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Original Article

Shear Bond Strength of Self-etch and Self-adhesive Resin Cements to Dentin after Cleansing Blood-Hemostatic Agent-Contamination with Different Techniques

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Abstract

The study aimed to evaluate the effect of cleansing protocols for blood – hemostatic agent contaminated dentin on the shear bond strength (SBS) of self-etch or self-adhesive resin cements. Flat 50 buccal or lingual dental surfaces obtained from intact human third molars were randomly allocated into two groups according to the type of resin cements (self-adhesive resin cement groups and self-etch resin cement groups). Specimens were then sub-divided into five subgroups according to contamination and cleansing protocols (Control, blood + aluminum chloride + water, blood + aluminum chloride + 17% ethylenediaminetetraacetic acid, blood + ferric sulfate + water, blood + ferric sulfate +17% ethylenediaminetetraacetic acid). In the self-etch resin cement group, Single Bond™ Universal adhesive in self-etch mode was used. Cylindrical RelyX™ Ultimate or RelyX™ Unicem was built up by using SBS jig. After three-month in distilled water storage at 37°C, all samples were performed SBS test. F-test one-way ANOVA followed by Tukey HSD post hoc test was used to analyzed data in the self-etch resin cement and Welch's ANOVA followed by Games-Howell was used in the self-adhesive resin cement. Level of confident was set at 95%. Failure mode of fracture specimens was evaluated with a stereomicroscope. In the self-etch group, mean values of SBS in all investigated groups demonstrated no statistically significant difference compared to the control group (p=0.537). In the self-adhesive group, all test groups showed significantly decreased mean values of SBS compared to the control group (p<0.001). Cleansing with either water or 17%EDTA is suitable for blood-hemostatic agent contaminated dentin when using resin cement combined with self-etch universal adhesive. However, both methods are not recommended for cleaning such contamination when self-adhesive resin cement is used.

Keyword: Blood contamination, Hemostatic agent, Resin cement, Shear bond strength

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Introduction

Bonded restoration is a well-known treatment providing both minimally invasive concept and esthetic restoration. To achieve a successful treatment, high quality of bond which is a key factor, requires a clean tooth substrate and a good moisture control[1] such as rubber dam application.[2] However, rubber dam isolation is impractical in some clinical situations, especially when the restoration oper operative field and optimal gingival displacement[3], topical hemostatic agents are utilized. The complexion of blood and hemostatic agents are more often found in clinical situations than either only blood or hemostatic agents alone. However, studies used such complexion were scanty.

The topical hemostatic agents possess different mechanisms of action to achieve hemostasis. 20-25% aluminum chloride ($AlCl_3$), a popular agent used in restorative dentistry, precipitates blood protein and provides vasoconstriction[4] whereas 15-20% ferric sulfate ($Fe_2(SO_4)_3$) chemically forms ferric or sulfate ion-protein complex in acidic condition and occludes the damaged vessels mechanically.[5] Such agents alter the quality of smear layer and underlying dentin due to their acidity (pH ~ 0.7-2.0). Besides, the remnants of blood-hemostatic complex are left behind in the smear layer.[6,7] Alteration of substrate quality consequently hampers the bond strength of adhesive systems[8-10] especially in 'no water rinsing' systems. The resin cement that is used along with self-etch adhesive or self-adhesive resin cement which incorporates partially dissolved smear layer into resin-dentin interfacial layer[11] may also demonstrate the impaired bond efficacy. However, controversial results of bond performance of self-etch adhesive to hemostatic-contaminated dentin were proposed by previous studies[12,13], whereas bond efficacy of self-adhesive resin cement has still been questionable when bonded to hemostatic agent-contaminated dentin.[14,15] Therefore, the effect of blood-hemostatic agent contaminated dentin on bond efficacy of such 'no water rinsing' adhesive or cement requires the investigation.

To recover hemostatic agent-contaminated dentin surface back to optimal level before bonding procedure, different cleansing protocols were proposed, including rinsing with water only or with ethylenediaminetetraacetic acid (EDTA).[14,16] EDTA, a well-known chelating agent, was shown to restore the bond strength of simplified self-etch adhesive to aluminum chloride-contaminated dentin to the comparable bond strength of that with normal dentin.[16] EDTA, which has pH=4.7, probably chelates aluminum compound in dentin resulting in optimal surface to obtain the high quality of bond. However, studies of cleansing method for dentin contamination with complexion of blood and hemostatic agent before bonding to self-adhesive resin cement or to resin cement combined with universal adhesive in self-etch mode are scarce.

