Effects of Cleansing Methods on Shear Bond Strength of Nanohybrid Composite Resin to Enamel after Saliva and Blood Contamination during Bonding

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Abstract

Composite resin is a sensitive material that needs a dry and clean operative area. This study aimed to determine the effects of saliva and blood contamination after bonding on shear bond strength of composite resin to enamel. It also compared several cleansing methods. Twenty-five samples of human premolars, mounted in acrylic blocks, were divided randomly into five groups (n = 5) based on type of contamination and cleansing procedures. The greatest shear bond strength was seen in the control group (14.78±0.89) while the blood-alcohol group showed the lowest bond strength (9.02±1.10). Contamination groups cleaned by water rinsing had greater shear bond strength than groups cleaned by alcohol swabbing. Significant differences ($p < 0.05$) were seen between the control group and the contamination groups. In summary, contamination of saliva and blood after bonding decreased the shear bond strength of composite resin to enamel. Water rinsing is more effective than alcohol swabbing in restoring bond strength.

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Introduction

Nanohybrid composite resin is a restorative material commonly used in dental practice because of its advantages over other types of composite.[1] Retention of composite resin is obtained from adhesion to the hard tissue of the tooth through a bonding system.[2] These days, bonding systems have improved significantly. This progress began with an enamel etching technique that was introduced and improvements have continued over several generations of bonding agents and include the total etch technique, which is most suitable for enamel, the self-etch technique, which is tolerant to dentin moisture, and the development of all-in-one adhesives that can be applied simply and easily.[3-5] The choice of a bonding agent will affect the bonding strength of an adhesive to tooth structure.[6]

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Successful adhesive bonding to enamel is affected by several factors, one of which is contamination.[7,8] Composite resin necessitates sensitive restoration, and its manipulation requires a dry and clean operative area in order to obtain adequate bonding strength. However, the oral cavity is a moist environment, and there will always be liquid of varying consistencies, such as saliva, gingival fluid, and blood from tissue inflammation, complications, and iatrogenic causes.[9] In some clinical situations, resin cannot be well-isolated because of the limitations of its location, such as cervical caries lesions near the gingiva or posterior region. Given the longer time needed to place and polymerize composite resin, it is more difficult to control and avoid contamination in such circumstances.

Based on previous research, it is expected that liquid contamination, such as saliva or blood, could decrease the bond strength of composite resin to tooth structure by 33–70%.[8] If it is neglected, composite resin's adhesion to tooth tissue may be weakened and cause a microleakage in a restoration. Failure of restoration can progress, resulting in secondary caries, dentin hypersensitivity, and pulpal inflammation.[10] In order to prevent them, a method is needed to remove contaminants from enamel

surfaces. The cleansing methods commonly utilized by dentists in clinical practice include using water, drying, and using a cleansing agent.[11-14] The cleansing agents or antiseptics commonly owned by dentists are 70% alcohol. The advantage of alcohol is its ability to effectively cleanse the contaminant and quickly evaporate without leaving residue. This makes alcohol the most common cleansing agent used in dental practice.[15]

A few authors have examined the effect of contamination on shear bond strength of composite resin. But most of them investigated the effects of saliva and blood contamination in different circumstances, such as in the use of an etch primer or of another substrate, such as dentin. Also, most reports have used a self-etch type of bonding agent or an all-in-one adhesive to tolerate dentin moisture; see, for instance, the work of Hedge et al. (2008), Soares et al. (2007), Tachibana et al. (2011), Neelagiri et al. (2010), and Koppolu et al. (2012).[7,9,11,14,15] Due to these limitations, a study is needed that will analyze the effects of saliva and blood contamination after the two-step application of a bonding agent of the total etch type on shear bond strength of composite resin to enamel. The study must also compare shear bond strength of composite resin that has been contaminated by saliva and blood and determine the best contaminant cleansing method to restore the shear bond strength of composite resin to enamel, especially water rinsing and alcohol swabbing.

Materials and methods

This study was an in vitro experimental laboratory study. The variables included independent variables, such as contaminant cleansing methods, and dependent variables, such as shear bond strength of composite resin to enamel. The control variable was etched enamel, to which was applied a bonding agent that was then contaminated by saliva or blood. The specimens used in this study were 20 human premolars that were extracted, cleansed by running tap water, and immersed in sterile saline solution (NaCl 0.9%). Inclusion criteria were that each tooth must be an upper or lower premolar from the right or left region; must have been extracted for orthodontic treatment; must have an intact buccal surface; and must have no fillings. Exclusion criteria included premolar teeth that were extracted because of caries or crown fractures.

These specimens were cleansed and mounted in acrylic blocks, and the buccal surface was ground using 800, 1000, and 2000 grit silicon carbide paper under running water until the flat superficial enamel surfaces were exposed. Operative areas were marked using a permanent fine tip marker and adjusted to a composite resin diameter mold. Then the specimens were divided into five random groups based on contamination type and cleansing method.

