Does Rubbing of Universal Adhesive Reduce the Negative Effect of Saliva on Adhesion?

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Purpose: To evaluate in vitro the effect of saliva contamination on bond strength of a universal adhesive applied with actively (with rubbing motion) and passively (without rubbing motion).

Materials and Methods: A total of 144 bovine dentin samples were used, and the universal adhesive, Clearfil Universal Bond Quick, was either applied in accordance with the manufacturer's instructions (passive application) or applied with rubbing motion for 10 s (active application). These 2 groups were divided into 6 subgroups according to saliva contamination (n = 12): 1. control: etch-and-rinse (no saliva); 2. control: self-etch (no saliva); 3. etching/saliva/bonding; 4. etching/bonding/saliva; 5. saliva/bonding; 6. bonding/saliva. A resin composite, Filtek Ultimate, was filled into a polyethylene mold (0.9 mm diameter, 1.2 mm height) on the surfaces. Samples were subjected to microshear bond strength testing, and five specimens from each group were examined using SEM. Resin-dentin interfaces were also observed using transmission electron microscopy (TEM). All failure modes were determined using light microscopy. Statistical analyses were performed with two-way ANOVA, the Kruskall-Wallis test, and the Mann-Whitney U-test ($p < 0.05$).

Results: Active-application groups showed statistically significantly higher bond strengths than did passive groups, regardless of adhesive strategy and saliva contamination ($p < 0.05$). Application of Clearfil Universal Bond Quick in self-etch mode with rubbing motion improved the μSBS among control groups ($p < 0.05$). The active application did not make a significant difference among the active groups ($p > 0.05$), except in group 2. Groups 2 and 5 showed significantly higher μSBS than group 3 among the passive groups ($p < 0.05$).

Conclusion: Rubbing the universal adhesives without any prior etching may increase the dentin bond strength. Following etching, passive application of the universal adhesive (without any additional rubbing motion) could affect the bonding to dentin in the presence of saliva.

Keywords: universal adhesive, saliva contamination, rubbing motion, etch-and-rinse, self-etch.

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One of the objectives of restorative dentistry is to develop adhesive materials that can provide an effective seal at the tooth-restoration interface. Such an ideal adhesive material can strengthen the tooth structure while preparing the cavity more conservatively.[7] However, the adhesion of these materials to dentin is highly sensitive and can be easily affected by contamination of the dentin surface. Moisture contamination adversely affects the interface between the adhesive and the tooth.[40]

Isolation with rubber-dam or cotton rolls/saliva ejector to prevent saliva and moisture contamination has an important place in clinical success. On the other hand, difficulties in providing moisture control, especially when rubber-dam use is not possible to prevent saliva contamination, is one of the biggest problems encountered in daily dental routine. Successful esthetic restorations are only possible with good adhesion. Therefore, the effects of environmental factors such as moisture, blood, saliva, and oil contamination from air-water syringes should be minimized in order to determine the influence of both oral and environmental factors on adhesion and to eliminate possible adverse effects.[32] It has been reported that these factors can lead to microleakage, secondary caries, discoloration, and post-operative sensitivity by affecting the quality of the bonding.[32]

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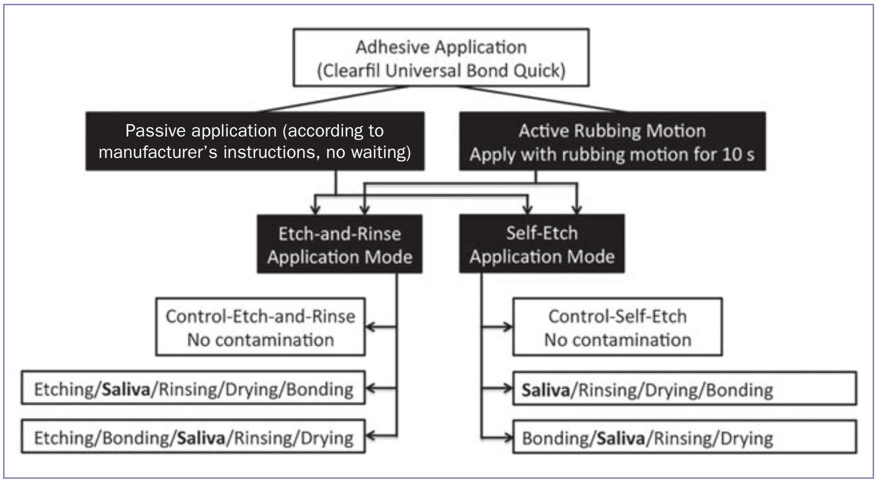

Fig 1 Schematic representation of bonding and saliva contamination procedures.