Altogether, the present study aimed to investigate the effect of different cleansing methods on the shear bond strength (SBS) of resin cement, either self-etch or self-adhesive to dentin contaminated with complexion of blood-hemostatic agent. The null hypothesis test was that the SBS values after different cleansing protocols were similar to non-contaminated dentin.

Materials and Methods

Sample collection and preparation

The present study protocol was approved by the Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2021-010). Twenty-five intact human third molars were extracted from patients between 16 to 40 years with consent forms. All collected teeth were evaluated under stereomicroscope (SZ61, Olympus, Tokyo, Japan) to confirm that they were free from caries, cracks, and fractures. All teeth were disinfected with 1% chloramine-T solution for 7 days and stored in distilled water at 4°C with a maximum of 6 months until testing procedure.

Roots of all collected teeth were cut off at 2 mm apically to the cementoenamel junction. Then, each crown was sectioned mesiodistally into two parts by using a low-speed diamond saw under water cooling (Isomet® 1000, Buehler, Lake Bluff, IL, USA). The fifty sectioned teeth were embedded in formatray acrylic resin in polyvinyl chloride rings (diameter 21.4 mm, height 21 mm) with exposing buccal or lingual surfaces upward. The tooth surface was ground with #150-grit silicon carbide (SiC) paper (TOA DCC Waterproof Abrasive Paper, Kobe, Japan) under water cooling to remove enamel and get a flat dentin. All ground samples were checked with a stereomicroscope at 15X magnification to confirm that no enamel was present on the surface. The dentin surface was further ground with #600-grit SiC paper under water cooling by polishing machine (NANO 2000T, Pace Technology, AZ, USA) at speed of 200 rounds/minute for 1 minute and was cleaned by water spray from triple syringe at pressure 2065 - 2585 mmHg and at distance 10 mm for 10 seconds to create a standardized smear layer.[17] Next, bonding area of the dentin surface was defined by three-millimeter hole at the center of blue tape (Scotch blue™, 3M, St. Paul, MN, USA) as shown in Figure 1.

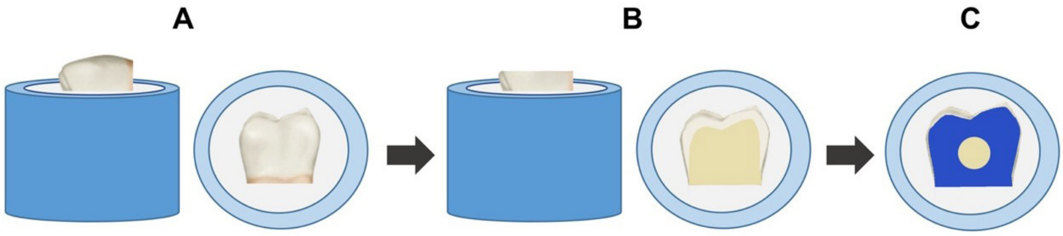

Figure 1 *Diagram illustrates the sample preparation; (A) Sectioned tooth was embedded in formatray acrylic resin in polyvinyl chloride rings with exposing buccal or lingual surfaces upward. (B) Tooth was ground with #150-grit SiC paper under water cooling to get flat dentin followed by grinding with #600-grit SiC paper to create smear layer. (C) Bonding area of dentin was defined by three-millimeter hole at the center of blue tape*

Conventional resin cement (RelyX™ Ultimate, shade A1, 3M ESPE, St Paul, MN, USA) combining with universal adhesives in self-etch mode (Single Bond™ Universal adhesive, 3M ESPE, St Paul, MN, USA) and self-adhesive resin cement (RelyX™ Unicem, shade A2 Universal, 3M ESPE, St Paul, MN, USA) were used in this study. The specific compositions of all materials were shown in Table 1. The specimens were randomly allocated into two groups according to the type of resin cements (n=25) and further subdivided into five sub-groups (n=5) according to the hemostatic agents and cleaning protocols. The summarized diagrams of all groups were shown in Figures 2 and Figure 3.