The specimens were cleansed by prophylaxis paste with non-fluoride pumice powder for 20 seconds, and then a total etch two-step type of adhesive was applied, containing 37% phosphorous etch acid (Magic Acid, Coltene, Switzerland) and a bonding agent (Magic Bond, Coltene, Switzerland). The contaminants used in this study were chicken blood preserved with anticoagulant (heparin) and Fusayama Meyer artificial saliva (made by the biochemistry laboratory of the Faculty of Medicine, Universitas Indonesia) with a composition of KCl 1.2 gr/l, NaCl 0.7 gr/l, KSCn 30.33 gr/l, Urea 0.13 gr/l, Na_2HPO_4 0.26 gr/l, KH_2PO_4 0.2 gr/l, and $NaHCO_3$ 1.5 gr/l.

The samples were divided into five groups. Group A (Control Group, n = 5) were specimens were etched by 37% phosphoric acid for 15 seconds, then rinsed by water for 10 seconds, and dried with an air syringe for 5 seconds (in accordance with the manufacturer's instructions). A bonding agent was applied for 10 seconds, further thinned with the air syringe, and then cured by LCU (Coltolux LED Pen, Coltene Whaledent) for 20 seconds. None of the specimens were given any contaminants. Group B (Saliva Contamination) comprised of Group B1 (Saliva-Water Rinsing, n = 5) which were specimens were etched, and the bonding agent was applied as with the control group. After bonding agent photopolymerization, the enamel surfaces were contaminated by artificial saliva using a microbrush for 15 seconds. Then the enamels were rinsed by water for 10 seconds and dried for 5 seconds. Moreover, Group B2 (Saliva-Alcohol Swab, n = 5) were specimens were etched, and the bonding agent was applied as with the control group. After bonding agent photopolymerization, the enamel surfaces were contaminated by artificial saliva using a microbrush for 15 seconds. Then the enamels were cleansed

by a cotton pellet swab that had been immersed in alcohol for 10 seconds and dried for 5 seconds. Group C (Blood Contamination) comprised of Group C1 (Blood-Water Rinsing, n = 5), which has procedures were the same as in group B1, but the specimens were contaminated by blood using a microbrush for 15 seconds after adhesive photopolymerization. Further, Group C2 (Blood-Alcohol Swab, n = 5) which procedures were the same as in group B2, but the specimens were contaminated by blood using a microbrush for 15 seconds after adhesive photopolymerization.

Then nanohybrid composite resin (Magic Fill NT Premium, Coltene, Switzerland) was applied to the plastic mold, 3 mm in diameter and 2 mm in height, which was placed on the operative area. The composite resin was then cured for 20 seconds using LED (Coltolux Pen, Coltene Whaledent). After curing, the molds were removed using a cutter. Then the specimens were immersed in a pot filled with aquadest and placed in an incubator at 37 ^{o}C for 24 hours to adjust in an environment similar to the oral cavity. On the next day, the specimens were tested for shear bond strength using a universal testing machine (Shimadzu Autograph 5000), with a crosshead speed of 0.5 mm/minute and load cell of 50 kgF.

Results

Table 1 shows that the highest mean values of shear bond strength were in group A (control) while the lowest mean values of shear bond strength were in group C2 (blood contamination-alcohol swab). All contaminated groups showed a decrease in shear bond strength values compared to the control group, but those contaminated with blood (group C) showed lower shear bond strength values than those contaminated with saliva (group B). When all of the contaminated groups were compared, the water rinsed groups (groups B1 and C1) had higher mean values of shear bond strength than the alcohol swab groups (groups B2 and C2). Results were parametrically, statistically analyzed using one-way ANOVA at significance = 0.001 ($p < 0.05$). Hence, H0 was rejected. This means there were significant differences in the mean values of shear bond strength in the five groups. In order to identify the mean differences in each group, further post hoc analysis using Tukey's multiple comparison test is needed.

Table 2 shows significant differences in mean values of shear bond strength in almost all groups ($p < 0.05$), except between group A (control) and group B1 (artificial saliva contamination-water rinse) (p-value = 0.776) and between group B2 (artificial saliva contamination-alcohol swab) and group C1 (blood contamination-water rinse) (p-value = 0.910).

Groups	Mean	Standard Deviation	Minimum Values	Maximum Values
A	14.78	0.89	13.86	15.99
B1	14.20	0.63	13.27	14.80
B2	12.40	0.82	11.42	13.54
C1	11.97	0.20	11.76	12.32
C2	9.02	1.10	7.32	9.96

Table 1. Results of descriptive statistics values of shear bond strength in each group (MPa). (A) Control group; (B1) Saliva-rinse-air dry group; (B2) Saliva-alcohol group; (C1) Blood-rinse-air dry group; (C2) Blood-alcohol group.

Groups	A	B1	B2	C1	C2
A	-	0.776	0.001*	0.001*	0.001*
B1	0.776	-	0.014*	0.002*	0.001*
B2	0.001*	0.014*	-	0.910	0.001*
C1	0.001*	0.002*	0.910	-	0.001*
C2	0.001*	0.001*	0.001*	0.001*	0.001*

Table 2. Significant differences in each group. (A) Control group; (B1) Saliva-rinse-air dry group; (B2) Saliva-alcohol group; (C1) Blood-rinse-air dry group; (C2) Blood-alcohol group.

