Assessment of post-contamination treatments affecting different bonding stages to dentin

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ABSTRACT

Objectives: To assess the effect of cleansing treatments following saliva and blood contamination at different bonding stages to dentin. **Materials and Methods:** Labial surfaces of 168 permanent maxillary central incisors were ground flat exposing superficial dentin. Specimens were divided into: uncontaminated control (A), contamination after etching (B), contamination after adhesive application (C), contamination after adhesive polymerization (D). Groups were further subdivided according to cleansing treatments into: rinsing (B1, C1, D1), re-etching (B2, D3), sodium hypochlorite application (B3), ethyl alcohol application (C2), acetone application (C3), rinsing and rebonding (D2), re-etching and rebonding (D4). Composite microcylinders were bonded to treated substrates and shear loaded micro-shear bond strength (μSBS) until failure and treated surfaces were examined with scanning electron microscope. Debonded surfaces were classified as adhesive, cohesive or mixed failure. The data were analyzed using one-way ANOVA and Tukey's *post hoc* test. **Results:** The μSBS values were ranked as follow; Group B: A > B3 > B2 > B1 > B, Group C: A > C3 > C2 > C1 > C, Group D: A > D4 > D1 = D2 ≥ D3. Debonded surfaces showed adhesive failure in Group B while cohesive failure in Groups C and D. **Conclusions:** Cleansing treatments differ according to bonding step; re-etching then rebonding suggested if etched substrate or polymerized adhesive were contaminated while acetone application decontaminated affected unpolymerized adhesive.

Key words: Acetone, bonding stages, contamination, dentin, re-etching

INTRODUCTION

Contemporary restorative dentistry relays on the durable adhesive joint for long survival of composite restorations. Although etch and rinse adhesives are considered the gold standards, they are technique sensitive. Thus, isolation of the working field via rubber dam application is a prime requisite.[1,2] Unfortunately, contamination of the adherent with saliva or blood represents a problem in adhesive dentistry. This occurs when rubber dam isolation is encroached in deep subgingival areas and while managing children with copious salivation.[3] When contamination of the bonding site occurs, several consequences take place as postoperative sensitivity, caries recurrence, discoloration, and restoration dislodgement.[4-7] With two-step etch and rinse adhesives, the procedure starts by etching, the adhesive application then polymerization. Most studies were directed toward contaminant-removing options of etched substrate while scanty focused on cleansing treatments if

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contamination occurred at subsequent stages.[8,9] Ari *et al*. reported that contamination should be avoided regardless the affected step to avoid a reduction in bond strength *t*.[8] Park and Lee suggested that blot drying of saliva could retain passable bond strength to etched dentin.[9] Others proposed that rinsing and adhesive re-application presumed reasonable bond strength.[8,10] Contrary researchers suggested that rinsing off the contaminant, particularly blood, although improved the bond strength, could not regain uncontaminated values.[11,12] This study conducted to assess saliva and blood effect on etch and rinse adhesive and to evaluate cleansing treatments via micro-shear bond strength (μSBS) and scanning electron microscope (SEM). The null hypothesis tested that no effect of postcontamination cleansing treatments on bonding to dentin at different stages.

MATERIALS AND METHODS

Adper™ Single Bond (SB) Plus (HEMA, Bis-GMA, Vitrebond™ copolymer, ethanol/water, photo-initiator) and nanofilled Filtek™ Z250 composite (3M ESPE, St. Paul, MN, USA) were used. Sound human 168 permanent central incisors stored in saline at 4°C until usage. Roots were removed 2 mm to cementoenamel junction. The crowns mounted horizontally in molds of 15 mm diameter and 18 mm height, using self-curing resin with labial surfaces upward. Surfaces were ground flat using diamond disc (Komet, Rock Hill, USA) in low speed under water to expose superficial dentin then polished using carbide paper 600-grit to obtain uniform smear layer.[13]

Specimens grouping

Specimens were randomly grouped (n = 12/group.) according to contaminated step and sub-grouped according to cleansing treatments [Table 1 and Figure 1]. All specimens, except Group A, were troughed inciso-cervically into two halves. Each mesial half received fresh human saliva (S) collected from the same donor 2 h after breakfast,[10] while the distal half received fresh venous blood (B) collected with a disposable needle from the same donor.[14] Plastic tubes 5-FR (Feeding Tube, Integral Medical Products, China) with 0.7 mm diameter and 2 mm height, were mounted on the dentin surface.[10] Bonding procedure and cleansing treatments were confined to the site of tubes placements. The composite was packed into each tube under gentle pressure over cellophane strip then light-cured according to manufacturers' guidelines using Elipar II unit (3M ESPE, St. Paul, USA). Each half received two tubes away by at least 3 mm whereas group A received two microcylinders only. All specimens were stored for 24 h in an incubator at 37°C and 100% humidity.

