

An *in vitro* study of the effect of blood and saliva contamination on the bond strength of self-etch and total-etch adhesive systems to dentine

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Abstract

Objectives: Evaluate and compare the effect of saliva and blood contamination on dentine during the bonding procedure on shear bond strength of self etch and total etch adhesives, and to Compare the difference between the two contaminants in bond strengths.

Methods: 38 freshly extracted human mandibular and maxillary molar teeth, intact and caries free. Occlusal surface of the teeth was reduced at 2 mm deep leaving a flat dentinal surface. The teeth's were mounted on plastic jigs, filled with self-cured acrylic. The samples were randomly divided into 2 main groups (n=19) Group1 (self-etch) and Group2 (total-etch). Each groups were further subdivided based on contamination (n=14) and non-contamination (control) (n=5). Each contaminated group was subdivided into contaminated with blood (n=7) and saliva (n=7).

Results: Self-etch group, Non Contaminated group (control) showed the highest mean (\pm SD) compressive strength (24.4326 ± 1.62734 MPa). The group contaminated with blood showed the least bond strength (6.2573 MPa), followed by contamination with saliva (18.6 MPa). In the total-etch group Non contaminated (control) b2 group showed the highest mean (\pm SD) compressive strength (23.7247 ± 0.81738 MPa). The group contaminated with blood showed the least (3.8380 MPa), followed by the group contaminated with saliva (19.6805 Mpa).

Significance: In the self etch and total etch group, blood contamination showed the least bond strength followed by saliva contamination which had a slightly better adhesion when compared to blood contamination.

Introduction

The increase in demand & use of esthetic restorations by patients and clinical practitioners in the present era has led to intensive research of adhesive materials. Contamination is one of the most common problems encountered during bonding restorative procedures, which can lead to mainly reduction in the bond strength of the composite especially when rubber dam isolation is not durable. Many carious lesions, which require the use of dentin bonding agents, are located on areas that are difficult to isolate especially when the site is near or at the gingival margin and, therefore, dentin contamination with saliva and blood is more likely to occur. In this context, the contamination of the operatory field is a recurrent reality that emphasizes the value of studies in this area. Contemporary adhesive systems can be classified into two groups: total-etching and self-etching systems.

Self-etching adhesive systems don't 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. Self etch adhesive systems supply increased user reliability with faster application as well as a decreased number of application steps and components. Although this reduces the risk of saliva contamination, it may sometimes be impossible to maintain a dry operative field after the self etch adhesive application. Total-etch systems combine the functions of the dentine primer and bonding agent

in one-bottle. Blood contamination of the adhesive surfaces may affect the bond strengths of adhesive resins. Recent studies have evaluated the effect of blood contamination of adhesives to dentin using different testing methods, showing a significant reduction in bond strength values. However, the effect of blood and saliva contamination on the adhesive properties of these newer adhesive systems has not been fully understood. Shear and Micro-tensile tests are the most common methods used for determining the bond strengths of adhesive systems, revealing valuable clinical information.

Therefore the purpose of this *in vitro* study is to evaluate and compare the effect of saliva and blood contamination on different steps of restorative procedures on shear bond strength of self-etch and total etch adhesives to dentin, and to compare the difference in bond strength between the two contaminants.

The null hypothesis tested was that blood and saliva contamination would not affect the shear bond strength of these newer adhesives to dentin.

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Key words: self-etch adhesive, total-etch adhesive, bond strength, saliva, blood, contamination

Received: April 28, 2017; **Accepted:** May 18, 2017; **Published:** May 20, 2017

Materials and methods

38 freshly extracted human mandibular and maxillary molar teeth, intact and caries free, were used in the study. The teeth's were stored in distilled water. Occlusal surface of the teeth was reduced at 2 mm deep leaving a flat dentinal surface and polished with 600-grit abrasive for 30 seconds. The teeth's were mounted on plastic jigs, filled with self-cured acrylic. The samples were randomly divided into 2 main groups (n=19) based on the adhesive used.



Group 1 was self etch using adhesive prime & bond one select self-etch and etch& rinse adhesive and **Group 2** was total etch using adhesive prime & bond one Etch & rinse Universal Etch & Rinse Adhesive. Each groups were further subdivided based on contamination and non-contamination (control) (n=5). Each contaminated group was again subdivided into those contaminated with blood and saliva (n=7).

