# Longitudinal evaluation of the effect of saliva contamination during the bonding protocol with a self-etch adhesive system

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## Abstract

**Aim:** To evaluate the influence of saliva contamination on the short- and long-term bond strength of a self-etch adhesive system. **Methods:** One hundred and twelve non-carious human molars were randomly divided according to: substrate (enamel/dentin); presence of saliva [none (control-C), before primer (BP), after primer (AP) and after bonding agent (AB)]; treatment of the contamination [none (1), rinsing + drying (2), drying (3) and primer re-application (4)] and specimen storage (24 h or 6 months). A self-etch adhesive system was applied to the dental surfaces followed by incremental insertions of composite resin. After storage in water at 37ºC, the specimens were perpendicularly cut into beams for microtensile bond strength testing. Data in MPa were compared by ANOVA followed by Tukey's test ($p < 0.05$). Micrographs were obtained by low vacuum scanning electron microscopy. **Results:** Control groups (G1 and G8) presented higher bond strength than all other groups. The factors *presence of saliva*, *treatments of the contaminant* and *specimen storage* showed no statistically significant results for the two dental substrates. Contaminants could be detected by LV-SEM. Six-month storage did not affect bond strength. **Conclusions:** The presence of saliva during the application of the self-etch system was deleterious to the bond to enamel and dentin, irrespective of the operative step in which the contamination occurred.

**Keywords:** saliva contamination, self-etch adhesive, bond strength, longevity.

## Introduction

During the application of adhesive systems none, of the steps in the operative protocol should be neglected in order to achieve clinical success, thus the use of a rubber dam is essential. In spite of its advantages[1], however, the majority of professionals do not use rubber dam isolation routinely in their daily clinic, and 45% of them have never used it while placing amalgam and composite resin restorations[2]. Moreover, some clinical situations hinder the use of the rubber dam, such as, uncooperative patients, subgingival margins, mispositioned teeth and partially erupted crowns.

The influence of saliva contamination during the application of adhesive systems has been evaluated both in enamel[3-16] and in dentin[4-7,10-12,17-32]. Nevertheless, controversial results have been observed. While some studies have found lower bond strength[3-6,9-10,12,17,21,25,29,31,33], others have found no deleterious effect on bond

strength when saliva contamination occurred[5,7-8,11,13,16,18-20,24,26-28,30,32,34]. It may be speculated that this controversy arises from the use of distinct test methods, experimental designs with different proposals, and the use of adhesive systems of different categories.

Most studies related to saliva contamination of the operative field have tested its influence on bond strength after 24 h of storage, but some authors have suggested the need of longitudinal studies[5-6] to establish long-term results. Until now only one study has evaluated the influence of saliva contamination on the bond strength to enamel after 1 year of storage[8]. Therefore, to the best of our knowledge the present study is the first to propose a longitudinal evaluation of bond strength to saliva-contaminated enamel and dentin. In addition to testing bond strength to both substrates, the present study was also aimed at detecting the presence of saliva on contaminated samples, but we understood that the chemical preparation for conventional scanning electron microscopy (SEM) could remove saliva and change the results. As low vacuum SEM (LV-SEM) does not require specific preparation with chemical substances or sputter-coating, it was selected for this study in an effort to detect the presence of saliva or the products of its reaction with adhesive systems deposited on dental tissues. This is the first time this microscopy has been used in contamination studies. Based on the controversial results of bond strength studies, lack of longitudinal evaluation and absence of LV-SEM experiments related to the influence of saliva contamination on bond strength to dental tissues, this study was conducted in an endeavor to add knowledge to the established paradigms of operative dentistry. Although it is not a clinical study, our expectation is to provide dental professionals with new ideas on how to deal with a normal clinical episode.

Briefly, the purpose of the present in vitro study was to evaluate the influence of saliva contamination on the bond strength of a self-etch system, according to the type of substrate (enamel/dentin), presence of saliva, and treatment of the contamination after 24 h and 6 months of storage, using microtensile bond strength test and LVSEM.

## Material and methods

### Sampling

One hundred and twelve non-carious human molars stored in distilled water at 4ºC were used within 3 months after extraction for the microtensile bond strength test (70 teeth) and LV-SEM (42 teeth). This project was approved by the FOUSP's Research Ethics Committee under docket number 103/05.

