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ORIGINAL ARTICLE

Effect of saliva contamination on the bond strength of single-step and three-step adhesive systems

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Abstract

The aim of this study was to evaluate the effect of saliva contamination on bond strength to dentin with an etch-and-rinse and a self-etch adhesive system. For each of these adhesive systems, the dentin surface of 24 human molars were allocated to one of four groups representing different saliva contamination scenarios. Saliva was applied at different stages in the bonding process, and was investigated to be remedied by water rinsing and/or air drying. Uncontaminated tooth surfaces were used as controls. Bonding procedures were performed according to the manufacturer's instructions, and a polymer-based composite was placed. The bond strength was measured by a micro-tensile test. Except for the etch-and-rinse approach having contamination with saliva after etching, followed by air drying, all salivary contamination regimens resulted in a substantial number of specimens not surviving the test, and the bond strength value of these was therefore set to 0 MPa for the purposes of the statistical analysis. Water rinsing after etching and salivary contamination did significantly reduce the bond strength. Contamination after priming showed the lowest bond strength. For the self-etch approach, saliva contamination before the adhesive procedure, followed by air drying, significantly reduced the bond strength, while contamination followed by water rinsing or air drying did not statistically significantly reduce the strength.

KEYWORDS
adhesives, decontamination, dental bonding, operative dentistry, saliva

INTRODUCTION

The options available for restorative dentistry have been essentially changed with the concept of adhesion between the tooth and resin-based restorative. Since the introduction of adhesives for dentin bonding in the 1980s, numerous adhesive products and procedures have been introduced [1,2].

Dental adhesives today can be divided in two main groups according to the adhesion strategy used: etch-and-rinse adhesive systems and self-etch adhesive systems. The etch-and-rinse adhesive system can be further subdivided into three-step adhesive approaches and two-step adhesive approaches. The self-etch adhesive system includes two-step adhesive approaches and single-step adhesive approaches [3]. The etch-and-rinse three-step approach involves three basic steps with successive applications of acid etchant (30-40% phosphoric acid), primer and adhesive, while the self-etch approach involves a self-etching primer and adhesive. Both strategies are represented by numerous products on the market.

It is well established that bonding between a polymer-based restorative material and dentin is based on physico-chemical interaction. Generally, the adhesives are complex mixtures of different promotors, usually substances that have amphiphilic properties [3-5]. The bonding quality depends on the surface properties of the substrate and the chemical, physical, and handling properties of the adhesive. An intimate contact between the substrate and the adhesive is decisive for a strong bond. The dentin substrate represents a challenging surface, being moist and prone to contamination with blood, gingival fluids or saliva, and accidental contamination of the prepared surfaces is a real risk in the clinical setting. There are inconsistent results regarding the effects of contamination on bonding, and the study designs differ [6–8]. According to Elkassas et al. [6], different post-contamination treatments are recommended for different bonding steps. They suggest to re-etch and re-apply the adhesive if the etched substrate or polymerized adhesive have been contaminated. If the contaminated adhesive layer is unpolymerized they suggest rinsing with acetone and then re-apply the adhesive. Re-application of adhesives without rinsing with acetone is recommended by others [9]. There is, though, a lack of scientific evidence regarding the effects on immediate bond strength of saliva contamination on dentin when using different adhesion strategies and cleaning procedures.

The purposes of this study were to evaluate i) the effect of saliva contamination on the bond strength to human dentin, ii) the effect of the adhesive system on the bond strength to human dentin, and iii) the effect on immediate bond strength of contamination from saliva on human dentin when using different adhesion strategies as well as the influence of different cleaning procedures. The null hypotheses were that i) saliva contamination and ii) adhesive strategy would not impact the bond strength, and that iii) different adhesion strategies and cleaning procedures would not impact the immediate bond strength.

MATERIAL AND METHODS

The design of this experimental study involved testing of the effect on the microtensile bond strength of salivary contamination at different stages during the bonding process of a polymer-based composite restorative material (Tetric EvoCeram; Ivoclar Vivadent) to the occlusal dentin of molar teeth using either a total-etch approach or a self-etch approach, respectively (Figure 1).