In **Group 2**, total etch, phosphoric acid 37% was used for 15 seconds then rinsed for 10 seconds with water stream, followed by a gentle blast of air for 10 seconds to dry the surface before bonding procedure.

Contamination Group 1A (1Aa blue n=7): The dentin surfaces of the specimens were contaminated with blood (**Group 1Aa**) for 20seconds using a micro brush. After the contamination, Surface rinsed for 10 seconds with water stream, followed by a gentle blast of air for 10 seconds to dry the surface.

Contamination Group 1A (1Ab green-red n=7): The dentin surfaces of the specimens were contaminated with saliva (**Group 1Ab**) for 20seconds using a micro brush. After the contamination, Surface rinsed for 10 seconds with water stream, followed by a gentle blast of air for 10 seconds to dry the surface.

Non-contaminated (control) Group1B (1B Black n=5): In control group, the specimens were not contaminated with either saliva or blood.

Contamination Group 2A (2Aa red n=7): the dentin surfaces of the specimens were contaminated with blood (**Group 2Aa**) for 20seconds using a micro brush. After the contamination, Surface rinsed for 10 seconds with water stream, followed by a gentle blast of air for 10 seconds to dry the surface.

Contamination Group 2A (2Ab 2green n=7): the dentin surfaces of the specimens were contaminated with saliva (**Group 2Ab**) for 20 seconds using a micro brush. After the contamination, Surface rinsed for 10 seconds with water stream, followed by a gentle blast of air for 10 seconds to dry the surface.

Non-contaminated (control) Group 2B (2B Black-red n=5): In control group, the specimens were not contaminated with either saliva or blood

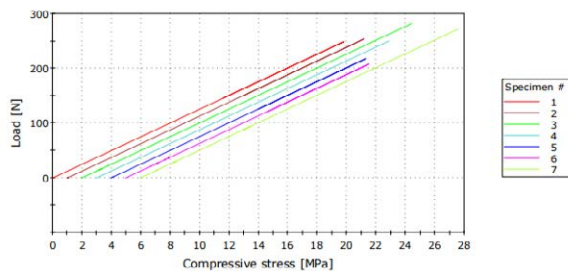
After all, Self-etch and total-etch adhesives were applied to dentin according to the manufacturers' instructions and light cured with a visible light curing unit for 20 sec, 4 mm diameter and 2 mm height vinyl polysiloxane tube placed on top of dentin surface and composite packed into it and light cured for 40 seconds. After complete

polymerization, the tube removed and specimens kept in distilled water, in incubator for 37°C then they were subjected for shear bond strength analysis using Instron machine result statically analyzed using scheffe's post-hoc analysis test.

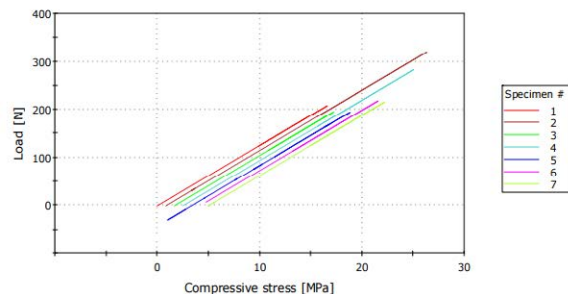
Results

The following are the results of the samples subjected to shear bond strength analysis.

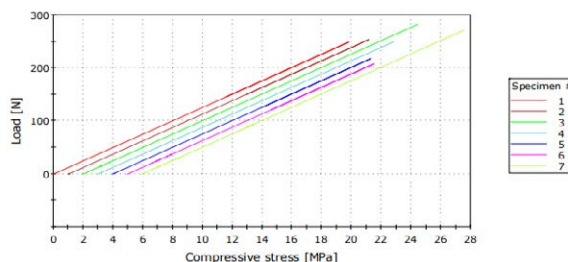
Group 2Ab (Contaminated with Slavia).



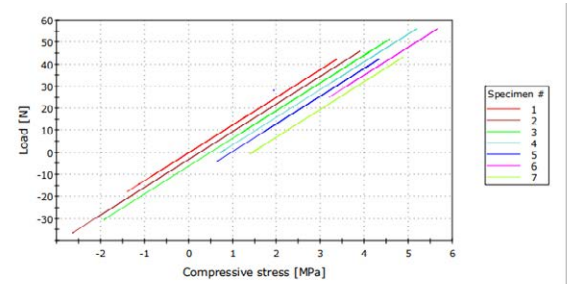
Group 1Ab (Contaminated with slavia).