### Preparation of Specimens

The occlusal enamel and superficial dentin were removed using a slow-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water cooling. A flat enamel/dentin surface was created perpendicular to the longitudinal axis of the tooth. The dentin surface was ground (Ecomet/Automet 2; Buehler Ltd.; FAPESP Proc. 03/12182-4) with 600-grit silicon carbide (SiC) paper under running water for 60 s to create a standard smear layer.

### Saliva Purchase and Use

A pool of human saliva was obtained from 3 donors, who were subjected to mechanical pre-stimulation by chewing on Parafilm® (American National Can Company, Chicago, IL, USA) for 5 min. The saliva was collected in a Beaker for 30 s, the pH of total saliva was recorded (6.8), and the saliva was divided into aliquots that were stored frozen (-80ºC) until use[21,35]. An aliquot of 4 mL[4,12] of purchased saliva was used to contaminate the adhesive procedures (Groups G2-G7 and G9-G14) for 15 s[6,18,22,30].

### Experimental Groups

Fourteen groups were formed according to the test substrate (enamel and dentin), presence of contamination (none, before primer, after primer, after bonding agent) and treatment of the contamination (none, rinsing + drying, drying, primer re-application) (Table 1).

**Table 1** - Experimental groups.

| Presence of saliva | No SC | SC before primer | | SC after primer | | SC after bond | |
|---|---|---|---|---|---|---|---|
| Treatment of the contamination | None | Rinsing + drying | Drying | Drying | Primer re-application | Rinsing + drying | Drying |
| Substrate **Enamel** | G1 | G2 | G3 | G4 | G5 | G6 | G7 |
| Substrate **Dentin** | G8 | G9 | G10 | G11 | G12 | G13 | G14 |

SC: saliva contamination.

The contaminant was treated according to the following descriptions: 1. Rinsing + drying: saliva was rinsed with air/water spray syringe for 15 s[13,16], followed by air stream for 10 s[4,31,33], at a distance of 10 cm[34] from tooth surface; 2. Drying procedure: air stream for 10 s[4,31,33] at a distance of 10 cm[34] from tooth surface; 3. Primer re-application: primer was re-applied for 20 s on the saliva-contaminated surface. This treatment was only performed in the group in which contamination occurred after primer application.

### Adhesive System Application

A self-etch adhesive system (Clearfil SE Bond; Kuraray, Kurashiki, Okayama, Japan) was used. Primer solution was actively applied on dry dental surfaces for 15 s and the excess was removed by blowing gently with an air syringe. Then SE bond was applied, dried by 5 s of gentle air drying, and the layer was polymerized for 10 s with a light-curing unit (Astralis 3, Ivoclar Vivadent, Amherst, NY, USA) with light intensity of 660 mW/cm², as confirmed by a curing radiometer (Demetron Curing Radiometer; Kerr, Orange, CA, USA).

## LV-SEM

For LV-SEM analysis, the specimens were not subjected to chemical preparation or sputter-coating with gold, as in the conventional SEM analysis. Specimens prepared according to experimental groups (Table 1) were mounted on stubs and SEM micrographs were obtained with a JEOL 6460-LV equipment (JEOL, Akishima Tokyo, Japan) at 20 kV.

Three specimens were allocated to each of the 14 groups (total of 42 specimens).

## Microtensile Bond Strength Test

The 70 molars were divided into two halves to obtain 140 tooth fragments. Ten specimens were allocated to each of the 14 groups. Each group was again divided into 2 subgroups according to the storage periods of 24 h (n = 5) and 6 months (n = 5).

After adhesive system application, as previously described, Clearfil APX (Kuraray) composite resin was inserted in 4 increments, each polymerized for 20 s with the same light-curing unit described above. All specimens were stored in water at 37°C, for 24 h.

The specimens were cut perpendicularly with a low-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) to obtain beams (1.0mmX 1.0mm cross-sectional dimensions) for microtensile strength testing. The final width and thickness of the bonded area were measured to the nearest 0.01 mm by means of a digital micrometer. Specimens were stored for 24 h at 37°C. At the end of this period, 5 molars were tested, while the other 5 were stored for up to 6 months to compose the longitudinal samples of the study.

Samples were attached to a Geraldelli jig with cyanoacrylate adhesive. The device was pulled under tension (Mini Instron 4442, Instron Co., Norwood, MA, USA) at 1.0 mm/min until failure. Bond strengths were calculated by dividing the load at failure by the cross-sectional bonding area. Using a light microscope at 40X magnification (Olympus SZ40, Shinjuku-ku, Tokyo, Japan), the failure mode of specimens was classified as adhesive if debonding occurred at the resin-dentin bond interface and cohesive if the failure occurred in the substrate (resin or dentin).

