# Effect of Saliva Contamination on the Microshear Bond Strength of One-step Self-etching Adhesive Systems to Dentin

HM Yoo • TS Oh • PNR Pereira

# **Clinical Relevance**

Saliva contamination significantly affects the bond strength of one-step self-etching adhesive systems to dentin; therefore, saliva contamination must be avoided when using these systems.

## **SUMMARY**

This study evaluated the effect of saliva contamination and decontamination methods on the dentin bond strength of one-step self-etching adhesive systems. Three commercially available "all-in-one" adhesives (One Up Bond F, Xeno III and Adper Prompt) and one resin composite (Filtek Z-250) were used. Third molars stored in distilled water with 0.5% thymol at 4°C were ground with #600 SiC paper under running

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water to produce a standardized smear layer. The specimens were randomly divided into groups according to contamination methods: no contamination, which was the control (C); contamination of the adhesive surface with fresh saliva before light curing (A) and contamination of the adhesive surface with fresh saliva after light curing (B). Each contamination group was further subdivided into three subgroups according to the decontamination method: A1-Saliva was removed by a gentle air blast and the adhesive was light-cured; A2-Saliva was rinsed for 10 seconds, gently air-dried and the was adhesive lightcured; A3-Saliva was rinsed and dried as in A2, then the adhesive was re-applied to the dentin surface and light-cured; B1-Saliva was removed with a gentle air blast; B2-Saliva was rinsed and dried; B3-Saliva was rinsed, dried and the adhesive was re-applied and light cured. Tygon tubes filled with resin composite were placed on each surface and light cured. All specimens were stored in distilled water at 37°C for 24 hours. Microshear bond strength was measured using a universal testing machine (EZ test), and data were analyzed by one-way ANOVA followed by the Duncan test to make comparisons among the groups (p<0.05). After debonding, five specimens

were selected and examined in a scanning electron microscope to evaluate the modes of fracture.

The A2 subgroup resulted in the lowest bond strength. For One Up Bond F and Adper Prompt, there was no significant difference between subgroup A1 and the control, and subgroup A3 and the control (p>0.05). Bond strengths of all B groups were significantly lower compared to the controls (p<0.05). For Xeno III, A1 subgroup showed the greatest decrease in bond strength as compared to the control (p<0.05). On the other hand, it showed more resistance to salivary contamination after adhesive curing. There was no statistically significant difference among the control groups (p>0.05).

### INTRODUCTION

With increased demand and the use of esthetic restorations by patients and clinical practitioners, contamination control has become an important topic, since dental adhesives and composites are very vulnerable to contamination. Achieving good moisture control and saliva contamination is a common problem encountered in restorative dentistry, especially when rubber dam isolation is not feasible. Many carious lesions 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.

Dentin bonding is extremely complex when compared to enamel bonding, and consequently, studies related to the bonding efficacy of saliva-contaminated dentin bonding agents are controversial. Several studies have suggested that "total-etching single bottle adhesive systems" are less sensitive to contamination with saliva than previous-generation bonding agents (El-Kalla & García-Godoy, 1997; Johnson & others, 1994; Xie, Powers & McGuckin, 1993; Abdalla & Davidson, 1998; Peschke, Blunck & Roulet, 2000; Taskonak & Sertgöz, 2002; Park & Lee, 2004). Others have reported that saliva contamination of dentin resulted in a reduction of shear bond strength (Hiraishi & others, 2003). In addition, saliva contamination did not show the same effect in different stages of the bonding process when modern adhesives were used (Hitmi, Attal & Degrange, 1999; Fritz, Finger & Stean, 1998).

Self-etching adhesive systems do not require acid etching and removal of the smear layer and smear plugs. This reduces the potential for post-operative sensitivity and bonding problems associated with movement of dentinal fluid through patent dentinal tubules. Technique-sensitivity associated with bonding to a dehydrated collagen matrix is also eliminated (Perdigão & others, 1999), since water is an essential component in these systems (Tay & Pashley, 2001). Self-etching adhesive systems provide increased user reliability,

with faster application and a reduced number of components and application steps. Even though this reduces the risk of saliva contamination, it may sometimes be impossible to maintain a dry operative field. Although this is a relevant topic, few studies have investigated the effect of saliva contamination on the bonding properties of self-etching adhesive systems.