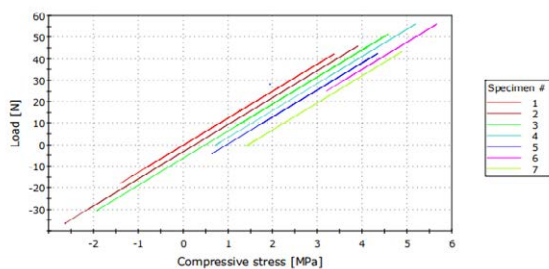
Group 1Aa (Contaminatedwith blood).



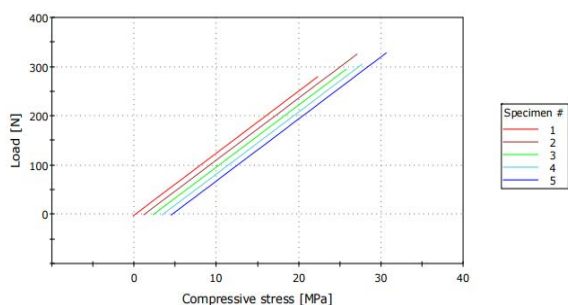
Group 1 Aa (contaminated with Blood)



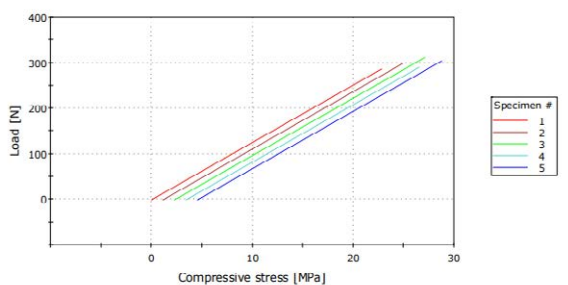
Group 2 Aa (contaminated with blood)



Group 1 B (non-contaminated)



Group 2 B (non-contaminated)



The data obtained in the present study was subjected to statistical analysis using one way ANOVA and scheffe's post hoc analysis test. In the self-etch group, Non Contaminated group (control) showed the highest mean (\pm SD) compressive strength (24.4326 \pm 1.62734 MPa). The group contaminated with blood showed the least bond strength (6.2573 MPa), followed by contamination with saliva (18.6 MPa) as mentioned in Figure 1.

In the total-etch group Non contaminated (control) b2 group showed the highest mean (\pm SD) compressive strength (23.7247 \pm 0.81738 MPa). The group contaminated with blood showed the least (3.8380 MPa), followed by the group contaminated with saliva (19.6805 MPa) as mentioned in Figure 2.

Scheffe's post-hoc test for multiple comparison showed a statistically significant difference between the groups in Table 2 ($p \leq 0.05$).

Table 1.

- **In self etch group**, statistically significant difference in the mean compressive strength was found between groups that contaminated with saliva 1Ab and blood1Aa ($p=0.000$); with saliva 1Ab and non-contaminated (control) group 1B ($p=0.002$); and group that contaminated with blood 1Aa and non-contaminated (control) 1B ($p=0.000$).

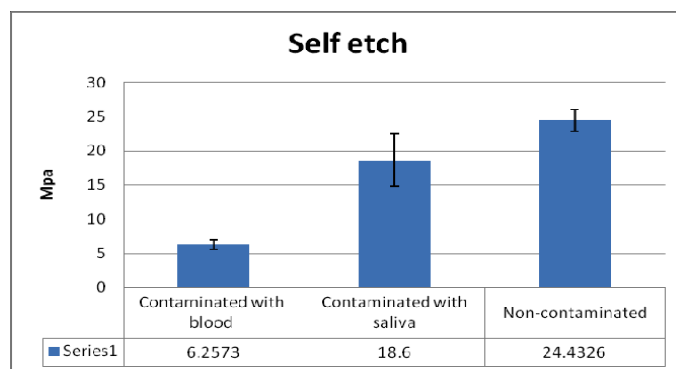


Figure 1. Mean (\pm SD) compressive strength for self-etch group.