The effect of saliva on the adhesion to dentin has been explained by various theories. One of these is that glycoproteins in saliva are absorbed by dentin, causing the dentin surface to become inappropriate for bonding.[24] Pashley et al[27] showed that glycoproteins could leak into dentin tubules and form a barrier that prevented the polymerization of the composite resin layer.[27] Another theory states that the water in saliva can dilute the primer and cause a weak hybrid layer, resulting in insufficient adhesion.[25] At the same time, it has been reported that enzymes in saliva can break down bis-GMA in composite resin, and this hydrolytic activity may cause deterioration at the adhesive interface.[25]

The tendency of today's adhesive technology is to reduce the application steps, application time, and technical sensitivity and facilitate the application of the bonding agent. Nowadays, adhesive technology is called universal or multimode, which is used with both self-etch and etch-and-rinse procedures, or combining the advantages of these procedures.[20] Many studies have been conducted to investigate the bond strength of universal adhesives.[4,10,12] In an attempt to increase the bonding efficacy of self-etch adhesives, Miyazaki et al[21] was the first to suggest that active application could help remove the smear layer in order to accomplish chemical and micromechanical interaction with dentin.[21] Some recent studies also investigated the effect of active application on the bonding efficacy of universal adhesives.[13,23]

To the extent of the authors' knowledge, there is no information regarding the effect of rubbing motion on universal adhesives bonding to dentin contaminated with saliva. The purpose of this in vitro study was to evaluate the effect of saliva contamination on the bond strength of a universal adhesive applied passively (by applying in accordance with manufacturer's instructions) or actively with a rubbing motion for 10 s. The first hypothesis was that applying the universal adhesive with rubbing motion would improve the bond strength, regardless of adhesive strategy and saliva contamination. The second hypothesis was that active application would reduce the negative effect of saliva contamination.

MATERIALS AND METHODS

One hundred forty-four bovine incisors were stored in 0.2% chloramine-T solution at 4°C before use. The use of extracted bovine teeth was approved by Hacettepe University Ethics Commission (Approval Number GO-19/437-52). Calculus and stains on selected specimens were removed by hand scaler and then were cleaned with pumice using rubber cups. Roots were separated from crowns using a low-speed saw (IsoMet 1000, Buehler; Lake Bluff, IL, USA) and a diamond-impregnated disk. The buccal surfaces of the teeth were ground using 240-grit silicon-carbide paper to form a flat dentin surface. Following this procedure, each tooth was embedded in cylindrical molds with a quick-set acrylic resin, the flat dentin surface exposed. The dentin surfaces were polished with 600-grit silicon-carbide abrasive papers under running water to form a homogeneous smear layer.

The teeth were randomly divided into 2 groups, and the adhesive, Clearfil Universal Bond Quick (Kuraray Noritake; Tokyo, Japan), was applied employing two different procedures: (a) "passive": applied on the adhesive (no waiting, no additional agitation) (n = 72), (b) "active": rubbing on the adhesive for 10 s (agitation for 10 s) (n = 72). These groups were randomly allocated to 6 subgroups according to the stage at which they were contaminated with saliva (n = 12) (Fig 1). Fresh saliva was collected from a healthy person at the same time of day before each application. The detailed adhesive procedures used in the six treatment groups were as follows:

- Group 1 (control: etch-and-rinse): Adhesive was applied to the surface in etch-and-rinse mode without saliva contamination.
- Group 2 (control: self-etch): Adhesive was applied to the surface in self-etch mode without saliva contamination.
- Group 3 (etching/saliva/rinsing/drying/bonding): Following the etching procedure, fresh saliva was applied to the etched dentin surface with a brush and left undis-

Table 1 Materials used in the study

Product name/Batch	Manufacturer	Composition	Application Mode	Rubbing motion
Etching agent Ultra-Etch (G017)	Ultradent Products; South Jordan, UT, USA	35% phosphoric acid		
Clearfil Universal Bond Quick (000001)	Kuraray Noritake; Dental, Tokyo, Japan	10- MDP, bis-GMA, 2-HEMA, hydrophilic amide monomers, colloidal silica, silane coupling agent, sodium fluoride, dl-camphorquinone, ethanol, water	Etch-and-rinse: Dentin surface was etched with phosphoric acid for 15 s. Conditioned surface was rinsed with water for 15 s and gently air dried.	Passive: Apply according to manufacturer's instructions, no waiting, mildly air dry (≥ 5 s) until the adhesive no longer moves and light cure for 10 s
			Self-etch: Etching with phosphoric acid was not performed.	Active: Apply with rubbing motion for 10 s, mildly air dry (≥ 5 s) until the adhesive no longer moves and light cure for 10 s
Filtek Ultimate (N214468)	3M Oral Care; St Paul, MN, USA	Bis-GMA, UDMA, TEG-DMA, bis-EMA, PEG-DMA, silica filler, zirconia filler		