The null hypothesis of this study was that saliva contamination would not affect the microshear bond strength of one-step self-etching adhesive systems to dentin. In order to test this null hypothesis, this study evaluated the effect of saliva contamination on the dentin bond strength of one-step self-etching adhesive systems contaminated during different bonding steps and identified the best decontamination method in order to re-establish bond strengths similar to the controls.

# **METHODS AND MATERIALS**

Three commercially available "all-in-one" adhesives and one resin composite were used. The bonding agents, composition and manufacturers are listed in Table 1. All teeth were restored with Filtek Z-250 (3M ESPE, St Paul, MN, USA).

Third molars stored in distilled water with 0.5% thymol at 4°C were used within one month of extraction. Dentin slices 2-mm thick were cut using a low-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under cooling water. The dentin surface to be bonded was ground with #600 SiC paper under running water to produce a standardized smear layer. Immediately prior to the bonding procedure, saliva was collected from a single individual, since fresh saliva is considered an acceptable material for use in saliva contamination testing (Eriksson & others, 2004; Hitmi & others, 1999).

The specimens were randomly divided into groups according to contamination method and subgroups according to decontamination methods (Figure 1). A description of all groups is as follows:

Control (C): Self-etching adhesive was applied to dentin according to the manufacturers' instructions and light cured with a visible light curing unit (VLC, Astralis 5, Vivadent, Liechtenstein, Austria) for 10 seconds. During all restorative procedures, light intensity, which ranged from 520 to 560 mW/cm², was measured periodically by a radiometer (Demetron/Kerr Corp, Orange, CA, USA).

Group A: This bonding procedure was carried out as in the control; however, fresh saliva was applied with a disposable brush to the dentin-bonded layer before light-curing and was left undisturbed for 15 seconds. Decontamination subgroups were as follows:

Subgroup A1–Saliva was removed with a gentle air blast. Adhesive was not reapplied. The adhesive was then light-cured with a VLC for 10 seconds.

Adhesive	Manufacturer (Lot #)		Composition
One Up Bond F	Tokuyama (U481183)	Bonding Agent A	Mac-10 methacryloyloxyalkyl acid phosphate, multifunctional methacrylic monomers
		Bonding Agent B	HEMA, water, fluoroaluminosilicate glass filler
Xeno III	Dentsply (0312000292)	Liquid A	HEMA, highly dispersed silicone dioxide, BHT (stabilizer), ethanol, water
		Liquid B	phosphoric acid modified polymethacrylate resin, mono fluoro phosphazene modified methacrylate resin, UDMA, BHT, CQ, ethyl-4-dimethyl aminobenzoate
Adper Prompt	3M ESPE (149338)	Liquid A	methacrylated phosphoric acid esters, initiators based on CQ, stabilizers
		Liquid B	water, HEMA, polyalkenoic acid co-polymer, stabilizers

Abbreviations

Mac-10: 11-methacryloxy-1, 1-undecanedicarboxylic acid, HEMA: 2-hydroxyethyl methacrylate BHT: butylated hydroxy toluene, UDMA: urethane dimethacrylate CQ: camphorquinone

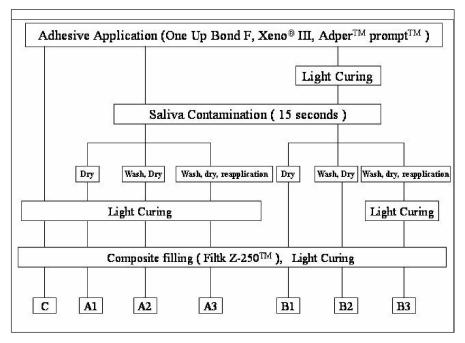


Figure 1. Schematic representation of the bonding procedures.

Subgroup A2–Saliva was rinsed with a water stream from an air-water syringe for 10 seconds and dried with a gentle air blast. The adhesive was then light cured with a VLC for 10 seconds.

Subgroup A3–Saliva was rinsed and dried as in A2; however, the adhesive was re-applied to the dentin surface and light cured with a VLC for 10 seconds.

Group B: The bonding procedure was carried out as in the control; however, the surface was contaminated with fresh saliva after light curing the adhesive. The saliva was left undisturbed for 15 seconds and decontaminated as follows:

Subgroup B1–Saliva was removed with a gentle air blast.

Subgroup B2–Saliva was rinsed with a water stream from an air-water syringe for 10 seconds and dried with a gentle air blast.