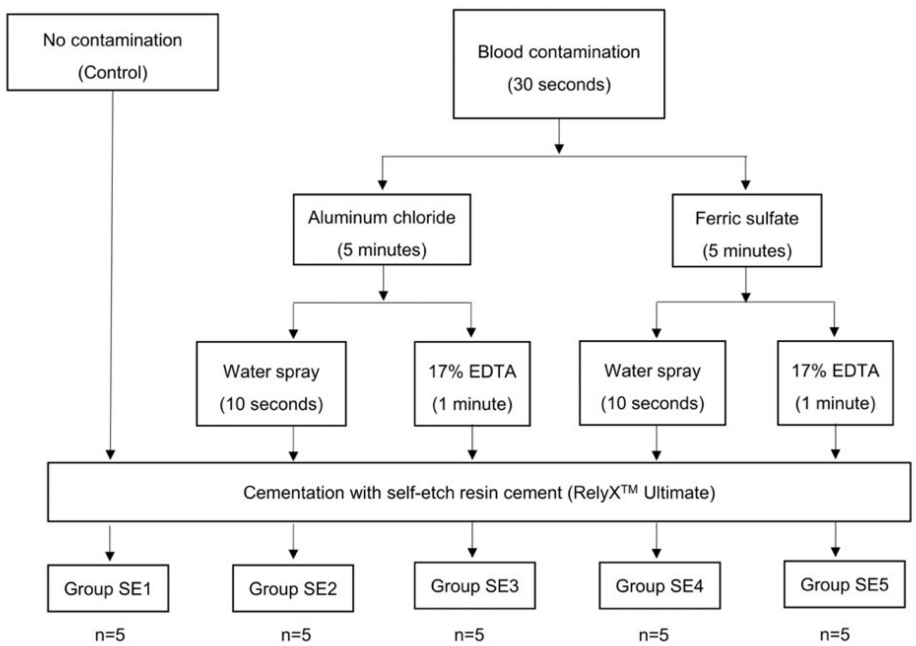

Figure 2 *Schematic diagram illustrates the experimental procedure of self-etch resin cement group*

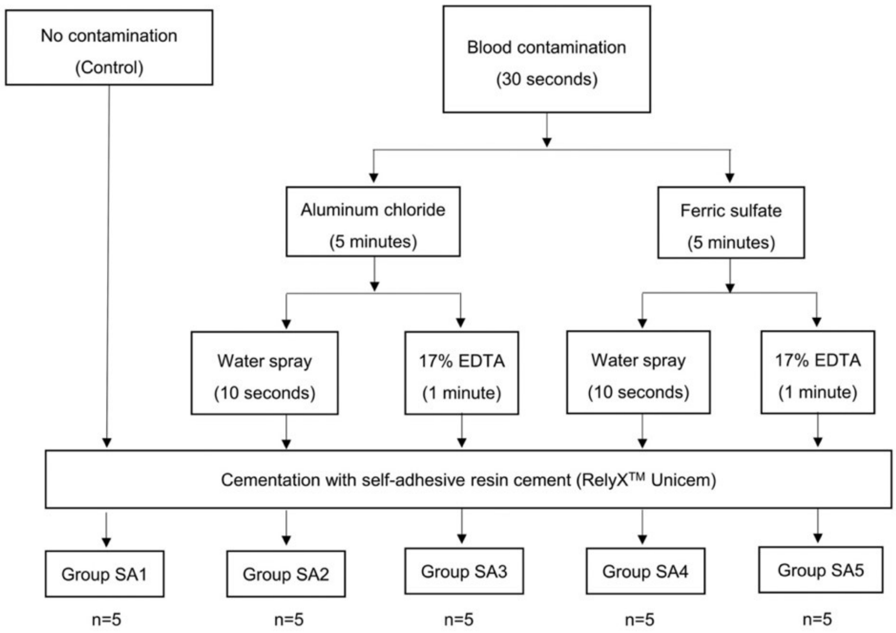

Figure 3 *Schematic diagram illustrates the experimental procedure of self-adhesive resin cement group*

pH measurement

pH measurement of two hemostatic agents and 17% EDTA agent (Chulalongkorn university, Bangkok, Thailand) were verified by pH meter (ORION™ Model 420A, Texas City, TX, USA). The pH information of these chemicals was shown in Table 1.