The teeth used in the study were 48 freshly extracted sound human third molars, which were approved as a biobank (Ref. 05/3191 TSP/PRE). The molars were stored in 0.9% saline, refrigerated, and used within six months from extraction. Before use, the teeth were cleaned with a curette to remove remnants of gingiva and jaw bone. The occlusal dentin of the teeth was exposed as a flat occlusal surface by wet grinding to a predefined roughness (silicon carbide paper, 220 grit). These tooth specimens were then randomly divided into eight groups (n = 6 in each group) (Figure 1) to receive the treatments detailed in the following list.

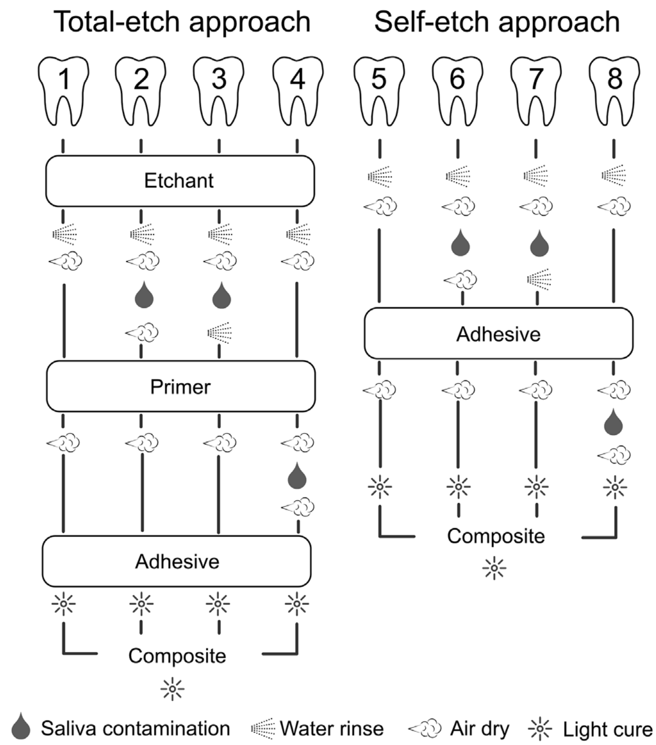

FIGURE 1 Saliva contamination at different stages of the bonding procedure. Group 1 and 5 serve as controls

The total-etch-approach with three-step etch-and-rinse system (Optibond FL; Kerr):

Group 1: etching, primer, adhesive, light-curing, composite, light-curing

Group 2: etching, **saliva, air drying**, primer, adhesive, light-curing, composite, light-curing

Group 3: etching, **saliva, water rinsing, air drying**, primer, adhesive, light-curing, composite, light-curing

Group 4: etching, primer, **saliva, air drying**, adhesive, light-curing, composite, light-curing

The one-step multimode system used in a self-etching mode (3M Scotchbond Universal Bonding; 3M):

Group 5: adhesive, light-curing, composite, light-curing

Group 6: **saliva, air drying**, adhesive, light-curing, composite, light-curing

Group 7: **saliva, water rinsing, air drying**, adhesive, light-curing, composite, light-curing

Group 8: adhesive, light-curing, **saliva, air drying**, composite, light-curing

The saliva used for contamination was fresh whole unstimulated human saliva collected from the first author of this study (JB) every morning at the same time. The saliva was used the same day within 30 min from collection.

TABLE 1 Materials used in the study

Product	Description	LOT no.	Manufacturer
Tetric EvoCeram	Composite, A3	W33365 W33510 V22848	Ivoclar Vivadent
Optibond FL Prime Refill	Primer	6259948	Kerr Italia
Optibond FL Adhesive Refill	Adhesive	6296138	Kerr Italia
Scotchbond Universal Bonding	Adhesive	658720	3M Deutschland
Etch gel	38% phosphoric acid	161219	DAB Dental

To standardize, no food, drinks, or other oral intakes were consumed prior to the saliva collection.

