Effect of saliva and blood contamination after etching upon the shear bond strength between composite resin and enamel

A S Armadi, M Usman* and E Suprastiwi
Department of Conservative Dentistry, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia
*E-mail: munyati_usman@yahoo.com

Abstract. The aim of this study was to find out the surface roughness of composite resin veneer after brushing. In this study, 24 specimens of composite resin veneer are divided into three subgroups: brushed without toothpaste, brushed with non-herbal toothpaste, and brushed with herbal toothpaste. Brushing was performed for one set of 5,000 strokes and continued for a second set of 5,000 strokes. Roughness of composite resin veneer was determined using a Surface Roughness Tester. The results were statistically analyzed using Kruskal-Wallis nonparametric test and Post Hoc Mann-Whitney. The results indicate that the highest difference among the Ra values occurred within the subgroup that was brushed with the herbal toothpaste. In conclusion, the herbal toothpaste produced a rougher surface on composite resin veneer compared to non-herbal toothpaste.

1. Introduction
Composite resin is one of several tooth-colored direct restoration materials. It was developed in 1962 by combining dimethacrylate (epoxy resin and methacrylate acid) with silanized quartz powder. Composite resin has become increasingly popular in material restoration because it has esthetic and adhesive properties that are superior to amalgam [1,2]. A new generation of composite resin is a nanohybrid composite. Nanohybrid composite resin has great mechanical properties as a result of combining different particle sizes in its filler (15–20 μm and 0.01–0.05 μm). Because of this property, composite resin can be used to restore a high occlusal-wear tooth [3].

One of composite resin’s properties is a good shear bond strength between the adhesive materials of the composite (bonding agent) with the tooth tissue [4]. This bonding is caused by micromechanical interlocking. However, in clinical practice it was found that composite resin can be broken. Because of this finding, this study aimed to find whether an improved bonding between composite resin and tooth tissue can be developed [2,5]. There are several factors which influence adhesion and retention of composite resin. These include gingival fluid, blood, lubricating oil from the handpiece, and an extension of caries itself [6]. One of the hardest factors to avoid under local conditions is the presence of saliva. Saliva is an exocrine fluid that consists of 99% water, plus electrolytes, immunoglobulins, polypeptides, and oligopeptides that are important for oral hygiene [7]. A study conducted by Yoo et al. in 2009 found an effect of saliva contamination on the shear bond strength of composite resin [6]. Glycoprotein of saliva decreases the efficacy of interaction between the composite resin and the tooth [6]. Saliva has a low surface energy which weakens the adhesive materials [8].

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Blood is another contaminant which could decrease the shear bond strength of composite resin. Blood has a high protein and macromolecule fibrinogen which forms a layer on the tooth surface and inhibits composite resin infiltration into the tooth structure and decreases shear bond strength between resin and tooth tissue by up to 70% [9]. If there is no contamination, a good bond may be formed between composite resin and tooth tissue. This bonding forms after the etching procedure is completed. Etching is the first step in composite resin restoration. Etching aims to get rid of the smear layer, after which is formed a rough surface that is microporous. Microporosity allows a resin tag to form between the bonding agent and the surface of the etched tooth. Contamination after the etching procedure weakens the resin tag because bonding is inhibited by the contaminant agent [10]. Therefore, etching becomes one of the most important steps before restoring a tooth, and should avoid any contaminating agent. If contamination occurs, there are several ways to overcome the problem, such as redoing the etching or washing away the contaminant with water [8]. De Calvalho et al. (2010) found that washing the tooth with water was effective in removing contamination but couldn’t restore the shear bond strength. A shear bond strength without any contamination is ±21 MPa [11,12].