MDP: methacryloyloxydecyl dihydrogen phosphate; bis-GMA: bisphenol A diglycidyl methacrylate; HEMA: hydroxyethyl methacrylate; UDMA: urethane dimethacrylate; TEG-DMA: triethylene glycol dimethacrylate; bis-EMA: ethoxylated bisphenol A dimethacryrlate; PEGDMA: polyethyleneglycol dimethacrylate.

turbed for 15 s. To decontaminate, the surface was rinsed with water for 5 s and dried with air for 5 s. Adhesive was applied and light cured with an LED device (Radii Plus, SDI; Bayswater, Victoria, Australia) for 10 s.

- Group 4 (etching/bonding/saliva/rinsing/drying): Following etching, the adhesive was applied, light cured for 10 s, and contaminated with saliva as in group 3.
- Group 5 (saliva/rinsing/drying/bonding): Following saliva contamination procedure, adhesive was applied in self-etch mode and light cured for 10 s.
- Group 6 (bonding/saliva/rinsing/drying): Adhesive was applied in self-etch mode, light cured for 10 s, followed by saliva contamination procedure.

Application procedures, including both etch-and-rinse/self-etch modes and passive/active modes, are detailed in Table 1. Following adhesive and contamination procedures, Filtek Ultimate (3M Oral Care; St Paul, MN, USA) was filled into polyetylene tubes (0.9 mm diameter and 1.2 mm height) on the sample surfaces and cured for 20 s. The specimens were kept in distilled water at 37°C for 24 h before the µSBS tests.

Microshear Bond Strength Test and Failure Mode Analysis

A universal testing machine (Instron, Lloyd Instruments; Bognor Regis, UK) was used at 0.5 mm/min with a shearing fixture (Test Base Clamp, Ultradent Products; South Jordan, UT, USA) for µSBS tests. µSBS in MPa were determined by dividing the maximum load at the time of failure by the bonded surface area. Following testing, the bonding areas between tooth surfaces and resin composites were observed under a light microscope (Olympus SZX7; Tokyo, Japan) at a magnification of 40X to determine the failure mode. Classification was made according to the types of failure observed in dentin/composite bonding areas: a) adhesive failure, b) cohesive failure in the dentin/composite, c) mixed failure.

Scanning Electron Microscopy (SEM)

To decontaminate the samples, they were soaked in 10% neutral buffered formalin solution for 8 h following debonding. Five representative specimens were then gold sputtered and examined in a scanning electron microscope (SEM, Tescan GAIA 3; Brno, Czech Republic) to examine the debonded interfaces and resin penetration.

Transmission Electron Microscopy Evaluation (TEM)

For TEM analysis, non-demineralized specimens were fixed in Karnovsky's solution, dehydrated in an ascending ethanol series, then embedded in epoxy resin. 90-nm-thick ultrathin sections were prepared (Leica UC6, Leica Microsystems; Wetzlar, Germany) and collected on 100-mesh formvar-coated copper grids. Without staining, they were observed in a TEM (Tescan GAIA 3) operated at 100 kV.

Statistical Analysis

The analyses were performed using the statistical software package IBM SPSS version 21 (IBM; Armonk, NY, USA). All data sets were subjected to normality testing using the Kolmogorov-Smirnov test. The mean µSBS for each group was analyzed using one-way ANOVA. Two-way ANOVA was used to analyze effects of adhesive application mode (etch-and-rinse/self-etch) and passive/active application. The independent and combined effects of saliva and adhesive strategy were evaluated with Mann-Whitney U- and Kruskal-Wallis tests. The distribution of failure modes among the groups was analyzed using the chi-squared test. Statistical significance was set at $p = 0.05$.