The adhesive materials (Table 1) were applied following the manufacturers' recommendations. Silicone tubes were used as a matrix for the composite resin, which was a polymer-based composite restorative material (Tetric EvoCeram; Ivoclar Vivadent). This was placed according to the manufacturer's recommendations (approximately 6 mm high) and applied with a horizontal layering technique. Light-curing was performed using the Bluephase G2 (Ivoclar Vivadent), set at low power (650 mW/cm^2). After the restorative procedure the teeth were stored in 100% air humidity overnight before being sectioned 24 h later using a low-speed saw (Isomet TM 11–1180; Buehler).

Each tooth was sectioned to produce test specimens (approximately 1.2 × 1.2 mm x 10 mm) for tensile testing, and each tooth provided 3–19 specimens for further testing. Specimens were discarded when enamel was present in the section or air bubbles were seen in the composite. Close relation to the pulp chamber was another reason to discard specimens due to dimensional limitations. In each group, more than 50 specimens could be tested. Each specimen was glued onto an attachment device which fitted the test device [10]. The test method used in this study was a micro tensile test (μTBS) (ISO/TS 11405:2015) [10]. The testing was performed in a biomechanical testing machine (MTS 858 Mini Bionix II with control and acquisition software; MTS Systems). The test device was suspended in a wire to accommodate the force direction. Force was applied at 1 mm/min perpendicular until the failure occurred. The fracture load (N) was recorded and the μTBS (in MPa) was calculated.

Two representative specimens from each group were examined with a scanning electron microscope (Phenom XL; Phenom-World). These specimens were handled as the other specimens and stored in 100% humidity in a refrigerator. The specimens were sputter coated with approximately 50 nm of gold-palladium alloy (Sputtercoater DSR1; VacTechniche). The interface between dentin and the filling material was observed at 10000x.

The following outcome variables were considered: The micro tensile bond strength (μTBS, in MPa) between the restorative material and dentin. Current μTBS guidelines from The Academy of Dental Materials were followed [10]. The failure mode was classified into one of five types following the microscopic evaluation of the fractured surfaces: A: Adhesive failure; fracture in the adhesive layer. C: Cohesive failure of the resin composite or dentin. M: Mixed failure where the fracture site involved more than one substrate. P: Pre-test failure; the fracture occurred before the specimen was tested. G: Failure outside the test region where the specimen was glued into the attachment device.

Statistical analysis

The data were subjected to a mixed-effects maximum likelihood linear regression analysis followed by Scheffe's test to statistically compare the groups. The two parts of the study, the etch-and-rinse and the self-etch approach, were analyzed separately. The statistical analysis was performed using Stata IC software (StataCorp). Specimens which exhibited pre-test failures were set to a bond strength value of 0 MPa in the statistical analysis.

RESULTS

There was a substantial difference in the total number of specimens evaluated in the groups with no or few pretest failures (groups 1, 2 and 5), each with 50–53 specimens, and the other groups, which had 70–86 specimens evaluated (Table 4). Hence, a considerable portion of the contaminated specimens failed during the mounting in the test jig, before the small preload was applied (< 0.2N) (Table 4), and these specimens were subsequently set to a bond strength value of 0 MPa.

The results show that any contamination with saliva weakens the bond strength regardless of the stage of contamination (Figures 2 and 3, Tables 2 and 3). The control groups (no contamination) showed the highest bond strength for both adhesive strategies, but the difference between the two adhesives was not statistically significant ($p = 0.382$).

