composite; group 2: saliva/drying/primer/bonding/composite; group 3: primer/saliva/rinsing/drying/primer/bonding/composite; group 4: primer/saliva/rinsing/drying/bonding/composite; group 5: primer/bonding (cured)/saliva/rinsing/drying/primer/bonding/composite; group 6: primer/bonding (cured)/saliva/removing contaminated layer with a bur/rinsing/drying/primer/bonding/composite. Shear bond strength was tested after specimens were stored in distilled water at 37°C for 24 hours. One-way analysis of variance and Tukey post hoc tests were used for statistical analyses. For CSE, groups 2, 3, and 4 and for OSE, groups 6, 2, and 4 showed significantly lower bond strengths than the control group (*p*<0.05). CSE groups 5 and 6 and OSE groups 3 and 5 revealed bond strengths similar to the control. When saliva contamination occurred after light polymerization of the bonding agent, repeating the bonding procedure recovered the bonding capacity of both self-etch adhesives. However, saliva contamination before or

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after primer application negatively affected their bond strength.

INTRODUCTION

The adhesive bond between dental adhesives and tooth structure is negatively influenced by fluid contamination.[1,2] In the oral cavity, such contaminants occur in the form of saliva, blood, or plasma. They present a common problem during clinical restorative procedures, especially when rubber dam isolation is not feasible, for example, with uncooperative patients, subgingival margins, malpositioned teeth, and partially erupted crowns.[3] In addition, most carious cavities are found in sites at or near the gingival margins where saliva contamination is most likely to occur.[1,4] Therefore, the effects of oral fluids on bond strength of bonding systems to enamel/dentin must be considered during the clinical application of these materials.

Several studies have evaluated the bond strength of bonding systems for normal enamel and enamel contaminated with oral fluids, such as plasma and saliva, and found that the oral fluids reduced the enamel bond strength.[3,5,6] Dentin bonding is particularly complex when compared with enamel bonding, and studies related to the bonding efficacy of saliva-contaminated adhesive systems to dentin are controversial. Some studies demonstrated that saliva contamination reduces the bond strength of dental adhesives to dentin,[1,2,4,7] while others reported contradictory results.[8,9] These differences are due to various influencing parameters, such as the composition of adhesive systems and the type of decontamination treatment used. Townsend and Dunn[9] suggested that recent self-etch adhesive systems, which used hydrophilic primers, were less sensitive to salivary contamination of prepared tooth surfaces than previous generations of adhesives.

Self-etching adhesives do not require acid etching and removal of the smear layer, which leads to a reduction of postoperative and technique sensitivity. These systems have become increasingly popular in daily practice.[10] However, few studies have been published on the influence of saliva contamination during the different application steps of self-etch systems to dentin.[1,2,4,9,11]

The purpose of this study was to evaluate the effects of saliva contamination at different bond application steps on the shear bond strength of two different two-step self-etch adhesive systems and to identify a decontamination method that reestablishes bond strengths comparable with the noncontaminated control group.

MATERIALS AND METHODS

One hundred eighty noncarious human molar teeth were stored at 4°C in 0.2% chloramine-T–containing distilled water after extraction. Soft-tissue remnants and calculus were removed, and the teeth were cleaned with fluoride-free pumice and a rubber cup. The occlusal tooth surfaces were cut slightly below the dentino-enamel junction with a low-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling to expose a flat area of dentin.

The teeth were then embedded in cylindrical molds with fast-set acrylic resin in a manner that exposed the flat dentin surface parallel to the base of the molds. The dentin surfaces were hand polished with 600-grit silicon-carbide abrasive paper for 60 seconds under running water to create a uniform surface and smear layer. The surfaces were then rinsed with distilled water for 15 seconds and gently air dried before application of the adhesives. The teeth were randomly divided into two groups of self-etch adhesive systems: Clearfil SE Bond (CSE) and Optibond Solo Plus SE (OSE). The self-etch adhesives were applied to the dentin surfaces according to the manufacturers' instructions (Table 1) under six surface conditions, as described in Table 2 (n=15/group).