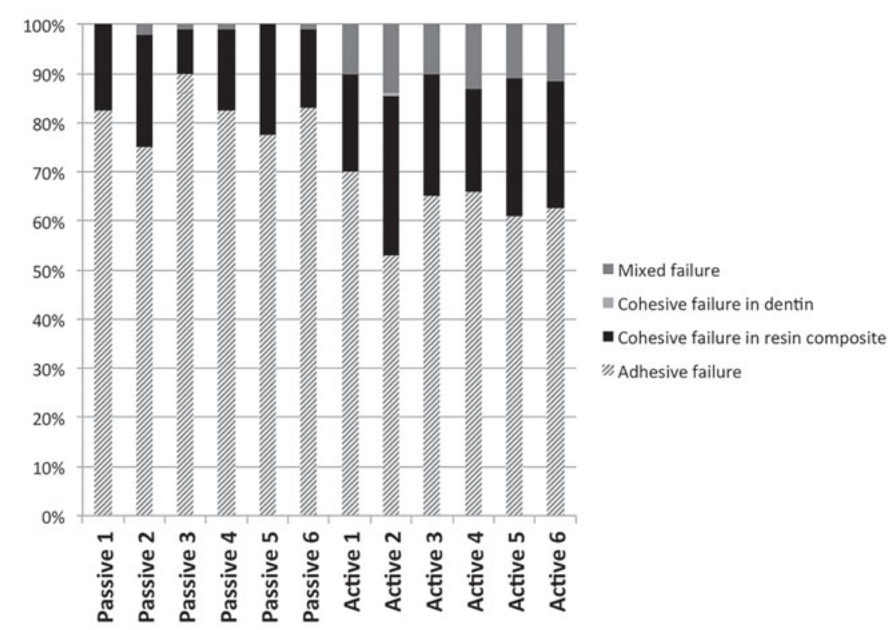

Fig 2 Frequencies of failure modes for passive and active application groups.

RESULTS

Active groups showed statistically significantly higher μSBS than did passive groups, regardless of adhesive strategy and saliva contamination ($p < 0.05$) (Table 1). Application of Clearfil Universal Bond Quick in self-etch mode with rubbing motion improved the μSBS among control groups ($p < 0.05$). Active application did not make a significant difference among active groups ($p > 0.05$), except group 2. On the other hand, group 2 (control: self-etch) and group 5 (saliva/rinsing/drying/bonding) showed significantly higher μSBS than did group 3 (etching/saliva/rinsing/drying/bonding) among passive application groups ($p = 0.04$).

Frequencies of failure modes are represented in Fig 2. Adhesive failure was commonly seen in all groups, regardless of adhesive strategy and saliva contamination. When comparing in terms of rubbing motion, passive application specimens showed more adhesive failures than did active application specimens ($p < 0.05$).

SEM and TEM Evaluation

Representative SEM images of the control groups are shown in Fig 3. For etch-and-rinse groups, it was observed that the smear layer was completely removed in both active and passive applications (Fig 3a and 3b). Rubbing the adhesive in self-etch mode dissolved the smear layer better than passive application of the adhesive (Fig 3a and 3b).

Representative SEM images of active application groups contaminated with saliva are shown in Fig 4. Although open tubules were observed following demineralization, they were also minimally sealed by adhesive monomers (Fig 4a). Figure 4b shows the effect of saliva and an example of fracture that occurred at the interface. Figure 4c shows the minimal effect of saliva contamination for group 5. On the other hand, deterioration at the top of the hybrid layer was observed in group 6 (Fig 4d).

Representative SEM images of passive application groups contaminated with saliva are shown in Fig 5. When samples were contaminated with saliva after etching, the destructive effect of saliva on adhesion was apparent (Fig 5a). While other passive application groups showed similar fracture patterns (Figs 5b to 5d), they differed from active application groups.

Representative SEM images of passive and active application groups with both etch-and-rinse and self-etch modes are shown in Fig 6. Resin tag penetration with passive application (Figs 6a and 6c) was less pronounced than with active application (Figs 6b and 6d). Representative TEM images are shown in Fig 7. In the case of passive application (Figs 7a and 7c), the morphological features of interaction or demineralization were not as clear as those observed for active application (Figs 7b and 7d).

DISCUSSION

In the present study, applying the adhesive with a rubbing motion improved the bond strength for all groups, and neither adhesive strategy nor saliva contamination affected these results. Therefore, the first hypothesis should be

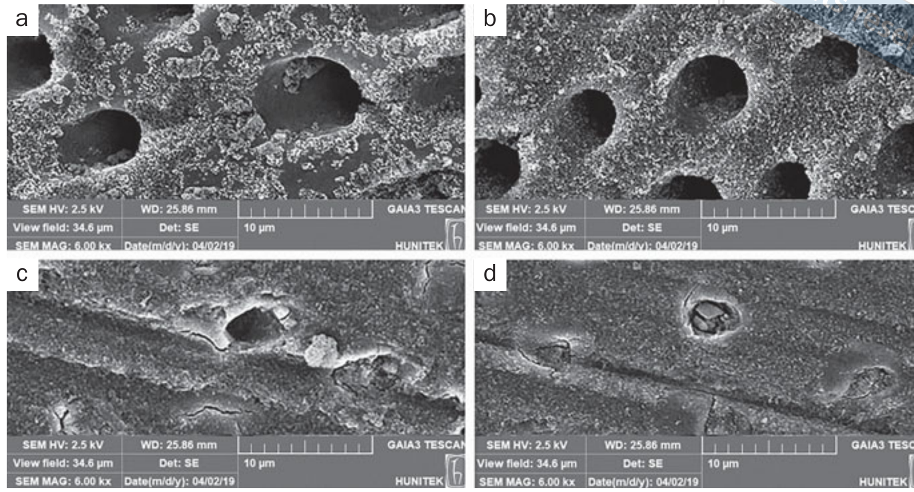

Fig 3 Representative SEM images of the control groups.