For the etch-and-rinse approach (Figure 2, Table 2), saliva contamination after etching followed by air drying did not markedly impair the bond strength ($p = 0.640$). On the

TABLE 2 The results of the mixed effect linear regression analysis of micro tensile bond strength in MPa according to the saliva contamination mode in the specimens treated according to the total-etch approach to bonding

Exposure	Regression coefficient, β	95% CI for β
No salivary contamination (reference)	24.47	18.84, 30.09
Salivary contamination before primer, air drying	−5.21	−13.07, 2.66
Salivary contamination before primer, water rinsing and air drying	−14.42	−22.15, −6.69
Salivary contamination after primer, air drying	−21.36	−29.06, −13.66

TABLE 3 The results of the mixed effect linear regression analysis of micro tensile bond strength in MPa according to the saliva contamination mode in the specimens treated according to the self-etch approach to bonding

Exposure	Regression coefficient, β	95% CI for β
No salivary contamination (reference)	18.26	12.76, 23.76
Salivary contamination before adhesive, air drying	−13.63	−21.26, −5.99
Salivary contamination before adhesive, water rinsing and air drying	−7.84	−15.50, −0.17
Salivary contamination after adhesive, air drying	−9.44	−17.07, −1.81

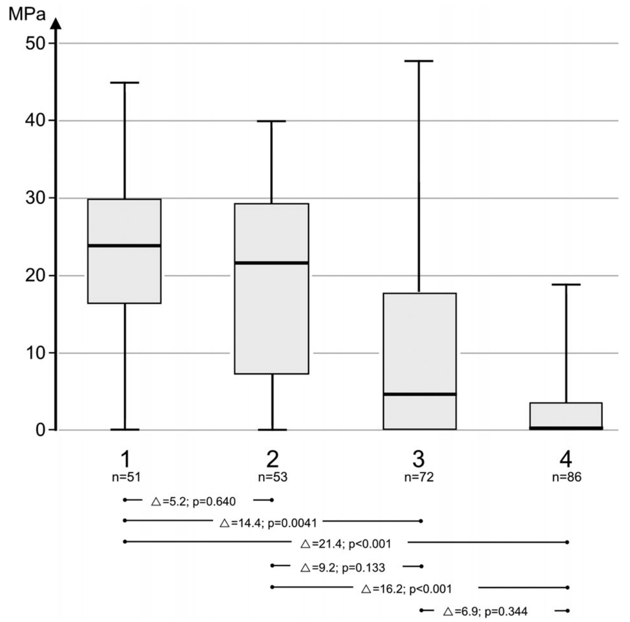

FIGURE 2 Box-plot of micro-tensile stress (MPa) for a total etch approach under different bonding conditions. 1: Control group without saliva contamination; 2: Saliva contamination after etching and then air drying; 3: Saliva contamination after etching and then water rinsing and air drying; 4: Saliva contamination and air drying after primer application. Box: 25 – 75 percentiles with median (horizontal line); whiskers: minimum and maximum values. Delta values show arithmetic means of micro-tensile stress (MPa) between the different groups and *p*-values

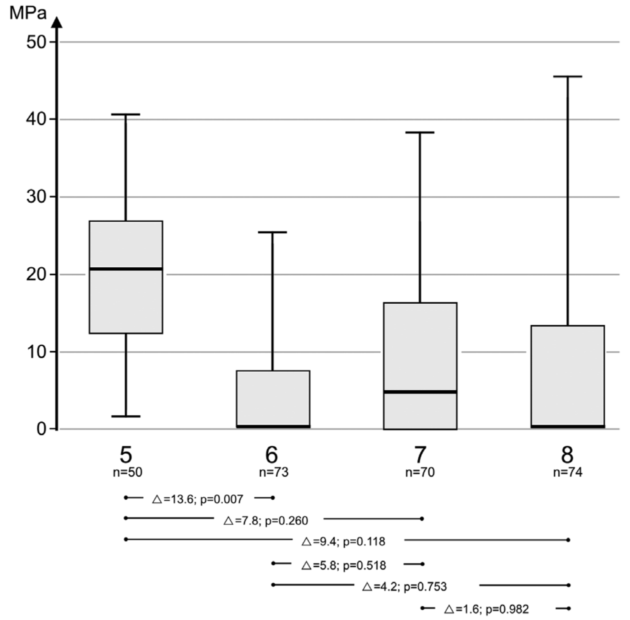

FIGURE 3 Box-plot of micro-tensile stress (MPa) for a self-etch approach under different bonding conditions. 5: Control group without saliva contamination; 6: Saliva contamination before adhesive then air drying; 7: Saliva contamination before adhesive and then water rinsing and air drying; 8: Saliva contamination and air drying after adhesive and light curing. For delta values see legend to Fig. 2