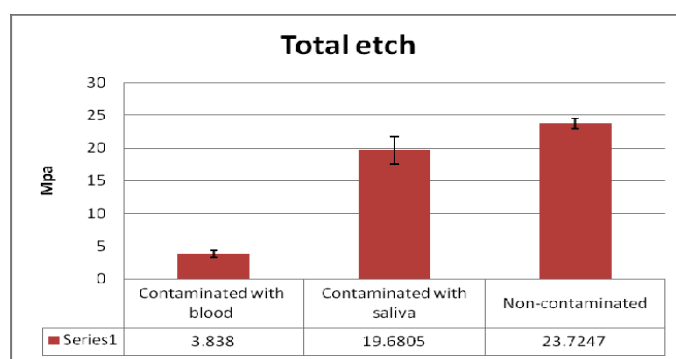


Figure 2. Mean (\pm SD) compressive strength for total etch group.

- **In the total etch group** statistically significant difference was found between groups that contaminated with saliva 2Ab and Blood 2Aa ($p=0.000$); and group that contaminated with blood 2Aa and non-contaminated/control B2 ($p=0.000$). Difference between group that contaminated with saliva 2Ab and non-contaminated/control B2 was not found to be statistically significant ($p=0.069$).

- **In comparison between the self etch and total etch group**, there was a mean difference between self etch saliva & total etch saliva (MD=2.4193), but not statically significant ($P=0.442$), the mean difference between self-etch blood & total etch blood group was 1.0805 but, again not statically significant ($p=.962$). In comparison between the control groups (non- contaminated self-etch and non-contaminated total etch) the main difference was .7079, and not statistically significant ($p=.997$), as mentioned in figure 3.

Discussion

Blood and saliva contamination is one of the major clinical problem during any restorative dental treatment [1] according to the basic principle of adhesion , closer the contact between the adhesive and the adherent, stronger is their junction [2].

Dentin surface contamination can occur at two critical situations during the bonding procedure, after the tooth surface has been etched, and / or after the adhesive system had been applied. Due to this, the bonding can be compromised at both these situations. In this study, the negative effect of blood and saliva contaminations on the bond strength to dentin and resin composite were significant in all contaminated groups. In this study, the influence of saliva and blood contamination of total etch adhesive system and self-etch adhesive system using a microshear bond strength test was conducted.

Table 1. Multiple Comparisons
Compressive strength-Scheffe

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group 2 CS Green	Group 1 CS Green-Red	1.0805	1.09050	.962	-2.7844	4.9454
	Group 1 B Blue	13.4231*	1.09050	.000	9.5582	17.2881
	Group 2 B Red	15.8424*	1.09050	.000	11.9775	19.7074
	Group 1 NC Black	-4.7521*	1.19458	.020	-8.9859	-.5183
	Group 2 NC Black-Red	-4.0442	1.19458	.069	-8.2780	.1896
Group 1 CS Green-Red	Group 2 CS Green	-1.0805	1.09050	.962	-4.9454	2.7844
	Group 1 B Blue	12.3426*	1.09050	.000	8.4777	16.2076
	Group 2 B Red	14.7619*	1.09050	.000	10.8970	18.6269
	Group 1 NC Black	-5.8326*	1.19458	.002	-10.0664	-1.5988
	Group 2 NC Black-Red	-5.1247*	1.19458	.010	-9.3586	-.8909
Group 1 B Blue	Group 2 CS Green	-13.4231*	1.09050	.000	-17.2881	-9.5582
	Group 1 CS Green-Red	-12.3426*	1.09050	.000	-16.2076	-8.4777
	Group 2 B Red	2.4193	1.09050	.442	-1.4456	6.2842
	Group 1 NC Black	-18.1752*	1.19458	.000	-22.4091	-13.9414
	Group 2 NC Black-Red	-17.4674*	1.19458	.000	-21.7012	-13.2335
Group 2 B Red	Group 2 CS Green	-15.8424*	1.09050	.000	-19.7074	-11.9775
	Group 1 CS Green-Red	-14.7619*	1.09050	.000	-18.6269	-10.8970
	Group 1 B Blue	-2.4193	1.09050	.442	-6.2842	1.4456
	Group 1 NC Black	-20.5945*	1.19458	.000	-24.8284	-16.3607
	Group 2 NC Black-Red	-19.8867*	1.19458	.000	-24.1205	-15.6528
Group 1 NC Black	Group 2 NC Black-Red	.7079	1.29030	.997	-3.8652	5.2809

*The mean difference is significant at the .05 level.