Bonding between composite resin on the enamel is different from bonding composite resin on the dentin. This difference is caused by the different humidity between enamel and dentin. Enamel is the outer surface of the tooth that consist of anorganic material such as hydroxyapatite crystal (95%–98% of total weight) with the remainder being water and organic material. In contrast, dentin has a greater amount of organic material and water (12% of total weight) [13]. Due to this greater amount of anorganic material in enamel, a resin tag formed in enamel is greater than the resin tag formed in dentin [4]. With these factors in mind, this study aims to analyze the effect of saliva and blood contamination on shear bond strength of composite resin on the enamel.

2. Materials and methods

2.1. Specimen preparation
Twenty-five premolar teeth which met the inclusion criteria (upper or lower right or left premolar and indicated for extraction due to orthodontic treatment), were stored in 0.9% NaCl solution (saline). The crowns were separated from the root using a Carborundum disc. The crowns were dried and planted by decorative resin into a PVC pipe with diameter of 2 mm and height of 1 cm, with the buccal aspect facing the upside. The surface of the enamel was ground to 0.5 mm using a LaboPol 21 rotary grinder with no. 1000 sandpaper under flowing water. The exposed enamel was marked using a permanent fine tip marker with the same diameter as the mould.

2.2. Grouping specimen procedure
Of the 25 specimens of enamel, each was cleaned by prophylaxis paste (pumice powder) for 20 seconds, then washed by water for 10 seconds and dried by air spray for 5 seconds. The etching procedure was then done on the working area with 37% phosphoric acid (Magic Acid, Coltene) for 15 seconds, then washed by water for 10 seconds and dried by air spray for 5 seconds. Then the specimens were divided into 3 groups: 1) the control group; 2) specimens that were contaminated by saliva; and 3) specimens contaminated by blood. This study used chicken blood as contaminant. Each of the two contaminant groups were further divided into two subgroups in which the specimens were treated with phosphoric acid. Thus there were 5 distinct groups that each contained 5 specimens. First group (control).

After the etching procedure, specimens underwent a bonding application using a bonding agent (Magic Bond DE, Coltene) on the working area for 10 seconds, then were dried by air spray for 5 seconds before being cured by a light cure unit for 20 seconds. Then nanohybrid composite resin (Fill Magic NT Premium, Coltene) was filled into a 2 mm-high plastic mould and lighted by a light cure unit for 20 seconds. Then the moulds were separated from the composite resin by a cutter.

1. First group (contaminated by saliva after etching procedure)
   a. Group 2A
After the etching procedure was done, saliva was applied to the enamel surface of each specimen using a microbrush for 15 seconds. Then the specimens were washed by water for 10 seconds and dried by air spray for 5 seconds. Bonding agent was applied (Magic Bond DE, Coltene) on the working area for 10 seconds and lighted by a curing light for 20 seconds. Then nanohybrid composite resin (Fill Magic NT Premium, Coltene) was filled into a plastic mould of 2 mm height and lighted by a light cure unit for 20 seconds. Then the mould was separated from the composite resin by a cutter.

b. Group 2B
After the etching procedure were done, saliva was applied to the enamel surface of each specimen using a microbrush for 15 seconds. Then the specimens were washed by water for 10 seconds and dried by air spray for 5 seconds. The etching procedure was redone by 37% phosphoric acid (Magic Acid, Coltene) for 15 seconds, then the specimens were washed by water for 10 seconds and dried by air spray for 5 seconds. Bonding agent was applied (Magic Bond DE, Coltene) on the working area for 10 seconds and lighted by a curing light for 20 seconds. Then nanohybrid composite resin (Fill Magic NT Premium, Coltene) was filled into a plastic mould of 2 mm height and lighted by a light cure unit for 20 seconds. Then the mould was separated from the composite resin by a cutter.

2. Second group (contaminated by blood after etching)
   a. Group 3A
   The procedure was identical to that done in group 2A. The only difference was that the contaminating agent was blood.
   
   b. Group 3B
   The procedure was identical to that done in group 2B. The only difference was that the contaminating agent was blood.