contrary, the bond strength after additional water rinsing is significantly lower than the uncontaminated controls ($p = 0.004$). Contamination after primer application resulted in the lowest bond strength ($p < 0.001$). These findings were confirmed by the SEM-analysis (Figure 4), showing a disintegrated hybrid layer and a gap between the primer and adhesive in group 4. No disintegration of the hybrid layer was observed in the other treatment groups.

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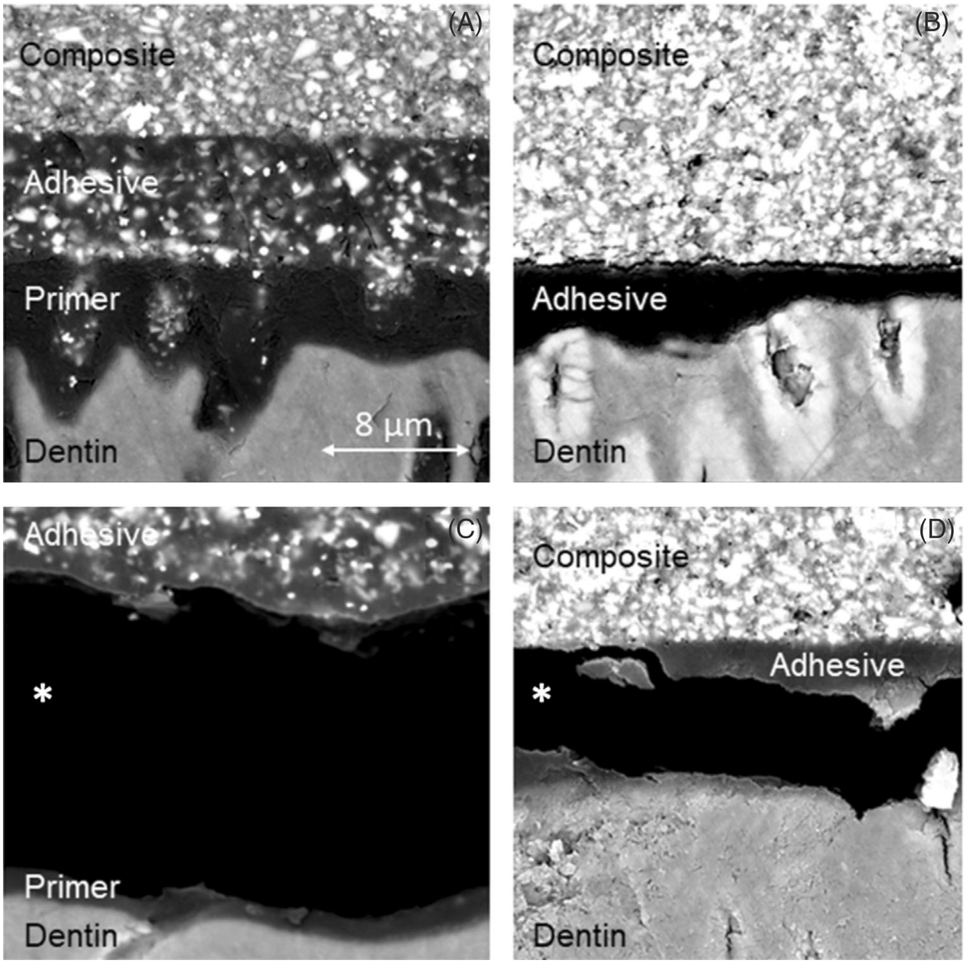