Discussion

The results in Table 1 show that mean shear bond strength in all groups was in the range of 9.02–14.78 MPa. This range is lower than the ideal shear bond strength of composite resin to enamel—that is, above 20 MPa.[2] Elements of the enamel's surface condition, such as roughness level and contamination, caused the decrease in the shear bond strength of composite resin in this study. Specimens were ground using 800, 1000, and 2000 grit silicon carbide paper. In the study by Hedge et al. (2008), in contrast, specimens were ground using 320–600 grit silicon carbide paper and had higher shear bond strength, ranging from 20.05-26.41 MPa.[7] Grinding using fine silicon carbide paper produced a decreasing roughness in the enamel surface; hence, the surface showed minimal retention. Loomans (2011) stated that a rough enamel surface would be more retentive than a smooth surface. Greater retention can be

obtained by micromechanical grinding using a bur or silicon carbide paper with a low grit number and micromechanically using etching or sandblasting.[16]

Decreases in shear bond strength values can also be influenced by saliva and blood contamination after bonding; this is demonstrated by this study's results (Table 1), which show low mean values of shear bond strength in contaminated groups (groups B and C) compared to the control group. These results prove the study's first hypothesis: saliva and blood contamination after bonding can decrease the shear bond strength of composite resin to enamel. There are two causes that can explain the reduction of shear bond strength caused by saliva and blood contamination after bonding: first, there was an adsorption of inorganic and organic substances contained in contaminants, including artificial saliva, mineral ions, and blood macromolecules like fibrinogen and platelets, onto poorly polymerized adhesive surfaces (the oxygen inhibiting layer). Second, contaminant rinsing after adhesive application and polymerization can remove the oxygen inhibiting layer, preventing adequate co-polymerization.[17]

The results also show that the blood contaminated groups had lower bond strength than the saliva contaminated groups. This may be because saliva and blood have different composition and consistency.[9] The saliva used in this study was a Fusayama Mayer artificial saliva with a composition of KCl, NaCl, KSCn, Urea, Na_2HPO_4, KH_2PO_4, and $NaHCO_3$. This saliva only contained mineral ions, and there were no organic components like proteins or enzymes; the consistency was more watery than blood or physiologic saliva. This meant the saliva contaminant could be rinsed easily without leaving a residue, so the shear bond strength produced had higher values than that of the blood contamination group. Clinically, physiologic saliva consists of 99.5% water and 0.5% inorganic and organic substances.[18] If there is contamination during clinical procedures, glycoprotein and enzyme deposits in substrate tend to form a thick film that is difficult to rinse. The decrease in bond strength is thus greater than that of artificial saliva.[14]

The blood used in this study was chicken blood preserved with heparin. Commonly, human and animal blood has the same composition: 55% blood plasma and 45% of blood cells, with 6.7% of its total weight made up of proteins.[8] High levels of organic matter (protein) and macromolecules, like fibrinogen and platelets, cause blood to be more viscous than saliva. Hence, blood cannot wet substrate surfaces as well as saliva and tends to form a solid mass. This solid mass is stickier on the substrate surfaces than saliva is, so it cannot be rinsed well.[8,9] The remaining blood protein that cannot be rinsed will disrupt adhesion between the adhesive layer and the composite.[9] This phenomenon provided an answer to the second hypothesis of this study: blood contamination causes a greater decrease in shear bond strength value than saliva contamination.

If we compare both rinsing methods, water rinsing (groups B1 and C1) had a higher mean value of shear bond strength than alcohol swabbing (groups B2 and C2). The decreasing value of shear bond strength in the alcohol swab group, due to force resulting from friction between the cotton pellet and the adhesive surface, was great enough to remove the oxygen inhibiting layer. The friction force from the cotton pellet directly contacting the substrate while rinsing the contaminant would, therefor, remove the thin oxygen inhibiting layer. In the water rinsing method, water was sprayed from a syringe a certain distance to the substrate surface. It is assumed that the contaminant was removed, but the oxygen inhibiting later was not dissolved as much as rinsed by the cotton pellet and alcohol.[11,19] This is also shown in Table 2, which displays no significant differences between the control group and group B1 (artificial saliva contamination-water rinsed) (p-value = 0.776). This means that saliva rinsing using water can restore shear bond strength values like those in the control group. This has been found in studies by Tachibana (2011), Brauchli (2010), Neelagiri (2010), and Khosravanifard (2010).[11-15] Thus, rinsing and drying is suggested for clinical application.

Conclusions

Saliva and blood contamination after bonding can cause a decrease in shear bond strength of composite resin to enamel. Blood contamination after bonding produced a greater decrease in this shear bond strength than did saliva contamination. Water rinsing followed by drying is more effective than alcohol swabbing in

restoring the shear bond strength of composite resin to enamel.

Declaration of Interest

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