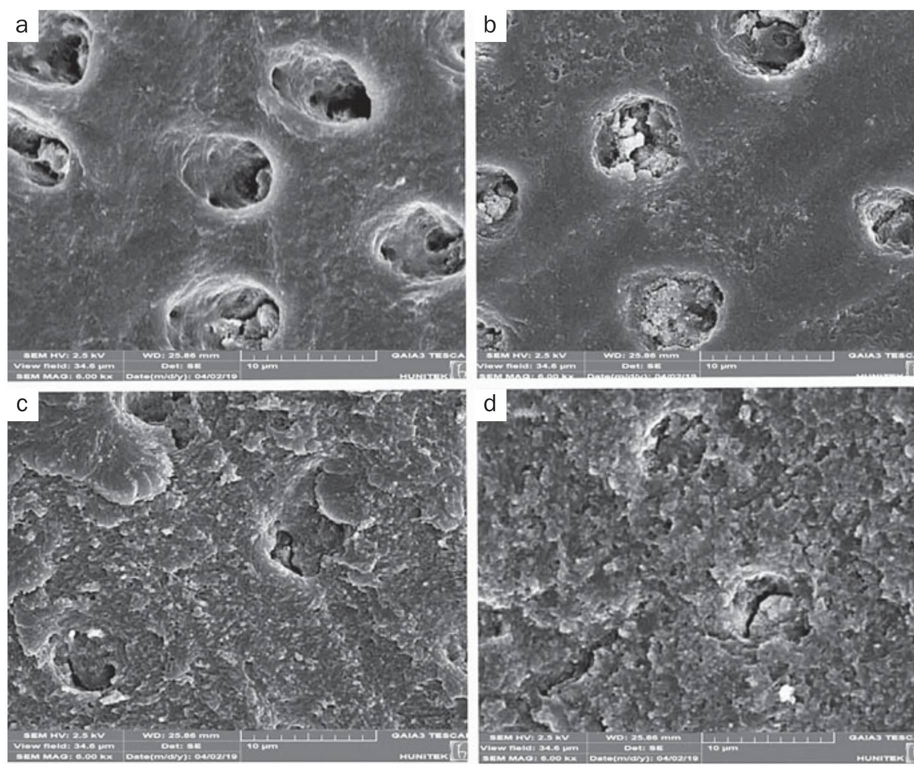

Fig 4 Representative SEM images of active application groups contaminated with saliva.

accepted. Moreover, the second hypothesis, that active application could reduce the negative effect of saliva contamination, should be accepted in part. Despite saliva contamination, rubbing the adhesive improved the µSBS. Saliva particularly affected the bonding performance when applied to dentin between the etching and bonding procedures without rubbing motion (subgroup 3, passive). Beside these results, the remaining passive groups showed acceptable µSBS.

Universal adhesives are distinguished from other adhesives by their ability to bond to enamel, dentin, metal alloys, and zirconia ceramics. However, there is still doubt that these adhesives may provide advantages over one-bottle self-etch adhesives.[6,28] When universal adhesives are applied to dentin in etch-and-rinse mode, there may be a reduction in the quality of the chemical bond between functional monomers and hydroxyapatite as a result of the lack of hydroxyapatite. Loguercio et al[19] and Imai et al[15]