## Statistical Analysis

The statistical analysis was made for enamel and dentin separately. The data were subjected to ANOVA and Tukey's test at a level of significance of 5%.

## **Results**

## LVSEM

Figures 1 (enamel) and 2 (dentin) are LV-SEM micrographs of the dental substrates representative of all experimental groups.

The enamel surface (G1) ground with 600-grit abrasive paper without saliva contamination showed a smear layer-
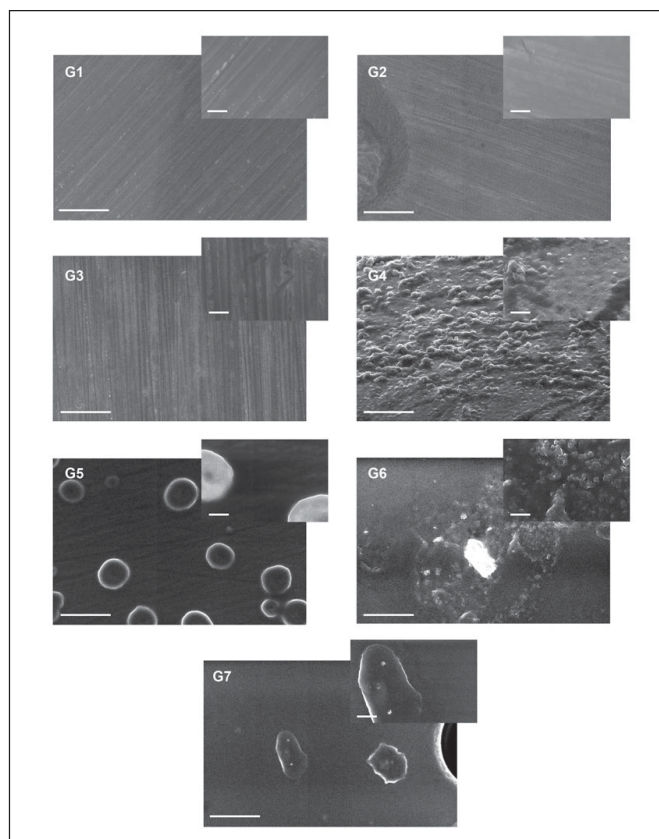


**Fig. 1.** LV-SEM micrographs of the enamel surfaces representative of all experimental groups. Main micrograph at 500X (bar = 50µm). Micrograph in detail at 2000X (bar = 10µm).
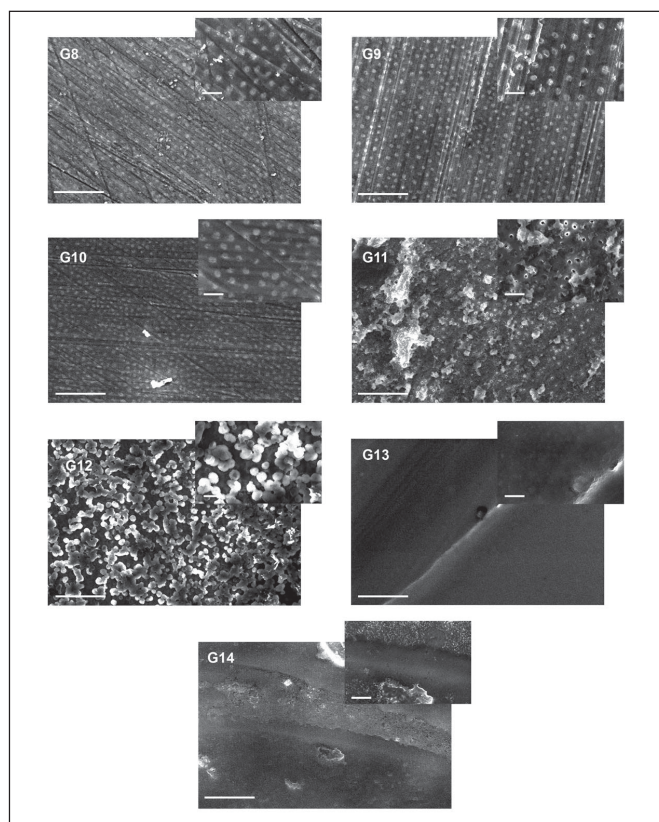