Before each application, fresh saliva was collected from the same individual at the same time of day. The detailed bonding protocols used in the five treatment groups and the control group were as follows:

- Group 1 (control): Adhesive systems were applied to the surface without saliva contamination.
- Group 2: Saliva (0.1 mL) was applied to the dentin surface with a micro brush for 20 seconds, and the surface was then dried with air for five seconds. The primer and bonding agent of the adhesive systems were then applied.
- Group 3: Following primer application, 0.1 mL of saliva was applied to the dentin surface with a micro brush for 20 seconds, the surface was rinsed with water for five seconds, and it was then dried with air for five seconds. The surface was reprimed, and bonding was applied.
- Group 4: After primer application, 0.1 mL of saliva was applied to the dentin surface with a micro brush for 20 seconds, the surface was rinsed with

Table 1: *Manufacturers, Primer Acidity, Components, and Application Procedures of the Bonding Agents Used in the Study.*

Bonding Agents	Composition	Primer Acidity	Batch No.	Manufacturers' Instructions
Optibond Solo Plus self-etch (OSE); Kerr, USA	Primer: HFGA-GMA, GPDM, ethanol, water, MEHQ, ODMAB ***Adhesive: Bis-GMA, HEMA, GDMA, GPDM, ethanol, CQ, ODMAB, BHT, filler (fumed SiO_2, barium aluminoborosilicat, Na_2SiF_6), coupling factor A174***	pH 1.5	205187, ***21168***	Primer was applied for 15 s, dried with air for 3 s; adhesive agent was applied for 15 s and dried with air for 3 s; adhesive was reapplied for 15 s and dried with air for 3 s and light cured for 20 s
Clearfil SE Bond (CSE); Kuraray, Japan	Primer: MDP, HEMA, hydrophilic dimethacrylate, photo-initiator, water Adhesive: MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, photo-initiators, silanated colloidal silica	pH 2	00195A ***00193A***	CSE primer was applied for 20 s; subsequently, the dentin was dried with a stream of air; then CSE Bond was applied and light cured for 10 s

water for five seconds, and it was then dried with air for five seconds. The bonding agent was applied afterward.

- Group 5: Following application of the primer and bonding agent, the dentin surface was contaminated with 0.1 mL of saliva using a micro brush for 20 seconds, rinsed with water for five seconds, and dried with air for five seconds. The primer and bonding applications were repeated.
- Group 6: Following adhesive system application and saliva contamination, as explained in group 5, the saliva-contaminated dentin surface was slightly removed for 10 seconds with a cylindrical diamond bur (No. 330, MANI, Dia-Burs, Japan), rinsed with water for five seconds, and dried with air for five seconds. The primer and bonding agent were reapplied.

All adhesives were applied strictly according to the manufacturers' recommendations. Corresponding composite resins (Clearfil AP-X, Kuraray, and Kerr Point 4, Kerr) were placed on the pretreated dentin surfaces by packing the material into cylinder-shaped plastic matrices with an internal diameter of 2.3 mm and a height of 3 mm (Ultradent, South Jordan, UT, USA). Excess composite was carefully removed from the periphery of the matrix with an explorer. The composite resin was cured with a curing light (Hilux 250, Benlioglu Dental, Ankara, Turkey) for 40 seconds. The intensity of the light was at least 400 mW/cm^2. The specimens were then stored in distilled water at 37°C for 48 hours before bond strength testing. The specimens were mounted in a universal testing machine (model 500, Testometric, Rochdale, Lancashire, UK). A stubby-shaped force transducer apparatus (Ultradent) attached to a compression load cell and traveling at a cross-head speed of 0.5 mm/min was applied to each specimen at the interface between the tooth and composite until

Table 2: *Saliva Contamination Conditions and Decontamination Procedures*

Group	Dentin Surface Treatments
1 (control)	Primer/bonding/composite
2	Saliva/drying/primer/bonding/composite
3	Primer/saliva/rinsing/drying/primer/bonding/composite
4	Primer/saliva/rinsing/drying/bonding/composite
5	Primer/bonding (cured)/saliva/rinsing/drying/primer/bonding/composite
6	Primer/bonding (cured)/saliva/removing contaminated layer with a diamond bur/rinsing/drying/primer/bonding/composite

failure occurred. The maximum load (N) was divided by the cross-sectional area of the bonded composite posts to determine the shear bond strength in MPa.

Failure modes were evaluated with a stereomicroscope and classified as adhesive if debonding occurred at the resin-dentin bond interface and cohesive if the failure occurred in either substrate (resin or dentin).

Statistical calculations were performed with SPSS 13.0 for Windows. In addition to standard descriptive statistical calculations (mean and standard deviation), the parametric one-way analysis of variance test was carried out to compare the adhesive groups. For the evaluation of subgroups, a Tukey post hoc test was performed. The results were evaluated with a 95% confidence interval. The statistical significance level was established at $p<0.05$.

RESULTS

Table 3 shows the mean shear bond strength and standard deviation values of the tested groups and failure modes (Figure 1). The bond strength values of CSE were found to be higher than OSE in all groups except for group 3. The two adhesive systems showed different sensitivities to contamination by saliva during the bonding application steps. The water rinsing of primer after saliva contamination and application of the bonding agent resulted in the lowest bond strength for both bonding agents.