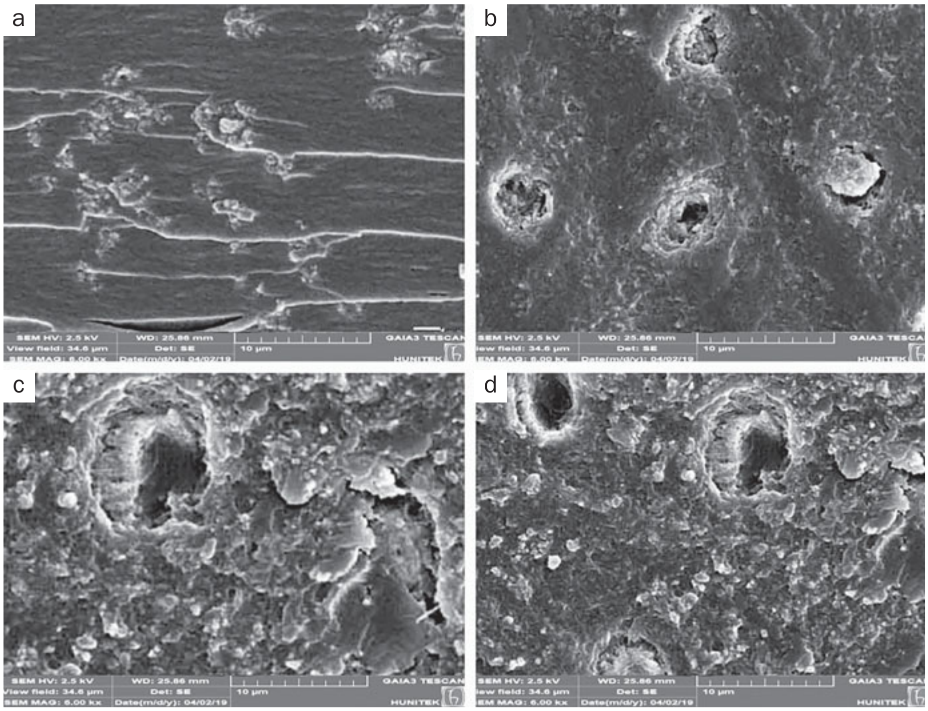

Fig 5 Representative SEM images of passive application groups contaminated with saliva.

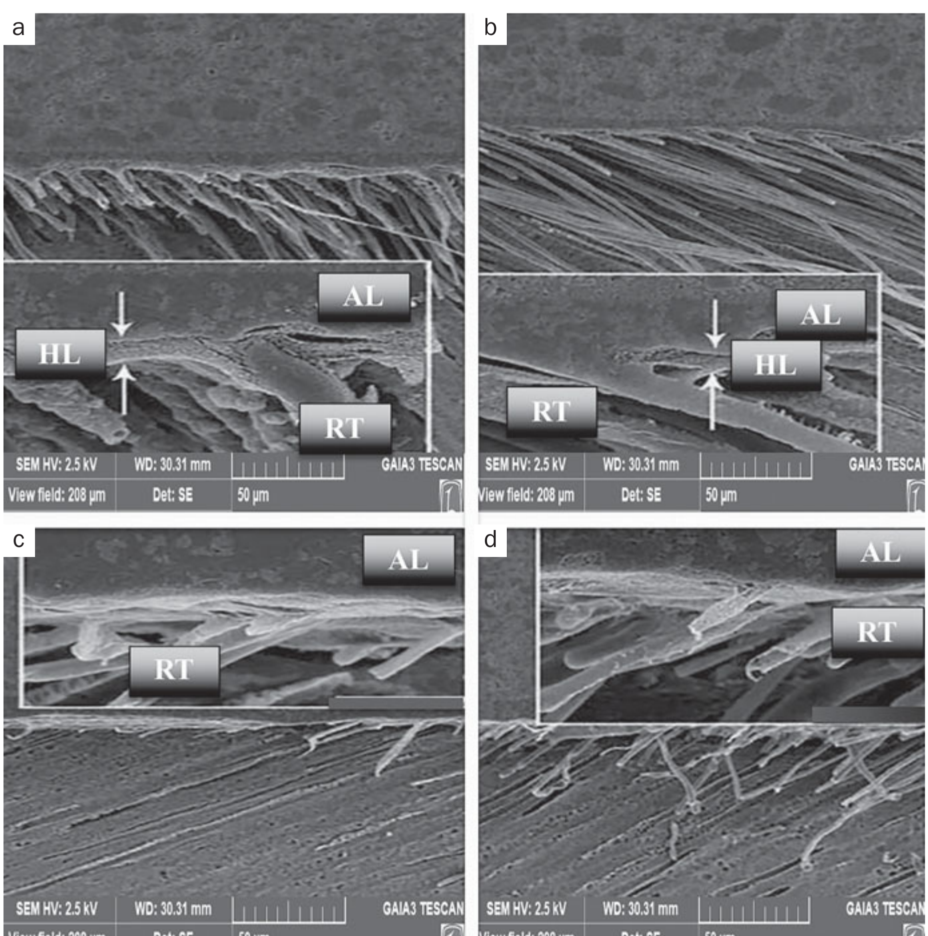

Fig 6 Representative SEM images of resin-dentin interfaces: (a) adhesive in etch-and-rinse mode with passive application; (b) adhesive in etch-and-rinse mode with active application; (c) adhesive in self-etch mode with passive application; (d) adhesive in self-etch mode with active application. AL: adhesive layer; HL: hybrid layer; RT: resin tag.