**Fig. 2.** LV-SEM micrographs of the dentin surfaces representative of all experimental groups. Main micrograph at 500X (bar = 50µm). Micrograph in detail at 2000X (bar = 10µm).

like image, in which grooves from the abrasive paper can be detected, at both magnifications (500 and 2000X). G2 and G3 present images similar to G1, suggesting that the water rinsing of saliva (G2) and/or primer application (G3) were able to remove the contaminant from enamel surface. However, when saliva contamination occurred after primer application (G4), a diffuse precipitate of amorphous structures could be detected on the entire enamel surface, probably as a result of the reaction between the primer and saliva. When the primer solution was re-applied on the contaminated enamel surface, rounded structures were seen, suggesting that the new layer of primer solution reacted with saliva and formed clusters of precipitate. It seems that the amorphous and diffuse structures observed in G4 were transformed into some limited and circumscribed spots on the enamel surface. A large number of amorphous structures could be detected on the adhesive resin pellicle (G6) adding a heterogeneous appearance to the enamel surface by means of a mixture of saliva and water. When dry saliva was present, smaller amounts of the same deposits could be easily observed in some areas of the specimen (G7) (Figure 1).

A typical smear layer with clearly demarcated smear plugs could be seen on the dentin surface without saliva contamination (G8). Furthermore, similar images were observed in the groups in which saliva was applied before primer and treated by a water rinsing (G9) or drying procedure (G10). In all these groups saliva could not be distinguished. However, when saliva contamination occurred after primer application, the presence of saliva could easily be detected as an irregular precipitate on the dentin surface with some open dentinal tubule apertures (G11) or a large amount of precipitate (G12), probably as a result of saliva and primer reaction. When contamination occurred after application of the light-polymerized bonding agent, but the contaminant had been washed and dried (G13), only a small amount of residues could be noticed. However, when saliva was only dried (G14), a larger amount of residues were observed, probably constituted by the salivary components that were deposited on the adhesive resin surface. (Figure 2)

### Microtensile Bond Strength Test

Table 2 shows the results obtained in the microtensile bond strength tests after 24 h and 6 months of storage. The bond strengths of specimens stored for 24 h were similar to those of specimens that were stored for 6 months for both tested substrates ($p = 0.064$, for enamel and $p = 0.115$, for dentin).

All saliva-contaminated enamel groups (G2, G3, G4, G5, G6 and G7) presented lower bond strength values than the non-contaminated group (G1), indicating that the presence of saliva reduced the self-etch adhesive system bond to enamel ($p \leq 0.001$). Moreover, when saliva contamination was only dried, the bonding performance of G3 (contaminated before primer application) and G4 (contaminated after primer application) showed similar and significantly lower values than bond strength results for the group in which saliva contamination occurred after the bonding agent (G7) was light polymerized ($p \leq 0.001$).

**Table 2 -** Micro-tensile bond strength of all experimental groups.

| Substrates | Groups | 24 h | 6 months |
|---|---|---|---|
| Enamel | G1 | 46.03±3.36 Aa | 46.53±3.13 Aa |
| | G2 | 32.15±5.20 Ab | 32.28±4.61 Ab |
| | G3 | 29.73±2.15 Ab | 30.22±3.49 Ab |
| | G4 | 30.39±2.47 Ab | 30.51±1.78 Ab |
| | G5 | 28.52±5.04 Ab | 26.63±2.15 Ab |
| | G6 | 35.92±3.04 Ac | 34.70±4.80 Ac |
| | G7 | 39.51±4.17 Ac | 35.84±3.73 Ac |
| Dentin | G8 | 54.90±4.70 Ad | 50.87±6.67 Ad |
| | G9 | 29.53±6.84 Ae | 32.12±12.20 Ae |
| | G10 | 35.40±5.39 Aef | 33.20±11.02 Aef |
| | G11 | 38.42±3.57 Ae | 36.80±2.02 Ae |
| | G12 | 37.42±3.69 Ae | 36.37±5.82 Ae |
| | G13 | 43.22±2.25 Ae | 43.44±7.37 Ae |
| | G14 | 43.00±2.14 Ae | 39.91±2.48 Ae |

Data are presented as mean ± standard deviation of the mean. Different uppercase letters in columns and lowercase letters in rows indicate statistically significant differences (ANOVA and Tukey's test; $p<0.05$).

