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ORIGINAL RESEARCH

Effect of Salivary Contamination on the Bond Strength of Total-etch and Self-etch Adhesive Systems: An *in vitro* Study

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ABSTRACT

Aim: To evaluate the influence of salivary contamination during dentin bonding procedures on shear bond strength and to investigate the effect of contaminant-removing treatments on the recovery of bond strength for two dentin-bonding agents.

Materials and methods: Seventy-seven human maxillary and mandibular molars were randomly divided into two groups for total-etch adhesive (Single bond-3M ESPE, USA) and self-etch primer (UniFil Bond-GC, Tokyo, Japan) and subjected to contamination with saliva.

The data for each group were subjected to one-way analysis of variance (ANOVA) followed by the Student Newman-Keuls test to make comparisons among the groups ($p < 0.05$).

Results: Salivary contamination had less adverse effect on the shear bond strength of single bond total-etch adhesive when it was blot dried or washed. UniFil bond was tolerant of salivary contamination, except when contamination occurred after application of the primer.

Conclusion: In single bond adhesive, when the etched surface is contaminated by saliva, blotting the surface and applying the primer can recover the bond strength. Complete drying of the salivary contaminated surface should be avoided. In the UniFil bond groups, the repriming treatment (UF-V and UF-VI) resulted in the recovery of shear bond strength in the specimens contaminated after priming.

Clinical significance: The results of this study showed that total- etch adhesive (single bond) was not affected by salivary contamination on the etched surface when the bonding surface was kept moist. Self-etch adhesive (UniFil bond) also tolerated salivary contamination except when the contamination occurred after application of the primer.

Keywords: Self-etch primers, Total-etch adhesive, Bond strength, Salivary contamination.

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INTRODUCTION

Dentin bonding has been known as one of the major challenges in adhesive dentistry mainly because of the inherent characteristics of this substrate. Dentin is an intrinsically wet organic tissue communicating with the pulp and the fluid flow in and outward direction making reliable bonding to dentin remarkably problematic.[1,2] The dynamic nature of dentin as a substrate has a major role in influencing bond strength.

Clinically, there are many factors that affect adhesion and retention of resin containing restorative materials. Moisture, such as gingival fluid, blood, handpiece oil and saliva in particular, can affect the quality of the bond, leading to microleakage at the interface.[3-5] However, many carious lesions which require the use of dentin bonding agents are found in areas that are difficult to isolate, especially when the site is near or at the gingival margin where saliva contamination is more likely to occur.[6,7]

Several studies have suggested that the clinical success of resin bonding systems to enamel could be jeopardized by contamination with oral fluids.[8] In one study, a 50% reduction in mean shear strength was demonstrated when composite resin was bonded directly to a saliva-contaminated etched enamel surface, as saliva when it comes in contact with conditioned enamel modifies it both chemically and physically.[9]

The concept of salivary contamination decreasing the bond strength is not universally accepted. However, Fritz et al[10] El-Kalla and Garcia-Godoy,[9] Vargas et al, Hitmi et al and Park et al[5] were unable to demonstrate statistically significant decrease in shear bond strength to tooth surfaces contaminated with saliva when testing modern adhesive systems that incorporated primer in the adhesive.

Recently developed adhesive systems, such as the 'one-bottle' system or the 'self-etching primer' system have a reduced number of components, application steps and thus reduce the risk of saliva contamination in the field of operation.

Even though contemporary dentin adhesive systems are easier to use and less technique sensitive, salivary contamination may still occur during bonding procedures resulting in a reduced bond strength and marginal seal. But, there are only few studies on the effect of treatment methods of saliva contaminated dentin surfaces for the recovery of bond strength for contemporary adhesive systems.

The aim of this present study was to evaluate the influence of salivary contamination of dentin during the bonding procedure on shear bond strength and to investigate the effect of contaminant-removing treatments on the bond strength of two dentin bonding agents (Single bond and UniFil bond).