FIGURE 4 Scanning electron micrographs (back-scatter mode) (10,000X) of the dentin-adhesive/composite interface. (A) Control group of the total-etch approach. (B) Control group of the self-etch approach. (C) Saliva contaminated and air dried after primer application (group 4). (D) Saliva contaminated before adhesive application and air dried (group 6). *The degradation of the adhesive layer

Saliva contamination before application of adhesives in the self-etch adhesive group (Figure 3, Table 3), followed by air drying, resulted in a significantly lower bond strength (p = 0.007), which was not remedied by additional water rinsing (p = 0.260). Salivary contamination and air drying after application of the adhesive resulted in a lower bond strength than seen in the uncontaminated group, but the difference was not statistically significant (p = 0.118). SEM-analysis of two randomly selected specimens from each group showed an internal disintegration of the adhesive layer in group 6, corresponding to the lower bond strength in this group. The other groups did not show any disintegration.

A comparison of all the contaminated specimens in the etch-and-rinse groups with all the contaminated specimens in the self-etch groups indicated no statistically significant differences in bond strength (p = 0.349).

The fracture mode distribution of specimens is reported in Table 4. Adhesive failure was the predominant mode for both control groups. A smaller number of specimens displayed mixed failures (M), failures outside the test region (G), and cohesive failures (C).

DISCUSSION

There are several methods for testing adhesive strength, and additionally the results are dependent on factors such as composite materials, adhesive systems, sample size, study design, and methodological standardization [11–13]. The micro tensile test (μTBS) used in this study is reported to have high discriminative power [14]. Although the μTBS testing method is considered a reliable adhesion test, it is time-consuming and technique sensitive [15,16]. This sensitivity may explain the rather high standard deviation in all groups. This is supported by the observation that pre-test failures were rather numerous in the contaminated groups, where many specimens did not survive the sectioning of the teeth. This is probably a result of work being done by freehand, whereby the hand pressure can traumatize the already weak adhesive layer. The sectioning of

TABLE 4 Number of specimens (%) according to fracture mode in each group: Adhesive failures: fracture in the adhesive layer. Cohesive failures of the resin composite or dentin. Mixed failure where the fracture site included more than one substrate. Pre-test failures: fracture occurred before the specimen had been tested. Failure outside the test region where the specimen was glued into the attachment device

	Total-etch approach				Self-etch approach			
		Saliva contamination				Saliva contamination		
Type of failure	Control	Air drying	Water rinse+air drying	After primer+air drying	Control	Air drying	Water rinse+air drying	After adhesive+air drying
Adhesive	43	31	31	24	42	32	36	30
	84.3%	58.5%	43.1%	27.9%	84.0%	43.8%	51.4%	40.5%
Cohesive	5	7	3	–	5	–	3	1
	9.8%	13.2%	4.1%		10.0%		4.3%	1.35%
Mixed	–	–	–	–	–	3	–	–
						4.1%		
Pre-test	–	10	34	62		38	30	42
		18.9%	47.2%	72.1%	–	52.1%	42.9%	56.8%
Outside test region	3	5	4		3		1	1
	5.9%	9.4%	5.6%	–	6.0%	–	1.4%	1.35%
N	51	53	72	86	50	73	70	74

- Not observed.

the tooth and the vibration of the cutting blade could also have an impact, as well as the presence of air bubbles and voids in or near the adhesive layer [17]. This could weaken the bond strength and lead to pre-test failure and lower bond strength values. The many pre-test failures and their distribution across the groups necessitated their representation as 0 MPa values in the statistical analyses, to obtain accurate results.

The present study showed that bond strength to dentin was negatively affected by saliva contamination, regardless of rinsing method. The first null hypothesis was rejected because all the groups that were contaminated with saliva showed lower bond strength values than the corresponding control group. All differences were statistically significant, except in group 2, where saliva contamination before the primer in the total-etch approach was followed by air drying only.