Saliva contamination decreased the bond to specimens in all the dentin groups when compared with non-contaminated group (G8) ($p \leq 0.001$). When saliva was air dried, the worst bonding performance could be observed in the group in which contamination occurred before primer application (G10) ($p = 0.003$). Water rinsing and primer re-application were not able to counteract the decreased bonding performance caused by saliva contamination.

The majority of the specimens (93.2%) presented adhesive failures. Cohesive failures in the substrate were 6.8% and no resin cohesive failure was observed.

## Discussion

Adhesive systems are widely used in clinical practice, especially associated with esthetic procedures. Bond to dental structures is complex and highly technique-sensitive, thus use of the rubber dam is always indicated. Rubber dam isolation[1]: prevents aspiration or deglutition of instruments or remnants of cavity preparation; retracts the lips and tongue; protects soft tissues; provides visual accuracy of the operative field; is a protective barrier preventing cross-infection of patients, professionals and technical assistants; and is a demonstration of excellence in treatment of patients.

Unfortunately, Joynt et al. (1989)[2] demonstrated that 45% of professionals do not use a rubber dam in daily clinical practice when placing composite resin restorations. Moreover, some manufacturers recommend that adhesive systems can be used with cotton rolls[10], which could confuse clinicians as regards choosing an adequate isolation procedure. This study emphasizes the essential involvement of the rubber dam in order to achieve excellence in restorative dentistry.

The findings of the present study showed that saliva contamination significantly decreased the self-etch system bond to dental tissues, irrespective of the step of the operative procedure in which saliva contamination occurred, and the treatment adopted for the contamination. This result is in agreement with previous studies[3-4,6,9-10,12,17,21-22,25,29,31,33]. Conversely, other authors[5,7-8,11,13,16,18-20,24,26-28,32,34] have reported

that saliva contamination does not affect the bonding performance of adhesives.

From the abovementioned literature, one could think that the influence of saliva contamination on bonding to dental tissues is not an essential topic to explore, but it is fundamental to point out that most studies related to contamination of the operative field, deal with bonding orthodontic brackets and the use of sealants. Thus, information on how to manage saliva contamination during adhesive procedures of contemporary adhesive systems can still make a significant contribution to clinicians understanding of the issues involved. Moreover, none of the aforementioned studies performed a longitudinal evaluation of the effect of saliva on adhesive procedures.

There are few studies addressing the influence of saliva contamination during the application of self-etch systems to enamel and dentin[5-6,21-22,24-25,34] using different methodologies, such as microleakage[6], shear bond strength[5,24], micro-shear[22,25] and micro-tensile[21,34]. One must consider that among the microtensile studies, Eiriksson et al. (2004)[34] studied the influence of saliva contamination on the resin-resin interface, thus, only Sattabanasuk et al. (2006)[21] conducted a study that could be considered similar to this one, using the same methodology, but a different self-etch adhesive system and different treatments.

It is thus suggested that the operative field must be protected against saliva contamination during all steps of the bonding protocol to avoid any deleterious effect to bonding performance of restorative materials. A possible explanation for this fact resides in the ability of saliva to buffer the weak acid of the self-etching primer and impair its perfect interaction with dentin. Almeida et al. (2008)[35] pointed out that the buffer capacity of stimulated saliva is strongly related to the carbonate-bicarbonate buffer system, differently from that of non-stimulated saliva, which is more related to phosphate buffer.

SEM studies have reported hybrid layer formation in the presence of saliva[9,28], while other authors[27,31] have shown that under this condition, the hybrid layer presents gaps and broken resin tags. Although the SEM evaluation of the present study did not include observation of the adhesive interface, our bond strength results support the assumption that saliva decreases adhesion. LV-SEM was employed to support the bond strength data, but unfortunately our findings could not be correlated to all steps of the adhesive application.

In the LV-SEM micrographs of groups in which saliva was present before primer application, whether they were treated with water rinsing (G2 and G9) or only air dried (G3 and G10), the contaminant could not be detected, nor was it detected in the non-contaminated specimens (G1 and G8). However, when comparisons were made considering the bond strength results, the bond strength was decreased in all the groups in which saliva was present. Pashley et al. (1982)[36] demonstrated a 65% decrease in dentin permeability when saliva is present because of adsorption of salivary glycoproteins by dentinal tubules, which could not be detected in SEM studies[27]. Conversely, other morphological studies[14] showed that one second of contact between saliva

and etched enamel is enough to noticeably modify enamel topography. Furthermore, Taskonak and Sertgoz (2002)[18] reported that etched enamel absorbs salivary components decreasing surface energy and impairing potential adhesion.