MATERIALS AND METHODS

Shear bond strength of two dentin adhesives were tested in this study (Table 1); Single bond (Lot No. 20041215, 3M ESPE, St Paul, MN, USA) and UniFil bond (Lot No. 0410081, GC, Tokyo, Japan). Filtek Z-350 (Universal restorative) and Solare-P hybrid resin composite was used respectively.

Specimen Preparation

For the shear bond test, 77 extracted human permanent maxillary and mandibular molars that were intact and caries free were collected and stored in isotonic saline at 4ºC. No more than 3 months time elapsed between the extraction of the molars and their use in the study. The teeth were cleaned of soft tissue debris by ultrasonics and then embedded in cylindrical moulds with self-curing acrylic resin, up to their cervical region.

The occlusal surfaces of the teeth were reduced perpendicular to the long axis on water cooled, model trimming wheel to create flat superficial dentin surfaces. To standardize the specimens, the occlusal surfaces were cut 1.5 mm apical to the deepest occlusal pit. The surfaces were evaluated under a stereoscopic microscope for the presence of any remaining enamel, which was removed by grinding when observed.

To create a uniform smear layer, the exposed dentin surfaces was abraded by 400, 600 and 800 Grit silicon carbide abrasive papers in running water for 60 seconds. After exposing dentin, all teeth were stored at room temperature in deionized water.

Table 1: Chemical composition of adhesive systems used in the study

Bonding systems/composition	
Single bond	*UniFil bond*
Etchant 35% phosphoric acid Bonding agent Bis-GMA HEMA Polyalkenoic acid copolymer Camphorquinone Solvent Ethanol and water	Self-etching primer 4-META HEMA Adhesive TEGDMA HEMA UDMA Camphorquinone Solvent Ethanol and water

Bonding Procedures

The teeth were randomly divided into five groups for single bond (seven specimens for each group) (Fig. 1) and six groups for UniFil bond (seven specimens for each group) (Fig. 2) .

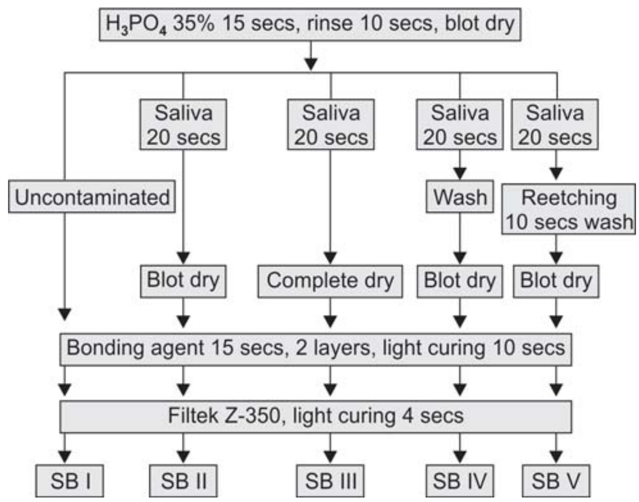

Fig. 1: Schematic representation of the experimental protocol used for single bond groups

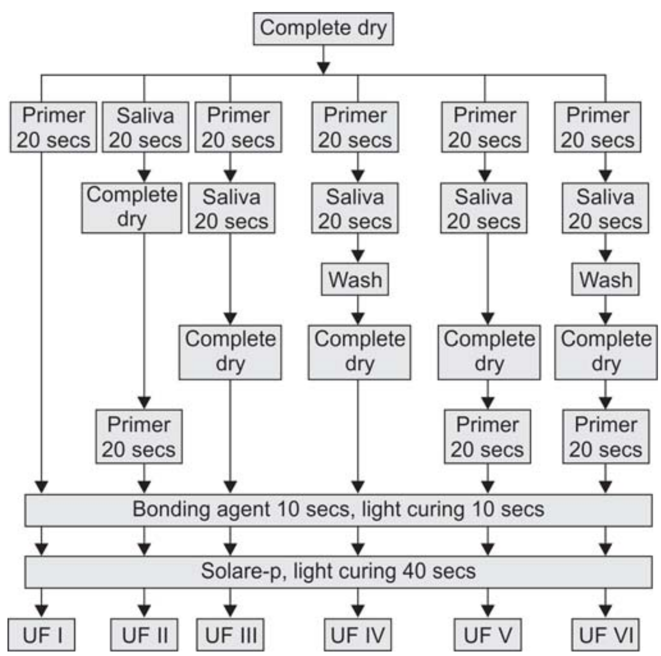

Fig. 2: Schematic representation of the experimental protocol used for uniFil bond groups