Table 1: Treatments at different bonding steps

Contamination	Symbol	Treatment
Uncontaminated	A	15 s Scotchbond™ 35% phosphoric acid (3 M ESPE, St. Paul, MN, USA), 10 s rinsing, blot drying, two adhesive coats agitated for 15 s using saturated microbrush, 5 s air thinning, 10 s light-curing
Contaminated etching	B	20 s uniform contaminant application
	B1	20 s rinsing
	B2	20 s rinsing, 15 s re-etching, 20 s rinsing
	B3	20 s rinsing, 5.25% sodium hypochlorite 15 s microbrush application (chlorox, cleanser company, Egypt), 20 s rinsing
Unpolymerized adhesive	C	20 s uniform contaminant application
	C1	2 s rinsing, rebonding
	C2	20 s rinsing, 70% ethyl alcohol microbrush application for 15, rinsing, rebonding
	C3	20 s rinsing, 15 s acetone microbrush application (ElSalam Chemicals, Egypt) rinsing, rebonding
Polymerization adhesive	D	20 s uniform contaminant application
	D1	20 s rinsing
	D2	20 s rinsing, rebonding
	D3	20 s rinsing, 15 s re-etching
	D4	20 s rinsing, 15 s re-etching, rebonding

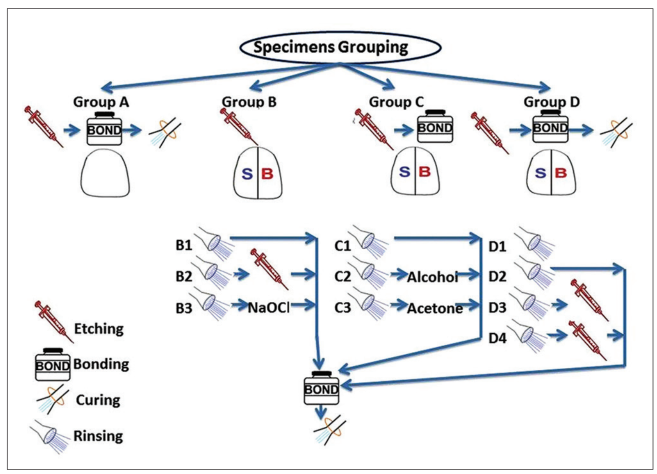


Figure 1: Diagram depicting experimental protocol among the test groups

Micro-shear bond strength testing

The plastic tubes were removed using sharp blade[14] then each specimen was screwed to the lower fixed compartment of testing machine (LRX-plus; Lloyd Instruments Ltd., UK) with 5 kN load. A loop wire, 0.014 in, wrapped around each microcylinder flushing with the resin-dentin interface and aligned with the loading axis of the machine upper movable compartment. A shearing load applied to each assembly at 0.5 mm/min crosshead speed until failure. The average of two microcylinders' values per half represents specimen value. The μSBS expressed in MPa.

Debonded surfaces were examined using stereomicroscope ×25 (Olympus/DeTrey, Germany). Failures classified as adhesive if occurred at the interface, cohesive as observed within dentin substrate or composite resin, and mixed when adhesive and cohesive fractures detected simultaneously.

Scanning electron microscope examination

Two representative specimens per group were examined. Specimens were gold sputtered under vacuum (Ladd sputter coater, BAL-TEC, SCD005, Germany) then examined under SEM (Philips, Holland).

Statistical analysis

The data were analyzed using SPSS (version 16.0) software package (SPSS Inc., Chicago, IL, USA) with significance level set at $P \leq 0.05$. One-way ANOVA evaluated cleansing treatments' effects and Tukey's *post hoc* test for multiple comparisons. The impact of saliva or blood contaminants assessed using independent *t*-test.