Subgroup B3–Saliva was rinsed with a water stream from an air-water syringe for 10 seconds, then dried with a gentle air blast. An adhesive was reapplied to the dentin surface and lightcured with a VLC for 10 seconds.

In order to test the microshear bond strength of the groups described above, the protocol described by Shimada and others (2002) was used. A Tygon tube with an internal diameter of .03 inches was cut into .04-inch-long pieces using a gauge and blade to ensure parallel ends (TGY-030, Small Parts Inc, Miami Lakes, FL, USA). Resin composite was filled into the iris of the Tygon tube and inspected to discard any defect within the composite. The tubes filled with composite were then placed on the

dentin bonded surface and were light cured with a VLC for 20 seconds. If resin a cylinder showed any gap formation, bubble inclusion or any other defect, it was excluded from this study. Three to five resin cylinders were attached on each dentin surface and a minimum of n=15 specimens were made for each subgroup. All specimens were stored in distilled water at 37°C for 24 hours.

# **Microshear Bond Strength Test**

Figure 2 shows the microshear bond test apparatus. The Tygon tubes were carefully removed with a scalpel blade prior to testing to ensure close contact of the ligature wire and cylinder/dentin junction. The dentin slice with the resin cylinders was attached to the

testing device with a cyanoacrylate adhesive, which, in turn, was mounted in a universal testing machine (EZ test, Shimadzu Co, Kyoto, Japan) for shear testing. The ligature wire was carefully looped around one resin cylinder at a time, ensuring close contact with half of the cylinder/dentin junction and was then held flush against the resin/dentin interface. The resin/dentin interface, the wire loop and the center of the load cell were aligned as straight as possible to ensure that the desired orientation in shear stress was maintained. A shear force was then applied to each specimen at a crosshead speed of 1.0 mm/minute. The microshear bond strength was calculated by dividing the maximum load at failure by the cross-sectional surface area of the bonded surface. If a spontaneous

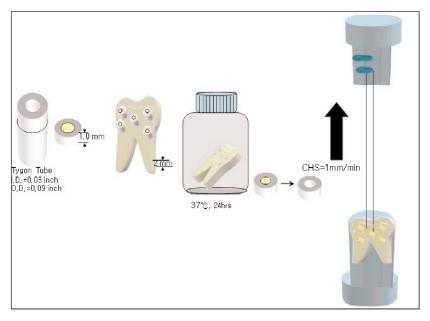


Figure 2. Illustration of specimen preparation and the test design of microshear bond strength test.

Table 2: Mean Microshear Bond Strength for Each Subgroup (MPa ± SD; n)					
Groups	One Up Bond F	Xeno III	Adper Prompt		
С	26.09 ± 2.78 <sup>A</sup>	27.53 ± 5.14 <sup>†</sup>	24.18 ± 6.37 <sup>a</sup>		
	n=18	n=16	n=15		
A1	26.36 ± 4.15 <sup>A</sup>	19.69 ± 3.92 <sup>‡</sup>	20.84 ± 6.92 <sup>abc</sup>		
	n=15	n=15	n=15		
A2	11.43 ± 4.44 <sup>E</sup>	12.17 ± 2.99 <sup>§</sup>	5.25 ± 4.12 <sup>d</sup>		
	n=15	n=19	n=15		
A3	24.62 ± 3.73 <sup>AB</sup>	26.01 ± 5.09 <sup>†</sup>	21.52 ± 3.46 <sup>ab</sup>		
	n=19	n=17	n=16		
B1	23.11 ± 4.95 <sup>BC</sup>	26.23 ± 5.33 <sup>†</sup>	19.66 ± 2.78 <sup>bc</sup>		
	n=16	n=16	n=15		
B2	20.96 ± 3.91 <sup>cd</sup>	26.81 ± 8.73 <sup>†</sup>	17.27 ± 3.74°		
	n=16	n=15	n=15		
В3	18.95 ± 4.40°	28.40 ± 4.62 <sup>†</sup>	19.94 ± 5.58 <sup>bc</sup>		
	n=18	n=15	n=16		
The same supe	rscript within a column indicates no sta	tistically significant difference (Dunc	can test, p<0.05).		

debonding occurred before bond strength testing, the bond strength was recorded as 0 MPa.

The data were subjected to one-way ANOVA followed by the Duncan test to make comparisons among the groups (p<0.05). The statistical analyses were carried out with SPSS 10.0 for Windows software system (SPSS Inc, Chicago, IL, USA).