In this context, this study is in agreement with those of previous studies[14,18,27,36] and their rationalization for the lower bond strength results. Moreover, it is in agreement with Hiraishi et al. (2003)[25] when they propose that future studies should perform chemical analysis of contaminated surfaces in order to detect the presence of saliva with higher precision, and then correlate bond strength data with SEM adhesive interface images.

When saliva was applied after primer application, for both treatments and substrates, the LV-SEM micrographs showed contaminant deposition on dental surfaces, consisting of a physical barrier to monomer diffusion impairing adhesion. If saliva was present after light polymerization of the bonding agent, with the two treatments - rinsed and dried (G6 and G13) or only dried (G7 and G14), LV-SEM was able to detect the presence of saliva to a higher or lower degree. LV-SEM was effective in detecting the presence of saliva under some specific circumstances - after primer and after bonding agent - thus, we suggested a chemical investigation of these deposits in order to clarify this result.

It is fundamental to point out that in this study the treatments that tried to counteract the influence of saliva were water rinsing followed by drying and primer re-application. Drying alone was performed in order to keep a pellicle of saliva on dental surface to provide a negative control. This could create some difficulties when making comparisons with studies that tested the drying procedure as a possibility of removing the contaminant from the surface by means of a strong air stream[10,34].

Kidd and Beighton (1997)[37] considered saliva contamination a superficial phenomena that can be easily removed from surface by means of a water rinsing procedure capable of recovering lost adhesion[10,16]. Other SEM studies, although using different adhesive systems with different action mechanisms[9] demonstrated that 20 s after rinsing etched enamel, saliva is no longer present on the surface.

Rinsing and drying of saliva contamination was carried out for 20 s. In this study this procedure was unable to recover the bond strength, in agreement with authors of previous studies[3,17,22,33-34]. Suliman et al. (1989)[33] reported that to efficiently remove saliva from dental surfaces, water rinsing should be performed for at least 30 s. Nevertheless, Silverstone et al. (1985)[14] support the findings of the present study, stating that water rinsing is not able to remove saliva from etched enamel even if the contaminant remained in contact with the tissue for only a single second.

Another treatment of contamination suggested in the literature[4,10,12,16] is to re-etch the dental surface with phosphoric acid. Some authors do not indicate this procedure[25,33] considering that in addition to the removal of saliva, further etching could grind up to 14μm of superficial enamel[13] in which fluoride is incorporated. Other possible reason for not indicating re-etching is the creation of a more humid surface that would originate more gaps in a thicker hybrid layer[23].

In this study one of the saliva treatment of contaminations proposed was re-application of the primer solution already tested by other researchers[21,24-25,32]. The findings of the present study showed that this procedure was unable to recover adhesion, whereas other authors[21,24-25] reported that it is efficient in removing saliva. These differences in results could be due to some variables, such as, the use of artificial saliva[25], which does not contain protein and does not form a pellicle on dental tissues[37]; and the combined water rinsing and primer re-application treatment[21,24].

The major differential feature of this study is the longitudinal evaluation, which unfortunately, was unable to detect any influence of the storage period - 24 h or 6 months - on bond strength to both enamel and dentin. Frankenberger et al. (2000)[8] showed that one year storage of samples reduced bond strength in enamel. Perhaps a 6-month storage period was not sufficient to identify differences in bond strength, thus being in agreement with other authors[5-6]. Other longitudinal studies should evaluate the influence of saliva contamination on adhesive interfaces in longer periods of storage.

In conclusion, it could be stated that saliva contamination was deleterious to bond to enamel and dentin, irrespective of the operative step in which contamination occurred. Water rinsing of contaminant and primer re-application were not efficient to recover the bond reduced by the presence of saliva on enamel and dentin, irrespective of the step of the operative procedure in which the contamination occurred. The 6-month storage of specimens did not change the bond strength results when compared to the 24-hour storage. LV-SEM was efficient for detecting the presence of saliva on dental surfaces only when contamination occurred after primer application and after the light polymerization of the bonding agent.

## Acknowledgments

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