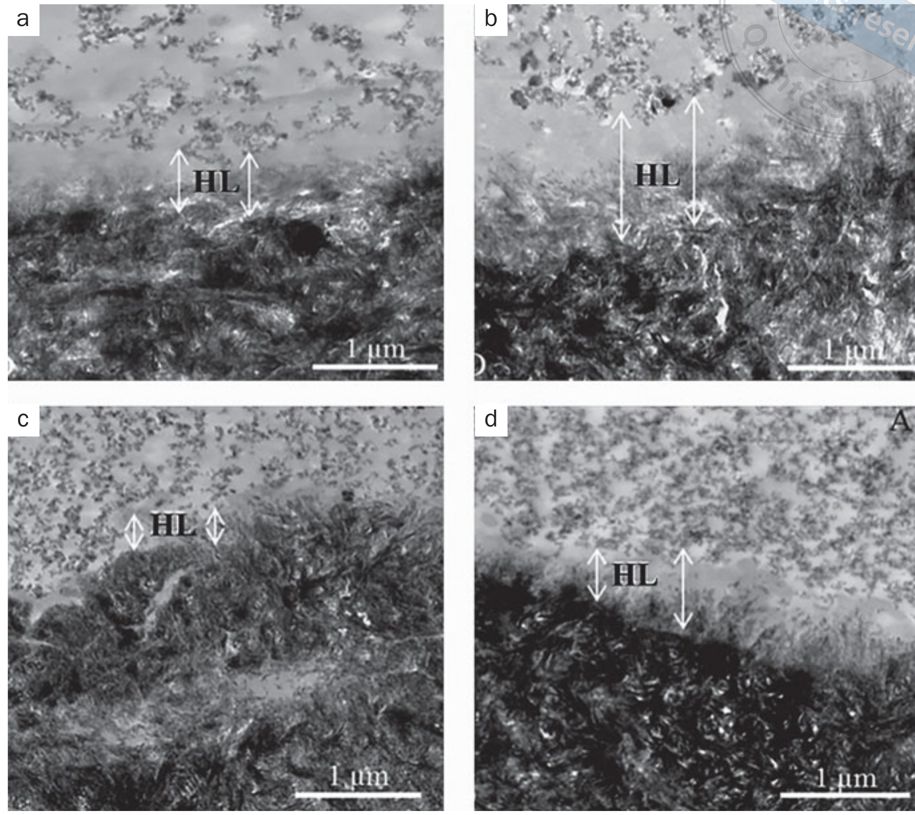

Fig 7 Representative TEM images of resin-dentin interfaces created by Clearfil Universal Bond Quick with 600-grit SiC paper: (a) adhesive in etch-and-rinse mode with passive application; (b) adhesive in etch-and-rinse mode with active application; (c) adhesive in self-etch mode with passive application; (d) adhesive in self-etch mode with active application. HL: hybrid layer.

recommended an active application technique to improve the bonding efficacy. A rubbing motion allows the transfer of acidic resin monomers to the smear layer, causing more aggressive demineralization.[5,16] Thus, the dissolution of the smear layer increases and the resin monomers penetrate dentin more readily.[33] In accordance with these results, the rubbing motion improved the bond strength to dentin when compared to passive application groups in the present study. SEM images of adhesive interfaces supported the increased bond strength. Moreover, rubbing the adhesive may accelerate solvent evaporation and may also cause a higher amount of monomer to diffuse into the smear layer.[21] While this application can provide better monomer diffusion inward, the solvents also diffuse outward.[8] On the other hand, Botelho et al[3] evaluated the rubbing time and bonding performance of one-bottle self-etch adhesives to dentin and reported that the bond strength of the etch-and-rinse adhesive was similar to that of the self-etch adhesives.

Some in vitro studies reported that acid etching of dentin prior to self-etch adhesive application had a negative effect on adhesion.[34,35] The self-etch approach partially dissolves the dentin surface and can provide adequate micromechanical interlocking.[37] Ikeda et al[14] suggested that phosphoric acid affected the chemical bonds between hydroxyapatite and functional monomers by dissolving the hydroxyapatite surrounding the collagen fibers. During application, the formation of an incomplete hybridized zone at the resin-dentin interface plays a crucial role in deterioration of self-etch adhesive bonding.[31] In this study, the µSBS values of the active self-etch application group without saliva contamination were significantly higher when compared to other groups. This result supports the conclusion of a review that the etching of dentin with phosphoric acid may be too aggressive for dentin collagens.[38] The presence of hydroxyapatite around the collagen ensures natural protection and thus increases the potential for chemical interaction of functional monomers in the adhesive.[42]

Contamination with saliva should be avoided during clinical procedures, as this may adversely affect adhesion, but if such a situation is encountered, strict decontamination procedures should be performed to achieve acceptable bond strength. Some studies reported that high molecular weight macromolecules in the saliva penetrated dentin tubules and prevented the formation of an adequate hybrid layer at the adhesive-dentin interface.[30,42] Other studies investigating other adhesives have also suggested that saliva contamination decreased bond strength to dentin.[26,41]