The bonding area on the dentin surface was delineated with a wheel diamond by marking a thin circular outline of diameter 4 mm into which the adhesive tested, were applied. For each adhesive before the placement of composites, the specimens were divided into noncontaminated and contaminated (experimental) groups. To contaminate the experimental groups, a dialable micropipette was used to deliver a standardized amount of fresh whole human saliva (0.06 ml) to contaminate the bonding surface. The saliva was gently agitated on the surface with an applicator tip and left undisturbed for 20 seconds. This was followed by contaminant removing treatments, wherever applicable. A stopwatch with digital readout was used to ensure accuracy of timed intervals used for the dentin bonding steps.

A metal disk with an internal ring 4 mm in diameter and 2 mm in height was placed against the tooth surface and the resin composite was packed into the mould in a single increment and light cured for 40 seconds. An Elipar 2500 curing light (3M ESPE) was used which had an output of 650 mW/cm^2. To ensure adequate polymerization of base, the disk were removed and additional 10 seconds of curing was done. After polymerization, the specimens were placed in 37°C distilled water.

Shear Bond Strength Testing

Twenty-four hours after storage in distilled water at 37ºC shear bond strength was tested with a Lloyd universal testing machine (model LR 100K, Hampshire, UK) using the series 1X software system to record the data. Each specimen was mounted in a shear testing apparatus and a chisel-shaped shearing rod with a crosshead speed of 1 mm/minute was used to load the specimens flush to the dentin-composite interface, delivering a parallel force until fracture (Fig. 3). Shear bond strength in Mpa was calculated from the peak load at failure divided by the specimen surface area.

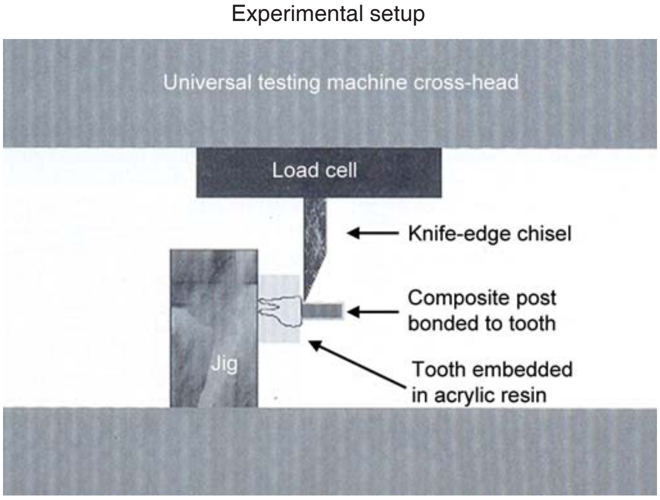

Fig. 3: Experimental setup of shear bond strength testing using Instron machine

Newton = Kg × 9.81

Bond strength (MPa) = Load (N)/Surface area (mm^2).

STATISTICAL ANALYSIS

The data collected were tabulated and subjected to statistical analysis using the Sigma stat software system (SPSS, Chicago, IL). The mean and standard deviation for each group were calculated and were tested for homogeneity of variances using Levene's test. The data for each group were then subjected to one-way ANOVA (independent variable; adhesive, outcome variable; SBS) followed by Student Newman-Keuls multiple range comparison tests to make comparisons among the groups ($p < 0.05$).

STATISTICAL ANALYSIS FOR SINGLE BOND GROUPS

Table 2 shows shear bond strengths of Single bond groups mean ± SD, MPa.

The same letters denote groups that are not significantly different ($p > 0.05$).

A = being the highest, B = the intermediate and C = the lowest bond strength.

One-way ANOVA was used to calculate the p-value

Student Newman-Keuls multiple range comparison tests was employed to identify the significant groups at 5% level.

