Avicenna J Dent Res. 2017 September; 9(3):e60711. doi: 10.5812/ajdr.60711.

Published online 2017 June 30. **Research Article**

Effect of Different Methods of Blood Decontamination on Resin-Resin Micro-Shear Bond Strength

Zahra Jaberi Ansari,[1] Maryam Moezzizadeh,[1] and Haleh Valizadeh Haghi[2,*]

[1]Associate Professor, Department of Operative Dentistry of Shahid Beheshti University of Medical Sciences, Tehran, IR Iran
[2]Assistant Professor, Department of Operative Dentistry of Ardabil University of Medical Sciences, Ardabil, IR Iran

[*]*Corresponding author*: Haleh Valizadeh Haghi, Department of Operative Dentistry, Dental School, Ardabil University of Medical Sciences, Ardabil, IR Iran. Tel: +98-9143515194, E-mail: hvh_haleh@yahoo.com

Received 2015 November 06; **Revised** 2016 July 08; **Accepted** 2016 December 08.

Abstract

Background: Blood contamination could interfere with the bonding of resin materials to different substrates.
Objectives: The purpose of this study was to evaluate the effects of various methods of removing blood contamination between composite resin increments on resin-resin micro-shear bond strength.
Methods: 90 composite blocks (Valux Plus, 3M ESPE) measuring 2 × 2 × 8 mm were prepared in 6 groups: one group was not contaminated as the control (group 1); for other groups the surface was contaminated with blood then rinsed and dried. One group only received this treatment (group 2) and others were etched with phosphoric acid (group 3), phosphoric acid and a bonding agent (Margin Bond, Coltene) (group 4), cleaned with 98% ethanol (group 5), and had 0.5 mm of their outer surface removed (group 6). Next, the second increments of composite were applied in the form of cylinders of 0.7 mm diameter and 1 mm height on the top surfaces of the specimens and were then light cured. Specimens underwent micro-shear bond strength testing. Data were analyzed by Kolmogorov-Smirnov and analysis of variance (ANOVA) tests.
Results: The mean value of bond strength was 23 ± 3.60 MPa for the control group, 17.89 ± 6.52 MPa for group 2, 19.40 ± 6.08 MPa for group 3, 20.20 ± 5.72 MPa for group 4, 20.01 ± 6.83 MPa for group 5, and 19.10 ± 6.20 MPa for group 6. There were no significant differences between the groups.
Conclusions: All the decontamination methods in this study could increase the resin-resin bond strength to the level of the control group.

Keywords: Blood Contamination, Decontamination Method, Resin-Resin Micro-Shear Bond Strength

1. Background

At present, considering people's fascination and obsession with beauty and esthetics, the popularity of tooth colored restorations has increased greatly, among which, resin composites have quickly gained fame and success to the extent that nowadays resin composites are among the most commonly used dental materials (1). One of the most important properties of resin composites is their adhesion to tooth structure, which is best done in a clean environment, free from any contamination. Clinically, various factors, such as surface moisture, gingival sulcus fluid, blood, and hand-piece lubricants could compromise the adhesion of resin to tooth structure and result in gap formation, post-operative hypersensitivity, color changes, recurrent carries, and eventually failure of the restoration (2-4). On the other hand, in order to improve the quality of composite restorations, incremental placement of composite is strongly recommended, which per se requires a contamination-free environment. However, the long time required for this technique, makes contamination control more difficult (5, 6). The effect of blood contamination on bond strength of resin composite to the tooth structure has been extensively studied in the literature (7-9). The influence of blood contamination on bond strength could be attributed to its high protein content that along with macromolecules, such as fibrinogen and platelets, could form a film on the dentin, obstructing the penetration of the adhesive system into dentin tubules (10). Some treatments have been proposed to reverse the contamination, such as resurfacing with rotary instruments, rinsing with water followed by air drying, rinsing with water plus primer reapplication (11, 12). However, there is a lack of information about the effect of blood contamination between resin increments and little is known about successful decontamination procedures.

2. Objectives

This study evaluated the effects of several decontamination methods after blood contamination on the microshear bond strength between resin composite increments. The tested hypothesis was that different microshear bond strength values would be observed among the study groups.

3. Methods

A total of 90 composite blocks were made using 2 × 2 × 8 mm metal rectangular molds. Valux Plus Composite (3M ESPE, St. Paul, MN) was filled in the molds that were placed on glass slabs. In order to achieve flat surfaces, another glass slab was pressed on the top of the molds and then it was removed to allow the formation of air-inhibited layer on the outer surface of composite, for simulation of clinical conditions. Next, specimens were cured using halogen light curing unit (ARIALUX, Tehran, Iran) with an intensity of 400 mw/cm^2, from top and bottom surfaces, each for 40 seconds. Specimens were divided to 6 groups (n = 15) based on the following categorization; sample size of 15 specimens in each group was calculated by conduction of a pilot study:

Group 1: This group was considered as the control group that was not contaminated with blood.