RESULTS

The effect of contamination at different bonding stages are shown in Table 2. Contamination reduced bond strength regardless the affected stage. Table 3 presents Group B cleansing treatments where re-etching showed the highest μSBS. Group C3 treatment offered the greatest μSBS in Table 4. In Group D, treatment D4 favored the highest μSBS values, [Table 5].

Figure 2 illustrates that predominant failure of Group B was adhesive mode while cohesive failure in composite prevailed Groups C and D. SEM used to understand cleansing treatments effect on substrate topography [Figures 3 and 4]. Saliva deposits observed in SB, SB1, SB3, C2 and SD (saliva contamination after adhesive polymerization) while blood remnants are notable in BB (blood contamination after dentin etching), BB1, BB3 and BD (blood contamination after adhesive polymerization). Red blood cells are detected at higher magnification in Figure 5.

DISCUSSION

Micro-shear strength test considered effective for measuring variation in bonding under different conditions. Small specimens allowed several readings from the single tooth and provided harmonious stresses yielding lesser data dispersion.[15] Bonding steps start with etching which selectively decalcify intertubular and peritubular dentin leaving collagen mesh for adhesive impregnation then polymerization.[16,17] This study declared that saliva and blood adversely influence bonding regardless affected step. However, others reported negligible moisture effect (particularly

Table 2: Means±standard deviation of microshear bond strength values (*MPa*) of contaminated bonding stages

Groups	Saliva	Blood	*P* value
A	32.3951±1.624^{a}		
B	9.4929±1.072^{b}	2.1461±1.260^{c}	≤0.001*
C	6.2533±1.280^{c}	3.0657±1.369^{b,c}	≤0.001*
D	7.1942±2.094^{c}	4.2714±1.298^{b}	≤0.001*

*Significant. Means with similar letters per column are not significantly different

Table 3: Means±standard deviation of microshear bond strength values (*MPa*) of contaminated etching cleansing treatments

Groups	Saliva	Blood	*P* value
A	32.3951±1.624^{a}		
B	9.4929±1.072^{b}	2.1461±1.260^{c}	≤0.001*
B1	19.4063±2.189^{d}	12.4304±1.802^{d}	≤0.001*
B2	29.5401±2.183^{b}	28.9975±2.260^{b}	0.5917^{NS}
B3	25.4445±1.998^{c}	23.9256±1.127^{c}	≤0.05*

*Significant. NS: non-significant. Means with similar letters per column are not significantly different

Table 4: Means±standard deviation of microshear bond strength values (*MPa*) of contaminated unpolymerized adhesive cleansing treatments

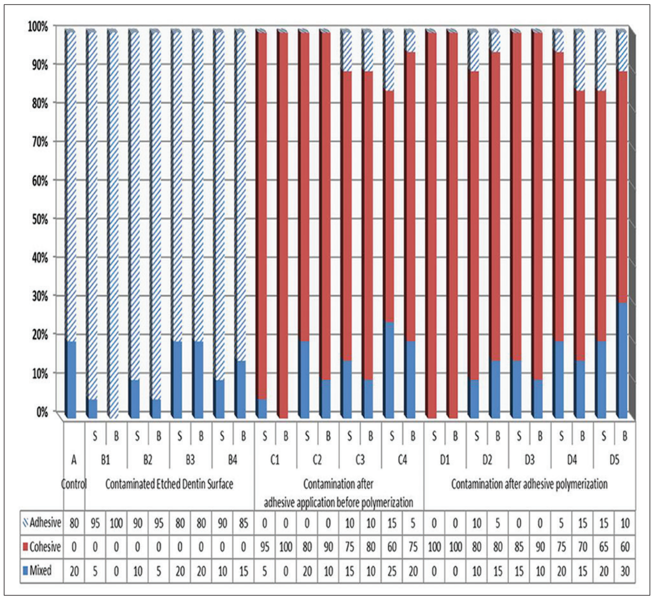
Groups	Saliva	Blood	*P* value
A	32.3951±1.624^{a}		
C	6.2533±1.280^{e}	3.0657±1.369^{e}	≤0.001*
C1	19.7394±1.322^{d}	17.5418±1.008^{d}	≤0.001*
C2	21.9153±1.393^{c}	19.1019±1.135^{c}	≤0.001*
C3	24.6775±1.593^{b}	22.5719±0.806^{b}	≤0.001*

*Significant. Means with similar letters per column are not significantly different

Table 5: Means±standard deviation of microshear bond strength values (*MPa*) of contaminated polymerized adhesive cleansing treatments