Table 2: Mean and standard deviation values for shear bond strength of single bond groups using Newman-Keuls test

Groups	*n*	*Shear bond strength (MPa)*	*Newman-Keuls test*
SB I	7	22.80 ± 1.37	A
SB II	7	18.39 ± 1.34	B
SB III	7	13.24 ± 1.44	C
SB IV	7	19.14 ± 1.31	B
SB V	7	21.11 ± 1.01	A

STATISTICAL ANALYSIS FOR UNIFIL BOND GROUPS

Shear bond strengths of UniFil bond groups mean ± SD, MPa.

The same letters denote groups that are not significantly different ($p > 0.05$).

A = being the highest and B = the lowest bond strength

One-way ANOVA was used to calculate the p-value.

Student Newman-Keuls multiple range comparison tests was employed to identify the significant groups at 5% level.

DISCUSSION

As shown in Table 2, in the single bond groups, there was a significant difference between the groups. One-way ANOVA showed that the mean shear bond strength of group

SB III that was dried with strong, oil free air after contamination (SB III-13.24 ± 1.44) was significantly lower than those of groups that were blot dried (Group II—18.39 ± 1.34) and just washed (group IV—19.14 ± 1.31) after contamination, in which the dentin surface was kept moist before priming.

Mean bond strengths of groups II and IV were not significantly different from one another, but both were significantly lower than those of groups I (uncontaminated) and V (reetching after contamination). Mean bond strengths of groups I (22.80 ± 1.37) and V (21.11 ± 1.01) were not significantly different from one another.

As shown in Table 3 UniFil bond groups with salivary contamination after primer application [UF III (14.21 ± 1.80) or UF IV (14.66 ± 1.57)] were significantly lower than the group that was not contaminated (UF I; 19.49 ± 0.97) or the one that was contaminated before primer application (UF II; 18.91 ± 0.94).

Table 3: Mean and standard deviation values for shear bond strength of UniFil bond groups using Newman-Keuls test

Groups	*n*	*Shear bond strength (MPa)*	*Newman-Keuls test*
UF I	7	19.49 ± 0.97	A
UF II	7	18.91 ± 0.94	A
UF III	7	14.21 ± 1.80	B
UF IV	7	14.66 ± 1.57	B
UF V	7	18.23 ± 1.16	A
UF VI	7	19.06 ± 0.77	A

The bond strengths of groups where the primer was reapplied after contamination (UF V and UF VI) were 18.23 ± 1.16 and 19.06 ± 0.77. These are similar to the uncontaminated UF I and UF II, 19.49 ± 0.97 and 18.91 ± 0.94 groups.

The results of this study on shear bond strength of samples with salivary contamination—which agree with the findings of Hitmi et al, Fritz et al[10] vargas et al and El-Kalla and Garcia-Godoy[9]—demonstrated that the newer generations of adhesive systems that use hydrophilic primers may be less sensitive to salivary contamination of prepared tooth surfaces than previous generations of adhesive systems.[5,11,12]

In the single bond groups (SB), when the contaminated surface was dried completely (SB III), bond strength decreased significantly. This result agreed with that of Fritz et al.[10] During air dry, the water-filled collagen layer collapses and dried protein film is adsorbed onto the dentin surface.[13,14] The protein adsorbing properties of hydroxyapatite are well known. Both phenomena prevent penetration of the adhesive into the exposed collagen mesh.[5,14]

However, if the salivary contaminated surface was blot dried (SB II), the decrease in bond strength of Single bond was less significant. The hydrophilic nature of the newer dentin bonding agents may allow them to function to some degree in the presence of saliva contamination.[8,9,13] This creates the transitional resin-reinforced ‘hybrid zone’, through micromechanical retention.[15] It has been shown that moist (although not saliva-contaminated) dentinal surfaces exhibited significantly higher bond strengths than dry surfaces.[13]

Moist dentin (SB II and SB IV) produced higher bond strength than dry dentin (SB III). The benefit of the moist bonding technique is derived from the ability of water to maintain the collagen framework and intertubular porosity patent for monomer infiltration.[13]

In group SB IV, careful rinsing of the contaminated etched dentin was apparently sufficient to remove all, or at least a sufficient amount of protein to let the adhesive monomer to diffuse and wet the hydroxyapatite.[7,10]

In this study, even though the shear bond strength of moist bonding groups (SB II and IV) are in the range of 17 to 19 MPa, they are slightly less than uncontaminated and reetching (SB I and V) groups. It is possible that the excess moisture diluted the primer, thus producing a weak hybrid layer.