# **Scanning Electron Microscopic Evaluation**

After debonding, the specimens were fixed in 10% neutral buffered formalin solution for at least eight hours in order to decontaminate the specimens. Five representative specimens were then mounted on stubs, gold sputter coated (SEM coating unit 5100, Polaron instruments Inc, Agawan, MN, USA) and examined in a scan-

ning electron microscope (SEM, model 6500, JEOL Corp, Peabody, MA, USA) to evaluate the modes of fracture.

# **RESULTS**

# **Microshear Bond Strength Test**

Table 2 summarizes the microshear bond strengths for the different groups and subgroups. For all adhesives, the A2 subgroup (saliva contaminated before adhesive curing and decontaminated by washing and drying) resulted in the lowest bond strength. For One Up Bond F and Adper Prompt, there was no significant difference between subgroup A1 (saliva contaminated before adhesive curing and decontaminated by slow drying) and the control, and subgroup A3 (saliva contaminated before adhesive curing and decontaminated by washing, drying and reapplication of adhesive) and the control (p>0.05). With the exception of Xeno III, bond strengths were significantly lower for all the B groups (saliva contamination after adhesive curing) as com-

> pared to the controls (p<0.05). Xeno III showed a different pattern in changes in bond strength as compared to the other adhesive systems. For Xeno III, subgroup A1 (saliva contaminated before adhesive curing and decontaminated by slow drying) showed significantly lower bond strength when compared to the control (p < 0.05). On the contrary, it showed more resistance to salivary contamination after adhesive curing. There was no statistically significant difference among  $_{
> m the}$ controlgroups (p>0.05).

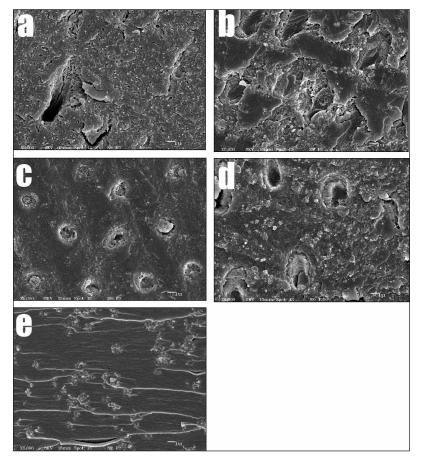


Figure 3. SEM images of fracture modes of debonded One Up Bond F specimens (5,000x, Bar = 1  $\mu$ m). (a) Control, fracture occurred at the top of the hybrid layer (b) When the saliva was applied and just dried on the surface (subgroup A1), there was a significant increase in failure within the adhesive as compared to the control. (c) On the other hand, when the saliva was washed and dried (subgroup A2), the etching pattern was extremely mild, indicating that the adhesive was rinsed out prior to proper demineralization of dentin. (d) When adhesive was re-applied after the washing and drying steps (subgroup A3), the fracture occurred at the hybrid, disclosing a very similar pattern as in the control. (e) When contamination occurred after light curing of the adhesive, washing, drying and reapplication of the adhesive (subgroup B3) was detrimental to the adhesion, as illustrated by fracture within the adhesive.

# Scanning Electron Microscopic Evaluation

In order to illustrate the fracture modes for the different subgroups, representative images of specimens with bond strengths closest to the mean bond strength of each subgroup were selected and are shown in Figures 3 through 5.

For the One Up Bond F group (Figure 3), significant changes were observed in failure modes as a function of contamination. In the control subgroup (Figure 3a), fracture occurred at the top of the hybrid layer, showing a typical fracture pattern for a control group. Subgroup A1 (Figure 3b) showed mixed fracture of the hybrid layer and adhesive; however, the pattern of fracture of the adhesive disclosed disruption caused by saliva. Subgroup A2 (Figure 3c) showed little demineralization of the dentin surface, which probably resulted

from rinsing the adhesive prior to proper demineralization of the dentin. Subgroup A3 (Figure 3d) showed a very similar pattern as the control group, which seems reasonable since the adhesive was re-applied. In Subgroup B3, fracture occurred within the adhesive; it indicated that saliva had a detrimental effect on adhesion (Figure 3e).