Groups	Saliva	Blood	*P* value
A	32.3951±1.624^{a}		
D	7.1942±2.094^{e}	4.2714±1.298^{e}	≤0.001*
D1	15.4382±2.885^{c}	6.0308±1.028^{e}	≤0.001*
D2	13.1060±2.134^{c,d}	11.7608±2.040^{c}	0.1668NS
D3	10.7495±1.957^{d}	9.0734±1.767^{d}	0.6060NS
D4	19.4446±3.014^{b}	16.7848±3.231^{b}	0.0731NS

*Significant. NS: non-significant. Means with similar letters per column are not significantly different



	Control	Contaminated Etched Dentin Surface								Contamination after adhesive application before polymerization								Contamination after adhesive polymerization									
	A	B1		B2		B3		B4		C1		C2		C3		C4		D1		D2		D3		D4		D5	
		S	B	S	B	S	B	S	B	S	B	S	B	S	B	S	B	S	B	S	B	S	B	S	B	S	B
Adhesive	80	95	100	90	95	80	80	90	85	0	0	0	0	10	10	15	5	0	0	10	5	0	0	5	15	15	10
Cohesive	0	0	0	0	0	0	0	0	0	95	100	80	90	75	80	60	75	100	100	80	80	85	90	75	70	65	60
Mixed	20	5	0	10	5	20	20	10	15	5	0	20	10	15	10	25	20	0	0	10	15	15	10	20	15	20	30

Figure 2: Failure mode distribution among the test groups

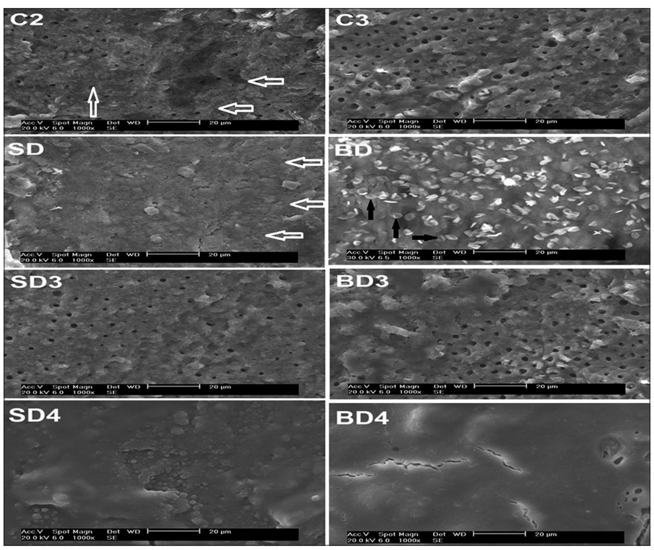


Figure 4: Scanning electron microscope photomicrograph of (C2) alcohol and (C3) acetone effect on contaminated adhesive before its polymerization, (SD/BD) saliva or blood contamination of adhesive after polymerization, (SD3/BD3) re-etching, (SD4/BD4) re-etching then rebonding. White arrows point deposits of saliva. Black arrows point red blood cells (×1000)

saliva) over bonding.[18,19] The difference attributed to different adhesives' composition[18] and experimental design using micro-leakage or diverse loading tests.[9,19] Contamination reduced μSBS despite using moisture-tolerant Vitrebond™ copolymer adhesive. This reduction attributed to biofilm adsorption[20,21] and monomer competing during hybridization. Hydrolytic enzymes of contaminants degraded Bis-GMA with subsequent adhesion impeding.[9,22] van Schalkwyk *et al.*, reported that blood affected bonding more adversely.[11] Chang *et al.*,[12] and de Carvalho Mendonça