Reetching is necessary to restore the surface to the proper condition for bonding.[5,16,17] Previous studies have shown that an additional 10 seconds of acid etching beyond the manufacturers’ recommendation is not detrimental to bond strength (Kanca,’ 1992). Therefore, in this study, the salivary contaminant was removed by washing and drying followed by a 10 second reetching using the original acid-etching agent. (SB V). The results revealed that there were no significant differences among the groups SB I and V.

The self-etching primers are mainly aqueous mixtures of acidic monomers, such as phosphate ester or carboxylic acid and hydroxy ethyl methacrylate.[18] With this process, there is no need to rinse off the reaction products or residual carboxylic acid ester.[3,6,19-21] Thus, in the UniFil bond groups, difficulty in finding an ideal humidification of the dentin is eliminated[21,22] and possible negative influences on adhesion are dramatically reduced.

In the UniFil bond groups, a significant decrease in bonding strength was seen for the UF III and IV groups, regardless of washing or drying, when there was salivary contamination after application of the primer. Therefore, it was not sufficient to dry or wash off the salivary contaminated surface after application of the primer to recover the bond strength. However, bond strength could be recovered after reapplication of the primer. It is likely that the bond strength was recovered, owing to the hybrid layer reformation after removing the unstable primer layer.[23]

The air-dried groups (UF III and IV) after salivary contamination show low shear bond strength values compared to other test groups. Dehydration causes collapse of the collagen scaffold and may impede penetration of liquid into the substrate.[11,24]

The pH of UniFil bond is about 2, which is categorized as 'mild' by Van Meerbeek.[12] He suggested that the advocation of 'mild' self-etch systems results in superficial demineralization that occurs only partially, keeping residual hydroxyapatite still attached to collagen. The preservation of hydroxyapatite within the submicron hybrid layer may serve as a receptor for additional chemical bonding.[25]

In addition, the thickness of the smear layer has no effect on the bonding of self-etching primers to dentin, at least in immediate or short-term intervals. Therefore, in the UF II, V and VI groups, no decrease in shear bond strength was expected, because some additional glycoproteins of saliva present on the smear layer would not inhibit infiltration of the resin monomers during the combined etch/primer step.[5]

CONCLUSION

Within the limitations of this study, it can be inferred that:

1. Salivary contamination had less adverse effect on the shear bond strength of single bond total-etch adhesive when it was blot dried or washed, i.e. when the bonding surface was kept wet before priming. In contrast, when saliva was removed by air-drying, there was a significant decrease in shear bond strength. To achieve the complete recovery of bond strength, reetching the contaminated surface is recommended.
2. UniFil bond was tolerant of salivary contamination, except when contamination occurred after application of the primer. However, the bond strength could be almost completely recovered by reapplying the primer.
3. The moist (although saliva contaminated) dentinal surfaces exhibited significantly higher bond strengths than dry surfaces, emphasizing moist bonding practise in clinical situations.