Salz et al[29] evaluated the hydrolytic stability of self-etch adhesives and suggested that cured self-etch adhesives

Table 2 Mean microshear bond strengths for each subgroup (MPa ± SD) (n = 12)

Groups	Passive	Active
Group 1 (control – etch-and-rinse)	13.88 ± 4.67^{aA}	20.94 ± 4.92^{aB}
Group 2 (control – self-etch)	17.43 ± 5.71^{bA}	28.81 ± 7.76^{bB}
Group 3 (etching/saliva/rinsing/drying/bonding)	7.25 ± 5.23^{cA}	21.72 ± 3.45^{aB}
Group 4 (etching/bonding/saliva/rinsing/drying)	12.86 ± 5.78^{aA}	20.96 ± 6.36^{aB}
Group 5 (saliva/rinsing/drying/bonding)	16.98 ± 3.93^{bA}	23.78 ± 4.65^{aB}
Group 6 (bonding/saliva/rinsing/drying)	12.11 ± 4.53^{aA}	22.98 ± 4.73^{aB}

*Different superscript lower case letters in each column and capital letters in each row indicate significant differences ($p < 0.05$).

acted like a permeable membrane, thus allowing dentinal fluid to pass through the polymerized adhesive. In order to prevent this, many self-etch adhesives contain HEMA; hence, the present study used an adhesive that contains HEMA. However, HEMA has some disadvantages, such as low polymerization efficacy and high water uptake.[36] Hiraishi et al[11] reported that HEMA monomer in the adhesives can be easily removed from dentin with water, in which case the collapse of the dentin collagen can be caused by drying after washing. The adhesive tested in the present study also contains MDP monomer, which enables bonding to the remaining hydroxyapatite in dentin and reducing nano-layering. The manufacturer of Clearfil Universal Bond Quick suggested that the adhesive does not require any waiting, multiple layers, or extensive rubbing. They attributed the superior shear bond strength values to its lower HEMA content, higher purity of the functional monomer 10-MDP, as well as the new hydrophilic amide monomer technology, which has extremely high hydrophilicity compared to hydrophilic HEMA monomers.[18] In a study conducted by Ahmed et al,[1] the microtensile bond strength of Clearfil Universal Bond Quick following a quick bonding mode was evaluated.[1] They found that the adhesive's immediate (1 week) bonding effectiveness was significantly better when an additional waiting period of 20 s was inserted before curing, compared to a quick bonding mode without waiting, for both etch-and-rinse and self-etch modes. On the other hand, no statistically significant difference was observed between groups for aged bonding effectiveness. That study's findings[1] for immediate bond strength supported our results. In general, immediate light curing without waiting can be considered a marketing benefit rather than a real advantage.

Our findings are in agreement with a study by Park et al,[26] who investigated the effect of salivary contamination on dentin adhesives' SBS. They reported that contamination after etching had a negative effect on adhesion, but that blotting and applying primer could retrieve the bond strength. Moreover, it has been reported that saliva contamination after etching reduces dentin bonding by 40% and re-etching should be performed to improve the bond strength.[39] Although no significant difference was observed in active application groups, lower shear bond strengths of passive groups were obtained when dentin was contaminated with saliva after etching.

"Only drying" after contamination with saliva was not preferred in the present study. In an in vitro study conducted by Sattabanasuk et al,[30] groups with saliva contamination and only air drying showed lower bond strengths when compared with groups with saliva contamination and rinsing+air drying. Some studies also found no difference between controls and saliva-contaminated and rinsed groups.[2,9,17] Drying without rinsing may not be able to remove some salivary glycoproteins and organic elements, preventing the composite resin from interacting with the oxygen inhibition layer.

The limitation of this study was that it did not perform thermocycling of the specimens to simulate clinical conditions. In the present study, the smear layer was prepared with 600-grit SiC paper, yielding a thinner smear layer than would be the case in clinical conditions. In order to make better comparisons with the literature, further studies should be done with additional groups evaluating thicker, bur-created smear layers. Moreover, adding drying-only groups will contribute to the literature and comparability of results.

CONCLUSION

It is noteworthy that rubbing the universal adhesive, Clearfil Universal Bond Quick, without any prior etching may increase the dentin bond strength. Moreover, following etching, applying the universal adhesive without any additional rubbing motion could affect bonding to dentin in the presence of saliva. If effective isolation cannot be achieved, rubbing the universal adhesive may be recommended to reduce the negative effect of saliva.

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Clinical relevance: The negative effect of saliva on adhesion can be reduced by applying the universal adhesives with a rubbing motion.