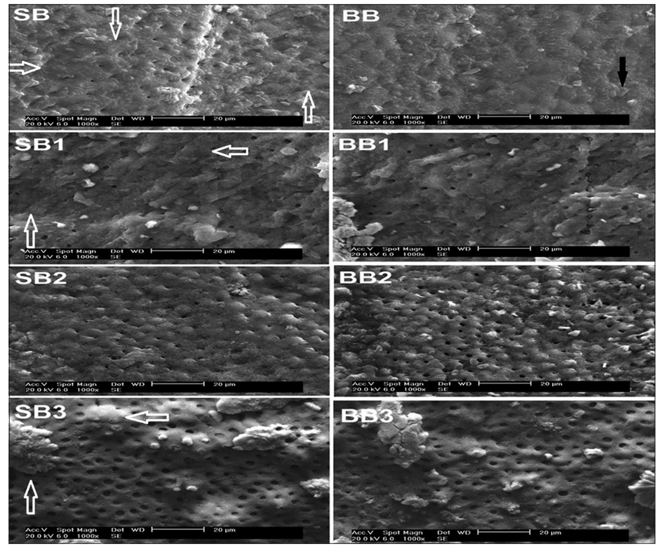


Figure 3: Scanning electron microscope photomicrograph of etched dentin substrate (Single Bond) contaminated with saliva, (BB) contaminated with blood, (SB1/BB1) rinsing of contamination, (SB2/BB2) re-etching, (SB3/BB3) sodium hypochlorite application. White arrows point deposits of saliva. Black arrow points red blood cell (×1000)

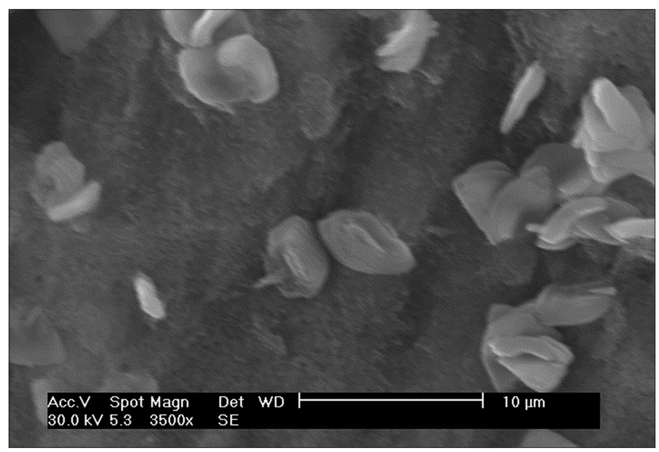


Figure 5: Scanning electron microscope photomicrograph depicting red blood cells (×3500)

et al.,[23] reported that rinsing failed to remove blood due to greater proteins macromolecules contents which resist rinsing and prevent adhesive permeation.[11] It was suggested that 17–20 MPa required to withstand stresses without gap formation.[24] Therefore, different treatments were suggested to counter contamination effect. NaOCl application, for <60 s, showed fractional reversing of contamination due to its nonspecific proteolytic action which eradicates organic remnants without negatively effecting bonding.[25-27] Whereby, re-etching regained adequate bonding due to acid denaturation of organic remnants rendering weak affinity to underlying substrate becoming easily washed.[28,29] When unpolymerized adhesive contaminated, its conversion becomes affected as a result of hydrophilic HEMA molecules which retain water within the adhesive limiting chain growth during polymerization, producing a plasticizing effect in polymer and oxidation of pendant C = C bonds. Further by-products release results in compromising bond polymerization.[30] In addition, higher blood viscosity diminish light permeation and adhesive polymerization.[31] According to the present result, acetone application successfully restored bonding strength when contamination affected unpolymerized adhesive. Both ethyl alcohol and acetone solutions are well known common solvents. However, acetone possess additional ability to remove monomer and denature plastics (polymers),[32] accordingly was able to remove contaminated unpolymerized adhesive leaving perspicuous bonding surface. Furthermore, contamination of polymerized adhesive permits glycoproteins adherence to air-inhibited adhesive surfaces forming a physical barrier preventing co-polymerization between adhesive and composite resin.[33] In agreement with Furuse *et al*.,[6] it was observed that etching of contaminated cured adhesive, created areas devoid from adhesive coverage since etching removed contaminant residue and peeled off adhesive coating [SD3/BD3, Figure 4]. Thus, rebonding after re-etching aided in the refurbishing of patent adhesive for bonding. Debonding of assemblies results from force propagation along lines of least resistance. Therapy, adhesive failures predominated etched contaminated substrate while cohesive mode prevailed affected adhesive stages.

CONCLUSIONS

In this study:

1. Contamination reduced bonding strength to dentin where blood yielded more negative effect than saliva
2. To enhance bonding; re-etching then rebonding are recommended with contaminated etching or polymerized adhesive while acetone and rebonding with affected uncured adhesives.

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Conflicts of interest
There are no conflicts of interest.

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