REFERENCES

1. Neelagiri K, Unadabala MK, Shashirashmi A, Thomas MS, Parolia A. Effect of saliva contamination and decontamination procedure on shear bond strength of self–etch dentine bonding system. An in vitro study. J Conserv Dent 2012;13:71-75.
2. Hegde MN, Hegde P, Chandra CR. Morphological evaluation of new total etching and self-etching adhesive system interfaces with dentin. J Conserv Dent 2012;15:151-55.
3. Chang SW, Cho BH, Lim RY, Kyung SH, Park DS, OH TS, et al. Effects of blood contamination on microtensile bond strength to dentin of three self–etch adhesives. Oper Dent 2010; 35:330-36.
4. Kuphasuk W, Harnirattisai C, Senawonges P, Tagami J. Bond strengths of two adhesive systems to dentin contaminated with a haemostatic agent. Oper Dent 2007;32:399-405.
5. Park J, Lee KC. The influence of salivary contamination on shear bond strength of dentin adhesive systems. Oper Dent 2004; 29-4:437-42.
6. Yoo HM, Pereira PN. Effect of blood contamination with 1-step self-etching adhesives on microtensile bond strength to dentin. Oper Dent 2006;31:660-65.
7. Eiriksson SO, Pereira PN, Swift EJ Jr, Heymann HO, Sigurdsson A. Effect of saliva contamination on resin–resin bond strength. Dent Mater 2004;20:37-44.
8. Johnson ME, Burgess JO, Hermesch CB, Buikema DJ. Saliva contamination of dentin bonding agents. Oper Dent 1994;19: 205-10.
9. EL-Kalla IH, Garcia-Godoy F. Effect of saliva contamination on micro morphological adaptation of single bottle adhesives to etched enamel. J Clin Pediats 1999;24(1):69-74.
10. Fritz UB, Finger WJ, Stean H. Salivary contamination during bonding procedures with a one-bottle adhesive system. Quintessence Int 1998;29:567-72.
11. Townsend RD, Dunn WJ. The effect of saliva contamination on enamel and dentin using a self-etching adhesive. J Am Dent Assoc 2004 Jul;135(7):895-901.
12. Hegde MN, Hegde P, Shetty SK. The influence of salivary contamination on the shear bond strength of two newer generation dentin bonding agent-An in vitro study. J Conserv Dent 2008;11:127-30.
13. John K. Resin bonding to wet substrate I. Bonding to dentin. Quintessence Int 1992;23:39-41.
14. Kumar P, Shenoy A, Joshi S. The effect of various surface contaminants on the microleakage of two different generation bonding agents. A stereomicroscopic study. J Conserv Dent 2012;15:265-69.
15. Nabuo N, Pashley DH. Hybridization of dental hard tissues. Quintessence Int 1992;23:39-41.
16. Barghi N, Knight GT, Berry TG. Comparing two methods of moisture control in bonding to enamel. Oper Dent 1991;16: 130-35.
17. Safar JA, Davis RD, Overton JD. Effect of saliva contamination on the bond of dentin to resin-modified glass-ionomer cement. Oper Dent 1999;24:351-57.
18. Toledano M, Osorio R, de Leonardi G, Rosales-Leal JI, Ceballos L, Cabrerizo-Vilchez MA. Influence of self etching primer on the resin adhesion to enamel and dentin. A J Dent 2001;14:205-10.
19. Hannig M, Reinhardt KJ, Bott B. Self etching primer vs phosphoric acid. An alternative concept for composite to enamel bonding. Oper Dent 1999;24:172-80.
20. Nakabayashi N, Saimi Y. Bonding to intact dentin. J Dent Res 1996;75(9):1706-15.
21. de Carvalho Mendonca EC, Vieira SN, Kawaguchi FA, Powers J, Matos AB. Influence of blood contamination on bond strength of a self-etching system. Eur J Dent 2010;4:280-86.
22. Tay FR, Gwinnett JA, Wei Shy. Micromorphological spectrum from overdrying to over wetting acid-conditioned dentin in water-free, acetone-based, single-bottle primers/adhesives. Dent Mater 1996 July;12:236-44.

23. Koppolu M, Gogala D, Mathew VB, Thangala V, Deepthi M, Sasidhar N. Effect of saliva and blood contamination on the bond strength of self-etching adhesive system–an in vitro study. J Conserv Dent 2012;15:270-73.
24. Pashley DH, Carvalho RM. Dentin permeability and dentin adhesion. J Dent Res 1997;25:355-72.
25. Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Abe Y, Fukuda R, et al. Bonding mechanism and micro-tensile bond strength of a 4-MET-based self-etching adhesive. J Dent Res 2000;79:249, Abstract 845.

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