There was no statistically significant difference in the bond strength values between the two adhesive strategies when bonded to uncontaminated dentin, and the second null hypothesis could therefore not be rejected.

The present study provides information on the effect of clinically common procedures in case of accidental saliva inflow onto the prepared surface and confirms that saliva contamination is detrimental to the immediate bonding performance. The third null hypothesis is therefore rejected. This is in concordance with other studies [18–20], although these have exclusively investigated self-etching and universal adhesives. Saliva consists of more than 99% water in addition to enzymes, glycoproteins, immunoglobulins, mucins, nitrogenous products, and electrolytes. According to Pinzon et al. [21], mucins do not affect the bond strength significantly. Others have suggested that dentin contaminated with saliva causes a reduction in adhesive strength due to the fact that glycoproteins create a barrier between the adhesive monomers and the collagen network of dentin [7,22,23]. Although not evaluated in this study, it is reported that the presence of saliva and its constituents affect the dentin-adhesive interface over time [24]. Enzymes like esterase from saliva have the ability to break down monomers by catalyzing the hydrolysis of ester bonds, and thereby degrade the dentin-adhesive interface [25,26].

Our study showed no significant difference between the etch-and-rinse control group and the group that was contaminated after etching and then air dried. Interestingly, water rinsing seemed to reduce the bond strength. One suggested explanation for this phenomenon is that moisture at the surface may dilute the primer that is subsequently applied and thereby impair the function [27]. When contamination occurred after primer application the hydrophilic nature of the primer retains water from the saliva with a lower degree of conversion as a result [6,28] (Figure 4). The results of the present study indicate that the priming step is the most critical step in the etch-and-rinse approach when it comes to saliva contamination.

The self-etch approach behaved differently than the etch-and-rinse approach, being most prone to deleterious effects of saliva contamination prior to adhesive application when only air drying was performed. It is noteworthy that water rinsing seemed to increase the μTBS, although not statistically significantly. When using the universal adhesive in a self-etch mode, the smear layer is still present and incorporated into the hybrid layer. The resin is brought to the same depth as the etching primer and when saliva and water, after rinsing, are present at the surface, the acidity of the adhesive will be lowered with reduced infiltration as a result [9]. Jacobsen et al. [29] has described how water will compete with the infiltrating resin space in the collagen mesh. Some studies have shown that re-application of the all-in-one adhesives after saliva contamination may improve the etching with further demineralization of the dentin [9,30]. The reduced sensitivity of water rinsing may be due to different chemistry [19], with the self-etch adhesive containing ingredients more tolerant to surface moisture. From a clinical view, when the etching and bonding are simultaneous it is less likely that saliva contamination takes place.

Adhesive failures predominated in the control groups. This shows that in the absence of saliva contamination, the weakest point with respect to bond strength is in the adhesive layer. A much more variable type of fracture mode was observed in the other groups, showing irregularity after saliva contamination. The pre-test failures that dominated the rest of the groups (except group 2), all occurred with fractures in the adhesive layer. This observation makes us conclude that the adhesive layer is the weakest link.

This study has not evaluated the effect of saliva contamination on the degree of conversion at the composite-adhesive interface, but the results of a recent study [31] indicate that saliva contamination does not influence the degree of conversion of a resin composite to an adhesive system.

In this study, saliva contamination significantly decreased the micro-tensile strength of the adhesive to dentin, regardless of adhesive strategy. However, the two different adhesive systems were sensitive to the non-mechanical decontamination procedures. These findings suggest that the stage at which saliva contamination takes place may be more critical than the actual presence of saliva, and that the two adhesive systems behave differently in this context. These aspects should be addressed in the directions for use.

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CONFLICTS OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

Conceptualization, Methodology, Validation, Formal analysis, Resources, Data curation, Writing – original draft preparation, Visualization: June Bolme, Nils R. Gjerdet, Torgils Lægreid; Investigation: June Bolme; Supervision, Project administration: Nils R. Gjerdet, Torgils Lægreid.

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