Figure 4 shows the fracture modes of Xeno III. For the control group, fracture occurred at the top of the hybrid layer and showed normal fracture patterns for a control (4a). Subgroup A1 showed less demineralization of dentin and less infiltration of adhesive. Fracture occurred at the interface, which showed evidence of the interference of saliva (4b). Subgroup A2 showed demineralization of the dentin surface and minimal adhesive infiltration resulting from the rinsing step (4c). In Subgroup B1, scratches from the specimen preparation can be seen, indicating that failure occurred at the top of the hybrid layer (4d). For Xeno III, all B groups showed similar failure patterns as the control, with a minimal effect of saliva contamination after light curing.

Figure 5 shows the fracture modes of Adper Prompt. The control showed failure within the hybrid layer (5a). Subgroup A1 showed demineralization and infiltration of the adhesive, but failure occurred at the interface and an unusual smooth surface can be seen (5b). Subgroup A2 showed aggressive demineralization of the dentin surface, disclosing open tubules, but incomplete monomer infiltration, collapsed collagen and rare resin tags (5c). Subgroup B2 showed a very flat adhesive surface, indicating that there was minimal interaction between the adhesive and resin composite (5d).

# DISCUSSION

Contamination of the operating field from inadvertent contact with saliva is a frequent problem in dentistry when rubber dam isolation is not used. Often, the use of a rubber dam may be difficult; thus, adhesive systems that bond effectively to dentin in spite of contamination would be highly desirable.

In this study, the authors evaluated the influence of salivary contamination of three "all-in-one" adhesive systems using a microshear bond strength test. For a better understanding of the mechanism of salivary contamination and to help interpret the results, the authors also examined the debonded surface with SEM.

It was reported that self-etching adhesive systems may be classified into mild, moderate and aggressive,

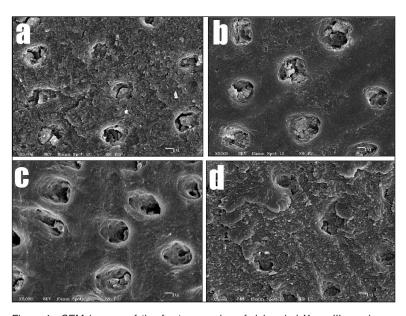


Figure 4. SEM images of the fracture modes of debonded Xeno III specimens (5,000x, Bar = 1 µm). (a) Fracture occurred at the top of the hybrid layer for the control group. (b) After the saliva was applied and dried (A1 subgroup), fracture occurred at the interface, which showed evidence of interference by saliva. (c) When saliva was applied and rinsed (Subgroup A2), the surface was demineralized and tubules opened; however, it was minimally infiltrated by adhesive monomers, probably due to the rinsing step. (d) There was a minimal effect of saliva contamination on Xeno III after light curing, and all failure modes were similar. This representative image (B1 subgroup) depicts failure within the hybrid layer.

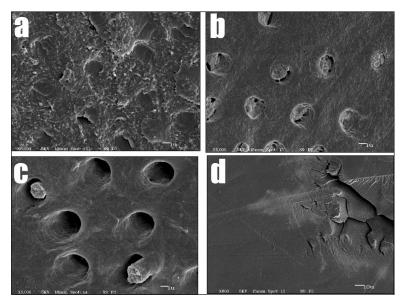


Figure 5. SEM images of fracture modes of debonded Adper Prompt specimens (5,000x, Bar = 1 µm). (a) The control showed failure within the hybrid layer. (b) When saliva was applied and dried (subgroup A1), although there was demineralization and infiltration of monomers, the specimen failed at the interface, resulting in a smooth surface. (c) When saliva was applied, rinsed and dried (subgroup A2), there was demineralization and incomplete infiltration of monomers, disclosing open tubules and rare resin tags. (d) When saliva was applied and dried after light curing (B2 subgroup), there was minimal interaction between the adhesive and resin composite.

based on their ability to solubilize dentin smear layers and demineralize surface dentin, and this aggressiveness is related to dentin permeability (Tay & Pashley, 2001; Tay, Pashley & Yoshiyama, 2002; Chersoni & others, 2004). Aggressiveness can be classified as One Up Bond F being mild, Xeno III being moderate and Adper Prompt being an aggressive self-etching adhesive. SEM imaging clearly revealed the difference in aggressiveness (Figures 3c, 4c and 5c).