All the specimens were then stored in a pot filled with aquades and incubated at 37 °C for 24 hours. On the following day, the shear bond strength was measured using a Shimadzu Autograph 5000 universal testing machine. Data were statistically analyzed using SPSS 17 with One-way ANOVA test followed by a post-hoc Tukey HSD test.

3. Results and Discussion
   3.1 Results
The results of the shear bond strength test on the 5 groups are shown in table 1. The values of average shear bond strength in the control group (without contamination) were the highest. Among the contaminant groups, the average shear bond strength was greatest in group 2A (contaminated by saliva then washed in water and dried). The lowest average shear bond strength was found in group 3B (contaminated by blood, then receiving a second application of etching solution). Standard deviation of shear bond strength in this study were the values of distribution values in each group. The greatest distribution values were found in the blood-contaminated group that underwent reapplication of etching solution: 6.59±1.18. The lowest distribution values were found in the saliva-contaminated group that underwent reapplication of etching solution; this value was 10.53±0.36. These results showed that saliva and blood contamination could affect the shear bond strength between composite resin and enamel, which were found to be lowest in the blood-contaminated group.

Hypothesis tests were done by parametric test using One-Way ANOVA. One of the criteria that should be met for using this test are data with normal distribution and data with homogeneity of variance. To find the distribution of data in specimens ≤50, each group was tested with the Shapiro-Wilk test. The result of the normality test was p=0.505 (significant value of p>0.05), meaning the distribution of data were normal. Homogeneity test of data variance was p=0.649 (significant value of p>0.05), meaning that the data were homogenous. These results showed that One-Way ANOVA could be done in this...
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study. One-way ANOVA test showed $p=0.0001$ (significant value <0.05), indicating there were no statistically different values of shear bond strength between the groups. Then a post hoc test was done (significant value <0.05) to learn which groups had significant differences.

### Table 1. Average Values and Standard Deviations of Shear Bond Strength (MPa)

| Group | Results Average | SD |
|-------|-----------------|----|
| 1     | 16.10           | 0.99 |
| 2A    | 12.05           | 1.16 |
| 2B    | 10.53           | 0.36 |
| 3A    | 8.26            | 0.98 |
| 3B    | 6.59            | 1.18 |

1 = Control  
2A = Saliva-contaminated, washed by water and dried  
2B = Saliva-contaminated, washed by water, then etching solution reapplied  
3A = Blood-contaminated, washed by water and dried  
3B = Blood-contaminated, washed by water, then etching solution reapplied

### Table 2. Statistically significant values between groups with post hoc test

| Group | 1  | 2A | 2B | 3A | 3B |
|-------|----|----|----|----|----|
| 1     | -  | 0.000* | 0.000* | 0.000* | 0.000* |
| 2A    | 0.000* | -  | 0.143 | 0.000* | 0.000* |
| 2B    | 0.000* | 0.143 | -  | 0.012* | 0.000* |
| 3A    | 0.000* | 0.000* | 0.012* | -  | 0.092 |
| 3B    | 0.000* | 0.000* | 0.000* | 0.092 | -  |

*groups with statistically different values

From the results of the post hoc test shown in Table 2, statistically different values appeared in group 1 (control), group 2 (saliva-contaminated), and group 3 (blood-contaminated), with values of $p=0.001$. There were no groups showing statistically different values: groups 2A and 2B had a $p$ value of 0.143, while between groups 3A and 3B there was a $p$ value of 0.092.

### 3.2 Discussion

The method of this study was the same as in the study conducted by Koppolu which implicated the oral cavity condition [14]. After incubation for 24 hours, shear bond strength was tested by using the Autograph AG 5000 universal testing machine. The average value of shear bond strength in the control group was 16.10 MPa, which was far from the normal value of ±21 MPa [12]. It was caused by a grinding procedure that used sandpaper no. 1000 on the enamel surface, thus forming a fine/smooth surface that weakened the retention of composite resin. It is therefore advised to grind with sandpaper no. 600. This grade has the same roughness as the Carborundum disc, and results in a rough surface that forms a greater retention.