Group 2: The surface of composite specimens was contaminated with human citrated blood using brushes (TPC Advanced technology Inc.), then, rinsed with a water syringe for 10 seconds followed by 20 seconds of air-drying at a distance of 15 cm.

Group 3: The surface was contaminated with human citrated blood, rinsed and dried as done for group 2 and next, the surface was etched with 37% phosphoric acid (Total Etch, Ivoclar vivadent) for 20 seconds, rinsed for 10 seconds, and air-dried for 20 seconds.

Group 4: The surface was treated as done for group 3, then a bonding agent (Margin Bond, Coltene, Batch number: MBO11) was applied and cured for 20 seconds.

Group 5: Once it was treated as group 2, the surface was cleaned with ethanol using cotton pellets, which were drawn on the surface 2 times.

Group 6: After it was treated as group 2, about 0.5 mm of the top surface was removed using 008 diamond bur, then rinsed for 10 seconds and air-dried for 20 seconds at a distance of 15 cm.

The summery of study groups is listed in the Table 1.

After these treatments, the composite cylinders (Valux Plus, 3M ESPE, St. Paul, MN) were made on the prepared surfaces of composite blocks using small tubes with internal diameter of 0.7 mm and height of 1 mm (Tygon, Norton Performance plastic, Cleveland, OH, USA). Composite was filled in the internal portion of tubes by pressing the material with a hand instrument ensuring of no void formation. Then the composite was cured for 40 seconds with the curing unit. Specimens were placed at room temperature for one hour and the Tygon tubes were removed.

Table 1. Summery of Study Groups

Group	Treatment
1 (control)	No contamination
2	Blood contamination/rinsing/drying
3	Blood contamination/rinsing/drying/etching
4	Blood contamination/rinsing/drying/etching/bonding
5	Blood contamination/rinsing/drying/applying alcohol
6	Blood contamination/rinsing/removal outer surface/rinsing/drying

The specimens were fixed in the microtensile tester machine (Bisco Inc, USA) using cyanoacrylate adhesive (Razi, Tehran, Iran) and a force at 0.5 mm/min was applied. To create shear forces, a modification was done on the testing portion of the machine. Cast metallic cylinders were vertically soldered to the jig of the microtensile tester machine. A wire ring prepared with orthodontic ligature wire was established around the base of casting and the composite cylinders of specimens, so that the tensile force of the machine was converted to shear force (Figure 1).

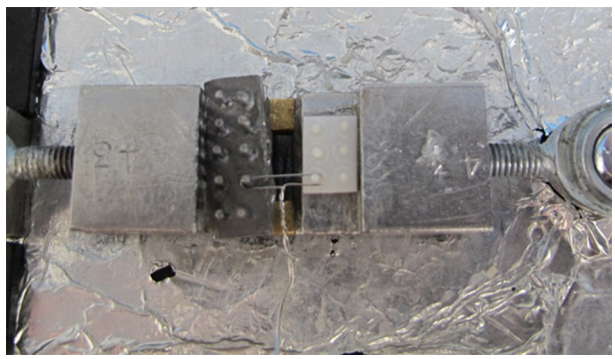

Figure 1. Microtensile Tester Modification

The values of forces at failure was translated to bond strength using this formula:

Bond strength = N/3.14r^2; r: radius of the tygon (mm); N: force (newton)

The data were analyzed using the SPSS 14 software. Distribution of data was checked using the Kolmogorov-Smirnov test. Analysis of variance (ANOVA) test was applied

for statistical analysis of data ($P < 0.05$).

3. Results

Mean and standard deviation of the micro-shear bond strength values of the groups are listed in Table 2. Kolmogorov-Smirnov test showed normal distribution between micro shear bond strength values of different groups, thus the ANOVA test was applied for the analysis of data. The results showed no significant differences between groups ($P = 0.29$).

Table 2. The Microshear Bond Strength Values

Group	Mean ± Standard Deviation	Minimum	Maximum
1 (control)	23.01 ± 3.60	18.15	28.15
2	17.89 ± 6.52	9.47	27.63
3	19.40 ± 6.08	11.05	31.31
4	20.20 ± 5.72	11.57	29.47
5	20.01 ± 6.83	10.78	32.63
6	19.10 ± 6.20	10.52	29.73

4. Discussion

The present study assessed the efficacy of different methods of removing blood contamination among composite increments on the micro-shear bond strength. Bond strength of the specimens was tested using the micro-shear technique that has some advantages compared to conventional shear and tensile bond strength tests. Conventional methods of bond strength tests require a larger surface area, which limits the assessment of the regional bond strength differences (13). However, the micro-shear technique uses small surface areas (0.5 - 1 mm^2), thus a few defects occur in the bond interface and this produces accurate results with relatively low standard deviations (13, 14).