For all adhesives, bond strength was lowest for the A2 Subgroups (saliva contaminated before adhesive curing and decontaminated by washing and drying). This result was probably caused by removal of the adhesive layer during washing and drying, leaving a surface that was demineralized but non-infiltrated by monomers. For Adper Prompt, four specimens spontaneously debonded in Subgroup A2. Figure 5c shows minimal resin infiltration on the fractured surface. Therefore, in a clinical scenario, if contamination occurs by saliva before light curing the adhesive, decontamination by washing and drying is not recommended.

One Up Bond F and Adper Prompt showed similar trends in changes of bond strength according to each group. There was no significant difference between Subgroup A1 (saliva contaminated before adhesive curing and decontaminated by slow drying) and the control, and Subgroup A3 (saliva contaminated before adhesive curing and decontaminated by washing, drying and reapplication of adhesive) and the control. In the B Group (saliva contamination after adhesive curing), One Up Bond F and Adper Prompt showed that salivary contamination of the cured adhesive layer significantly decreased shear bond strength. SEM images supported the decrease of bond strength. These results are in accordance with the results of previous studies. Fritz and others (1998) reported that salivary contamination of the cured adhesive layer had a detrimental effect on bond strength. Irrespective of the decontamination technique, shear bond strength was reduced to about 50% of control values. Hitmi and others (1999) also reported that salivary contamination of the cured adhesive layer decreased shear bond strength dramatically. The strong influence of salivary contamination at this stage may be partially explained by the adsorption of glycoproteins onto the poorly polymerized adhesive surface, which results in oxygen inhibition. In subgroup B1 (salivary contamination of the cured adhesive layer and slow dry), glycoproteins could prevent the necessary intimate contact between the adhesive and resin composite. Presumably, glycoproteins compromise copolymerization between the adhesive

and resin composite, causing flaws at the adhesive resin-composite interface (Hitmi & others, 1999). In Subgroup B2 (salivary contamination of the cured adhesive layer, and wash and dry), some other reason may be hypothesized as to the cause for the reduction of bond strength. The first hypothesis is the compromise of copolymerization with the subsequent resin layer by removal of the oxygen-inhibited, unpolymerized surface layer during rinsing and drying. However, this hypothesis is not clear, because investigations have shown that there is no difference in bond strength when resin composite is added to and polymerized on cured adhesive with or without an unpolymerized surface layer (Finger, Lee & Podszun, 1996). The second hypothesis is the insufficient filling of demineralized dentin mesh with adhesive or all-adhesive, or not any of the adhesive occupying the interstices of the collagen mesh being polymerized. Rinsing and drying after salivary contamination may result in a collapsed collagen zone deprived of resin (Fritz & others, 1998). The same hypothesis could explain the result of Subgroup B3 (salivary contamination of the cured adhesive layer, and wash, dry and re-application of adhesive). However, theoretically, a self-etching adhesive system would demineralize dentin and infiltrate it simultaneously with monomers that are polymerized in situ so that no gaps would be left between the primed surface and the organic dentin surface. To understand the exact mechanism, further research is needed.

However, Xeno III showed a different pattern in bond strength change compared to the other materials. In Xeno III, Subgroup A1 (saliva contaminated before adhesive curing and decontaminated by slow dry) showed significantly lower bond strength compared to the control. SEM revealed less demineralization of the dentin surface and less infiltration of the adhesive. On the contrary, it showed no statistically significant difference when salivary contamination occurred on the cured adhesive layer. SEM revealed predominant failures at the top of the hybrid layer. In addition, the composition of materials may have influenced the behavior of the different materials.

Although all-in-one adhesive systems are appealing, no long-term data exists on the clinical performance of self-etching adhesive systems or the effects of salivary contamination. Therefore, caution is recommended in interpreting these *in vitro* results, and it is not possible to extrapolate the results directly to the clinical situation. Further clinical research is necessary to confirm these results.

## **CONCLUSIONS**

The null hypothesis that saliva contamination would not affect the dentin microshear bond strength of one-step self-etching adhesive systems was rejected. Saliva contamination and decontamination methods significantly affected the bond strength of one-step self-etching adhesive systems to dentin, regardless of the materials evaluated. Within the limitations of this *in vitro* study, it can be concluded that if saliva contamination occurs at the adhesive surface before light curing, that washing, drying and reapplying the adhesive is recommended. If saliva contamination occurs to the adhesive surface after light curing, then re-application of the adhesive is not necessary. However, saliva contamination affects the bond strength of one-step self-etching adhesive systems to dentin. Therefore, routine use of the isolation technique is highly recommended.

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