The shear bond strengths in the two saliva-contaminated groups group were, respectively, 12.05 MPa and 10.53 MPa (Table 1). Both these values were lower than in the control group. They show that contamination can decrease the shear bond strength between composite resin and enamel. Khoroushi and Karimi (2006) declared that glycoprotein in saliva interferes with bonding between composite resin and tooth surface [8]. However, their study used synthetic saliva to conclude that elimination of the contaminant agent was not effective.

The shear bond strengths of the blood-contaminated, water-washed group was 8.26 MPa, while the blood-contaminated group with etching reapplication had a strength of 6.59 MPa (Table 1). These results
showed that blood contamination can decrease shear bond strength in a greater amount. Similar results were found in the study by Prasad, where the shear bond strength in from blood contamination was 5.02 MPa [10]. Our study had a lower value because it used chicken blood which has a higher viscosity than human blood, making it harder to wash away. A study conducted by Soares et al., which confirmed Koppolu et al.’s study, stated that blood contamination forms a macromolecule fibrinogen and platelets of blood clot that inhibits penetration of adhesive material into the enamel [14,15]. Soares et al. stated that blood contamination decreased shear bond strength of enamel and dentin by 30–70%. This study suggested that blood contamination should be eliminated in order to facilitate penetration of adhesive material into the enamel [15].

The shear bond strengths between the control and contaminated groups were statistically different (p=0.001). This result confirms the hypothesis that saliva and blood contamination decreases shear bond strength between composite resin and enamel. Sfondrini et al. (2004) stated that contaminant agents from oral cavities decreased the shear bond strength between composite resin and enamel, and was the cause of failure to form a good bonding between enamel and composite restoration [16]. Blood contamination decreases surface energy and forms a weak bond [17]. This study showed that there was an effect of saliva and blood contamination on the value of shear bond strength between composite resin and enamel [17]. There were no statistically significant differences in the shear bond strength values between the two saliva-contaminated groups with and without etching reapplication: the p value was 0.143. The same result was found in the two blood-contaminated groups, which had a p value of 0.092. These p values show that these two procedures (washed and dried, and etching reapplication) had no effect on shear bond strength. These results mirror the study of Yoo et al. which stated that washing the contaminant agent with water and drying it with air spray didn’t restore the value of shear bond strength between composite resin and enamel [6]. A study by Eriksson et al. showed that the protein in the blood cannot be completely eliminated by water washing and air drying [18]. Soares et al. stated that re-etching of contaminated teeth was not advised because it didn’t significantly improve shear bond strength [15].

There were no statistically different values between the contaminated groups with and without etching reapplication, yet there were substantially different values of shear bond strength, where the lowest value was found in the blood-contaminated group that underwent etching reapplication. This result confirms the second hypothesis that there are different values of shear bond strength between the saliva- and blood-contaminated groups. This result was also found in the study by de Carvalho et al. (2010). These authors stated that washing the contaminant agent with water and drying it with air spray led to a higher shear bond strength because these methods eliminated the contaminant agent. The same results were found in studies conducted by Yoo and Pereira and Chang et al. [11,19,20]. Khoroushi and Karimi stated that the same methods could be done in saliva-contaminated dentin after the bonding agent was cured [8]. Dame suggested that washing and air drying were reliable for blood-contaminated teeth [21]. Etching reapplication didn’t eliminate the contaminant agent and could impair the microporosity, after which the adhesive couldn’t penetrate the tooth tissue to form a resin tag. As a result, there was no bonding between adhesive materials and tooth tissue [22]. A further study is needed to discover the effects of blood and saliva contamination with different applications.

4. Conclusion
Blood contamination after etching decreased the value of shear bond strength at a much greater rate than saliva contamination. Washing the tooth followed by drying gave a better result than etching.

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