Studies have suggested that the presence of blood plasma on the tooth surface could decrease bond strength by 30% to 70%. Proteins with large molecules are able to develop a layer on the surface and since blood contains 6% to 7% protein compared to the 0.2% protein content of saliva, the effect of contamination with blood seems to be higher than saliva (6). Since in clinical situations the possibility of maintaining blood is the least for the sake of its adverse effects on the esthetic, in the present study, no blood contamination group without rinsing was included. Therefore, only methods of removing contamination were analyzed and water rinsing was included in all groups (except the control group).

According to the results, rinsing blood contamination and drying prior to second composite increment, increased bond strength to the level of the control group. Eriksson et al. showed that rinsing blood contamination significantly restores bond strength of resin layers, however, SEM observations showed craters indicative of blood or water presence on the surface after rinsing and drying (6). Kaneshima et al. reported that rinsing the etched dentin surface after blood contamination decreases bond strength, unless the most outer surface layer (exposed collagen) is removed by hypochlorite solution before or after the contamination. The SEM morphological differences were not observed in the latter study. They concluded that large blood corpuscle elements are prone to complete removal (8); however, the reaction between blood protein components and exposed collagen network prevents primer penetration into dentin (8, 15). In the present study, the complete restoration of the bond strength was justified since the assessment was done among composite increments, with the lack of existence of the collagen network.

Brauchli et al. demonstrated that bond strength would be enough after cleaning the contaminated surfaces with water and the bond strength values were only slightly lower than the control group without re-etching the surface (16). Sayinsu also reported similar results (17). Their studies contradict previous reports (18).

According to Eiriksson et al. adhesive application significantly increased resin-resin bond strength (19). Also in the present study using Margin Bond showed similar bond strength to the control group. The use of Margin bond in this study was because its use at the absence of dentin was more effective and economical. Because of the different adhesive agents used in the mentioned studies, the results are not comparable. Studies have shown that adhesives with acetone or ethanol solvents are capable of removing additional moisture and increase the surface wetting by the resin (7, 16, 18, 19). In the study of Kaneshima et al. the contamination of primed dentinal surfaces decreased the bond strength even after rinsing, yet new application of a primer enhanced the bond strength values to the level of the uncontaminated group (8).

At the present study, re-etching after blood contamination provided sufficient resin-resin bond strength. Xi et al. showed that plasma contamination reduced bond strength values by about 33% to 70% in dentin and enamel, while re-etching restored bond strength values to the same level as the control group (20), yet, a comparison could not be done because of different methodologies. Tachibana et al. used cleansing agents including hydrogen peroxide, anionic detergents, and an antiseptic solution to remove blood contamination of tooth substrates. They showed

that there was no statistically significant difference among cleansing agents and they were as effective as only a water stream and concluded that water stream is sufficient to remove blood contamination from dental tissue before the application of a one-step adhesive (21), that is in accordance with the current study.

All the used methods of blood decontamination for restoring the values of bond strength were effective in this study and the null hypothesis was rejected. It could be stated that surface cleaning through re-etching and using alcohol, and surface removal and application of adhesive layer could enhance bond strength to the level of the control group, yet for the following reasons they are not cost-effective and are not recommended:

1. There were no significant differences between these methods and the one in which contamination was only removed by rinsing.

2. The possibility of gingival bleeding increases during these procedures.

3. They need for more time and higher expenses.

Therefore, it seems that in the case of composite surface contamination, the surface could only be rinsed and dried sufficiently with water-air syringe, and then the next increment of composite could be applied.

4.1. Conclusions

All decontamination methods in this study were effective to increase microshear bond strength of specimens to the level of the control group, however, surface rinsing and drying was the most cost-effective technique in the present study.

Acknowledgments

This article was based on the doctoral dentistry thesis of Atena Mohammadpour from the department of operative dentistry of Shahid Beheshti University of Medical Science. The authors wish to express their sincere appreciation to this department.