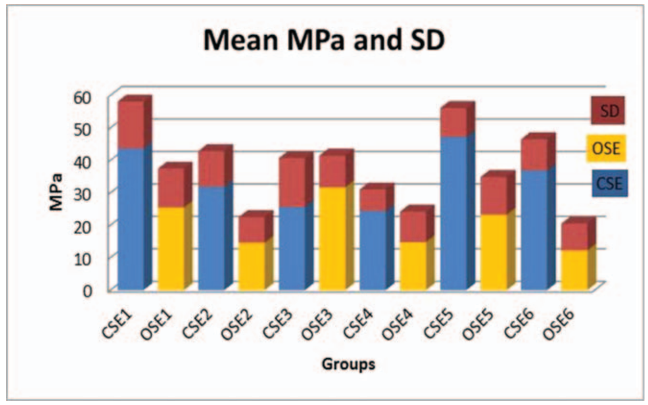

Figure 1. *Mean bond strength values and standard deviations (MPa).*

For CSE, group 2 (31.9 ± 11 MPa), group 3 (25.7 ± 15.1 MPa), and group 4 (24.4 ± 6.6 MPa) showed significantly lower bond strengths than the control group (43.6 ± 14.5 MPa; $p<0.05$). However, group 5 (47.2 ± 9 MPa) and group 6 (36.9 ± 9.6 MPa) had no adverse effect on the shear bond strengths ($p>0.05$).

For OSE, group 2 (14.6 ± 7.9 MPa), group 4 (14.8 ± 9.4 MPa), and group 6 (12.2 ± 8.2 MPa) showed significantly lower bond strengths than the control (25.5 ± 12 MPa; $p<0.05$). However, this self-etch adhesive system showed more resistance to salivary contamination in group 3 (31.7 ± 9.8 MPa) and group 5 (23.2 ± 11.6 MPa). There was no statistically significant difference among the bond strengths of groups 3 and 5 and the control group ($p>0.05$).

Table 3: *Mean Shear Bond Strength Values (MPa) and Failure Modes of the Tested Groups (n=15)*[a]

Test Group	CSE			OSE		
	Bond Strength Values (Mean ± SD)	Failure Modes		Bond Strength Values (Mean ± SD)	Failure Modes	
1 (control)	43.6 ± 14.5[cd]	80% A	20% CD	25.5 ± 12.0[bc]	93.3% A	6.7% CD
2	31.9 ± 11.0[ab]	86.6% A	13.4% CD	14.6 ± 7.9[a]	100% A	
3	25.7 ± 15.1[a]	93.3% A	6.7% CD	31.7 ± 9.8[c]	86.6% A	13.4% CD
4	24.4 ± 6.6[a]	93.3% A	6.7% CD	14.8 ± 9.4[a]	100% A	
5	47.2 ± 9.0[d]	80% A	20% CD	23.2 ± 11.6[b]	100% A	
6	36.9 ± 9.6[bc]	86.6% A	13.4% CD	12.2 ± 8.2[a]	100% A	

Abbreviations: A, adhesive; CD, cohesive dentin.
[a] *Same superscript letters indicate statistically similar values* ($p>0.05$).

Saliva contamination of the cured adhesive layer and reapplication of the adhesive system after rinsing (group 5) had no adverse effect on the dentin shear bond strengths for either bonding agent. When the contaminated surface was removed after saliva contamination of the cured adhesive layer (group 6), the bond strength of OSE significantly decreased ($p<0.05$).

The failure modes of all groups were found to be mainly adhesive in nature.

DISCUSSION

This study evaluated the effect of saliva contamination and possible decontamination procedures on the bond strength of two self-etch adhesive systems to dentin during different application steps. Saliva contamination is frequently encountered in clinical practice. Saliva consists mostly of water (99.4%) with 0.6% solids. The solids are composed of macromolecules such as proteins, glycoprotein sugars, and amylase; inorganic particles such as calcium, sodium, and chloride; and organic particles such as urea, amino acids, fatty acids, and free glucose.[12]

Self-etch adhesive systems are widely used in clinical practice because they eliminate the rinsing phase, which not only lessens the clinical application time but also significantly reduces the technique sensitivity and risk of contamination during the application.[13] Furthermore, self-etch adhesive systems contain acidic monomers that promote adhesion to smear-covered dentin. The acidic monomers infiltrate and incorporate the smear layer within the hybrid layer.[14,15] Once saliva contacts the dentin surface, salivary pellicle deposits on the surface and the thickness of the smear layer increase because of the evaporation of water, leaving a film of glycoprotein sugars on the surface. This thick smear layer may affect the bond strengths of self-etch adhesives by inhibiting penetration of the self-etching primer into dentin.