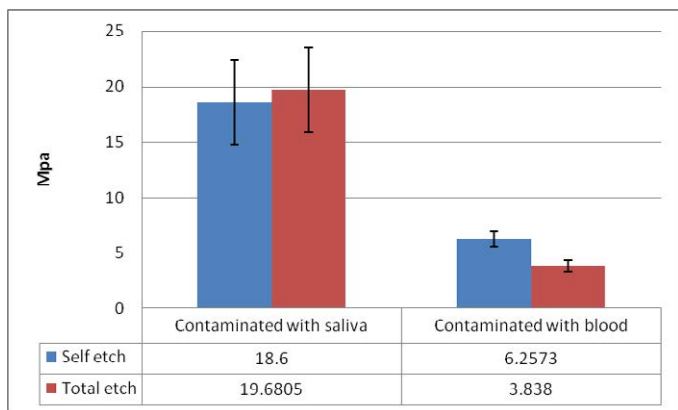


Figure 3. Comparison between self-etch and total etch.

According to our study, blood contamination has the highest effect on total etch adhesive systems. This might be due to the left over of blood contaminates in the dentin surface and also cost collagen fibers to collapse, preventing adhesive monomers to infiltrate. The blood protein components trapped on the dentin surface interferes with the bonding agent ability to form a uniform surface coating [1]. Resin tags formed on air-dried dentin surfaces were found to be fewer and much shorter than that in control conditions and blood contamination prevents adhesive infiltration into dentin tubules [3]. Our study is similar to the study done by Kaneshima *et al.* and Abdalla *et al.* [1,4], which showed decreased in shear bond strength when blood contamination occurred before the adhesive system application. Blood residues or reactants obstructing the dentin tubules can inhibit primer infiltration into dentin, thus interfering with hybrid layer formation [1,4].

Saliva contamination has the highest effect on self-etch adhesive system. This might be due to the adsorption of glycoproteins to the poorly polymerized adhesive surface, thus preventing adequate polymerization rinsing of the contaminants after adhesive application

could have disrupted the oxygen inhibited and un-polymerized layer, there by compromising the co-polymerization with subsequent resin layer [5-7] this is similar to this study done by Yoo *et al.* [8] which showed saliva contamination severely affected the bond strength to the self etch adhesive system.

The total-etch technique is still considered the gold standard for bond strength to enamel and dentin, and so, in our study self-etch adhesive systems have been compared to these to assess the relative bond strength with each.

Total-etch systems use a phosphoric acid step to dematerialize tooth surfaces. The acid present helps to create porosities, into which the adhesive can penetrate. On dentin, the acid etch removes the smear layer and exposes collagen fibers, allowing the adhesive to penetrate and interlock with the fibers. However, when using total-etch systems, there is a risk of over-drying which causes the collagen fibers to collapse. And if the primer and adhesive are applied to these collapsed fibers, instead of infiltrating into upright collagen fibers, the result can be poor adhesion [9].

Self-etching systems are generally less technique sensitive compared to systems that utilize separate acid conditioning and rinsing steps. Collapse of the air-dried, demineralized collagen is prevented as the smear layer-retained dentin is simultaneously demineralized and polymerized in situ. The material is designed to surround the collagen fibers in the dentin, enabling it to create a hybrid layer on wet or dry surfaces. One-bottle self-etch systems typically combine hydrophilic and hydrophobic agents in the same bottle; with pH levels ranging from 1.0 to 2.7 [10]. The lower acidity reduces the risk of over-etching and post-operative sensitivity.

The results from the present study clearly indicated that, when the dentin surface was contaminated with either blood or saliva, it adversely affected the bond strength.

It is most likely that the contaminants may have remained on the

dentin surface, thus interfering with the bonding of adhesive system to resin cement. Therefore, the most important factor for an optimal bonding is to avoid any type of contamination. However, further studies varying the conditions, specially the laboratory technique, using a bigger sample size and the adhesive systems used, are necessary for definitive conclusions [11-31].

Conclusion

Within the limitations of this *in vitro* study it can be conclude that:

1. In the self etch and total etch group, blood contamination showed the least bond strength followed by saliva contamination, which had a slightly better adhesion when compared to blood contamination.
2. In comparison between self etch and total etch groups, saliva contamination on self etch group caused a lower bond strength to dentin than total etch group; but with blood contamination, total etch group caused a lower bond strength to dentin than self etch adhesives.
3. The null hypothesis that saliva and blood contamination would not affect the dentine microshear bond strength of self-etch & total-etch adhesives systems was rejected except on total etch saliva contamination groups

Acknowledgments

We would like to thank King Saud University the Research center for the permission to use the universal testing (INSTRON machine) and also to Dr. Ashwin for helping with the statistical part.

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