References

1. Heymann HO, Swift Jr EJ, Ritter AV. Sturdevant's Art & Science of Operative Dentistry-E-Book. Elsevier Health Sciences; 2014.
2. Summitt JB, Robbins JW, Hilton TJ, Schwartz RS. Fundamentals of operative dentistry: a contemporary approach. Quintessence Pub.; 2006.
3. Hitmi L, Attal JP, Degrange M. Influence of the time-point of salivary contamination on dentin shear bond strength of 3 dentin adhesive systems. *J Adhes Dent.* 1999;**1**(3):219–32. [PubMed: 11725670].
4. van Schalkwyk JH, Botha FS, van der Vyver PJ, de Wet FA, Botha SJ. Effect of biological contamination on dentine bond strength of adhesive resins. *SADJ.* 2003;**58**(4):143–7. [PubMed: 13677688].
5. Price RB, Doyle G, Murphy D. Effects of composite thickness on the shear bond strength to dentin. *J Can Dent Assoc.* 2000;**66**(1):35–9. [PubMed: 10680332].
6. Eiriksson SO, Pereira PN, Swift EJ, Heymann HO, Sigurdsson A. Effects of blood contamination on resin-resin bond strength. *Dent Mater.* 2004;**20**(2):184–90. [PubMed: 14706802].
7. Dietrich T, Kraemer M, Losche GM, Wernecke KD, Roulet JF. Influence of dentin conditioning and contamination on the marginal integrity of sandwich Class II restorations. *Oper Dent.* 2000;**25**(5):401–10. [PubMed: 11203848].
8. Kaneshima T, Yatani H, Kasai T, Watanabe EK, Yamashita A. The influence of blood contamination on bond strengths between dentin and an adhesive resin cement. *Oper Dent.* 2000;**25**(3):195–201. [PubMed: 11203816].
9. Yoo HM, Pereira PN. Effect of blood contamination with 1-step self-etching adhesives on microtensile bond strength to dentin. *Oper Dent.* 2006;**31**(6):660–5. doi: 10.2341/05-1279. [PubMed: 17153973].
10. de Carvalho Mendonca EC, Vieira SN, Kawaguchi FA, Powers J, Matos AB. Influence of blood contamination on bond strength of a self-etching system. *Eur J Dent.* 2010;**4**(3):280–6. [PubMed: 20613916].
11. Sattabanasuk V, Shimada Y, Tagami J. Effects of saliva contamination on dentin bond strength using all-in-one adhesives. *J Adhes Dent.* 2006;**8**(5):311–8. [PubMed: 17080879].
12. Faltermeier A, Behr M, Rosentritt M, Reicheneder C, Mussig D. An in vitro comparative assessment of different enamel contaminants during bracket bonding. *Eur J Orthod.* 2007;**29**(6):559–63. doi: 10.1093/ejo/cjm052. [PubMed: 17873148].
13. Ogata M, Nakajima M, Sano H, Tagami J. Effect of dentin primer application on regional bond strength to cervical wedge-shaped cavity walls. *Oper Dent.* 1999;**24**(2):81–8. [PubMed: 10483444].
14. Burrow MF, Nopnakeepong U, Phrukkanon S. A comparison of microtensile bond strengths of several dentin bonding systems to primary and permanent dentin. *Dent Mater.* 2002;**18**(3):239–45. [PubMed: 11823016].
15. Wakabayashi Y, Kondou Y, Suzuki K, Yatani H, Yamashita A. Effect of dissolution of collagen on adhesion to dentin. *Int J Prosthodont.* 1994;**7**(4):302–6. [PubMed: 7993540].
16. Brauchli L, Eichenberger M, Steineck M, Wichelhaus A. Influence of decontamination procedures on shear forces after contamination with blood or saliva. *Am J Orthod Dentofacial Orthop.* 2010;**138**(4):435–41. doi: 10.1016/j.ajodo.2008.10.021. [PubMed: 20889048].
17. Sayinsu K, Isik F, Sezen S, Aydemir B. Effect of blood and saliva contamination on bond strength of brackets bonded with a protective liquid polish and a light-cured adhesive. *Am J Orthod Dentofacial Orthop.* 2007;**131**(3):391–4. doi: 10.1016/j.ajodo.2005.04.049. [PubMed: 17346596].
18. Silverstone LM, Hicks MJ, Featherstone MJ. Oral fluid contamination of etched enamel surfaces: an SEM study. *J Am Dent Assoc.* 1985;**110**(3):329–32. [PubMed: 3889092].
19. Eiriksson SO, Pereira PN, Swift EJ, Heymann HO, Sigurdsson A. Effects of saliva contamination on resin-resin bond strength. *Dent Mater.* 2004;**20**(1):37–44. [PubMed: 14698772].
20. Xie J, Powers JM, McGuckin RS. In vitro bond strength of two adhesives to enamel and dentin under normal and contaminated conditions. *Dent Mater.* 1993;**9**(5):295–9. [PubMed: 7995480].
21. Tachibana A, Castanho GM, Vieira SN, Matos AB. Influence of blood contamination on bond strength of a self-etching adhesive to dental tissues. *J Adhes Dent.* 2011;**13**(4):349–58. doi: 10.3290/j.jad.a19665. [PubMed: 20978637].