Some studies have reported that proteins in saliva are the main factors responsible for the improper formation of the dentin-adhesive interface when high-molecular-weight macromolecules in saliva may diffuse into dentin tubules[7,12,16] and compete with hydrophilic monomers during the hybridization process, reducing the bond strength.[17] Moreover, the salivary pellicle protects dental hard tissues against demineralization caused by acid attacks.[18,19] Similar to previous studies,[1,3,4] drying the contaminated dentin surface alone significantly decreased the bond strength values to dentin of both self-etch adhesives when contamination occurred before application of the primer (group 2).

Studies evaluating the effect of saliva contamination on the bond strength of self-etch adhesives demonstrated that contamination after primer application significantly decreased the bond strength, even when the surface was carefully dried.[1-4] Hiraishi and others[1] suggested that a hydrophilic monomer such as HEMA in the self-etching primer would be easily rinsed away with water from the demineralized dentin, which might result in collapse of the collagen when the dentin surface was air dried after rinsing. The monomers in the bonding agents were not able to effectively penetrate into the dentin because of collapsed collagen and therefore caused decreased bond strength values. Similarly, bond strength values to dentin declined in our study at the same rate (44%) for both bonding systems when the contaminated dentin surfaces were rinsed and air dried after primer application (group 4). The decreased bond strength values significantly increased after reapplication of OSE primer before bonding, but the reapplication of CSE primer did not increase the bond strength values (group 3).

There are two possible explanations for the increase in bond strength values with OSE. When bonding to air-dried dentin, the H-bonding capacity of solvents has been shown to reexpand the shrunken demineralized collagen network after dehydration.[20,21] Solvents that have a higher affinity to form H-bonds will be able to break the stabilizing H-bonds and other forces that keep the collagen in a shrunken state. Water and ethanol consist of a hydroxyl group that can form strong hydrogen bonds. The hydroxyl group of HEMA also provides hydrogen bonds.[21] However, the H-bonding capacity of HEMA is limited.[22] Therefore, after repriming, OSE primer, which contains water and ethanol, might have been able to reexpand the shrunken demineralized collagen more effectively than CSE primer, which contains water and HEMA. Moreover, water is a poor solvent for organic compounds, but the ethanol in the OSE primer may also have denatured the glycoprotein in the salivary pellicle and cleaned the surface.

The bond strength values of both adhesive systems in group 5 were not altered after saliva contamination of the bonding agent that was applied to and cured on the dentin surfaces. It seems that the salivary proteins could not penetrate into the dentin through the polymerized resin/dentin layer and were removed from the surface by the rinsing and reapplication of the self-etching primer. A suitable surface for bonding was formed again. These findings are consistent with the study by Arı and others.[23] Conversely, Fritz and others[24] have shown that salivary contamination of the polymerized adhesive layer caused a reduction of the bond strength of a total-etch one-bottle adhesive system in both reapplied and non-reapplied adhesive resin groups. However, acid etching was not repeated, while in our study, acid etching was repeated through the application of the primer. Yoo and others[4] also evaluated the influence of salivary contamination on three "all-in-one" self-etching adhesive systems. They indicated that when saliva contamination occurs after light curing of the adhesive bonding agent, reapplication of the adhesive decreases the bond strength.[13] The all-in-one adhesives prime and bond the surfaces at the same time. It can be assumed that even though the contaminated surface was treated again, the saliva proteins were not completely removed as they are during the separate priming procedure with two-step self-etch adhesives.

Several studies recommend that a saliva-contaminated cured adhesive layer must be removed with a bur.[17,23] We could not support these findings in our study as the bond strength values decreased with both adhesive systems when the saliva-contaminated surface was removed with a diamond bur (group 6). However, while the decrease in the bond strength values of CSE was not statistically significant, the bond strengths with OSE decreased significantly. This difference can be attributed to the thickness of the hybrid layer created during the first application of the bonding agent. It can be expected that the OSE primer with a lower pH of 1.5 may have formed a thicker hybrid layer than that formed by CSE (pH 2). Therefore, the hybrid layer created with OSE may not have been sufficiently removed by the slight cutting of the dentinal surface with the bur.

The influence of saliva contamination during different application steps and the differing results with two self-etch adhesives indicate the importance of this investigation and the need for further studies, which should include additional parameters and bonding agents.

CONCLUSIONS

Within the limitations of this *in vitro* study, the following conclusions were drawn:

1. The bond strength values of two-step self-etch adhesive systems are affected by saliva contamination at different adhesive application stages.
2. Saliva contamination before or after primer application significantly decreased the bond strength values of self-etch adhesive systems.
3. Saliva contamination after curing the bonding agent and repeating the bonding procedures did not affect the bonding performance of either self-etch system. However, the removal of the contaminated surface with a diamond bur decreased the bond strength values.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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