Table 1 *Chemical compositions, pH and batches of used materials*

Materials	Composition	pH	Batch/Lot
Racestyptine hemostatic solution agent, Septodont, Cedex, France	25% Aluminum chloride solution, 0.1% 8-Hydroxy quinoline sulfate, inert excipient	0.8	B24353BAD
Astringedent™, Ultradent Products, South Jordan, Utah, USA	15.5% ferric sulfate equivalent solution with inert binding agent in a viscous, aqueous carrier	1.1	BGN6F
17% EDTA, Chulalongkorn university, Bangkok, Thailand	Ethylene diamine tetraacetic acid-disodium salt, Sodium hydroxide, Cetrimide, Distilled water	7.7	075/64
Single Bond™ Universal adhesive, 3M ESPE, St Paul, MN, USA	MDP Phosphate Monomer, dimethacrylate resins, HEMA, Vitrebond™ Copolymer, filler, ethanol, water, initiators, silane	2.7	91212B
RelyX™ Unicem, 3M ESPE, St Paul, MN, USA	**Powder:** Glass powder, initiator, silica, substituted pyrimidine, calcium hydroxide, peroxy compound, pigment **Liquid:** Methacrylate phosphoric ester, dimethacrylate, acetate, stabilizer, initiator	-	7396460
RelyX™ Ultimate 3M ESPE, St Paul, MN, USA	**Base paste:** Methacrylate monomers, radiopaque silanated fillers, initiator components, stabilizers, rheological additives **Catalyst paste:** Methacrylate monomers, radiopaque alkaline (basic) fillers, initiator components, stabilizers, pigments, rheological additives, fluorescence dye, dark cure activator for Scotchbond™ Universal Adhesive	-	7373529

Contamination procedure and cleansing protocols

Flat dentin surface was dried by using clean air pressure. Then, ten-microliter blood was applied on dentin for 30 seconds. The cotton pellet soaked with 100 µl of either $AlCl_3$ (Racestyptine hemostatic solution agent, Septodont, Cedex, France) or $Fe_2(SO_4)_3$ (Astringedent™, Ultradent Products, South Jordan, Utah, USA) was applied on dentin surface for 5 minutes. After contamination, the dentin was cleaned by water or 17% EDTA. For water rinsing groups (SE2, SE4, SA2, SA4), the contaminated dentin was rinsed with water spray at pressure 2065 - 2585 mmHg, distance 10 mm for 10 seconds. For 17% EDTA rinsing groups (SE3, SE5, SA3, SA5), the contaminated dentin was rinsed with 17% EDTA 3 ml for 1 minute followed by rinsing with water spray at pressure 2065 - 2585 mmHg, distance 10 mm for 10 seconds. Samples in the control groups (SE1, SA1) were rinsed with water spray as performed in water rinsing group without contamination.

Bonding procedure

For the conventional resin cement groups (SE1, SE2, SE3, SE4, SE5), dentin was dried with gentle air blow, Single Bond™ Universal adhesive was used in self-etch mode according to the manufacturer's instructions. Briefly, Single Bond™ Universal adhesive was applied by one-millimeter applicator (Super fine dental micro applicator, Cotisen®, Huanghua promise dental, Hebei, China) on dentin surface with rubbing motion for 20 seconds. After application, the surface was dried with gentle clean air blow at pressure 2065 - 2585 mmHg, at 10 mm distance until absence of fluid movement. Then, dentin sample was assembled to the SBS jig which stabilized a silicone tube inside, RelyX™ Ultimate was insert in a silicone tube which was 3 mm in diameter and 3 mm in height by using silicone gun. One kilogram was put over a cover slip upon a silicone tube for 1 minute to simulate the finger pressure. Light polymerization was performed for 40 seconds (LED Curing light Demi™ Plus, Kerr, Orange, CA, USA) over the cover slip. Tip of light curing unit was passively touched to cover slip. After disassembled the jig, light cured polymerization was repeatedly performed at each lateral side for 20 seconds. Then, the tube was gently removed. Light intensity of light curing unit was calibrated at every 5 samples with Optilux Radiometer (L.E.D. radiometer by Demetron®, Kerr Corporation, Danbury, CT, USA).

For the self-adhesive resin cement groups (SA1, SA2, SA3, SA4, SA5), dentin surface was blot dried by using absorbent paper (Kimwipes®, Kimtech Science, Kimberly-Clark, Irving, TX, USA) to prepare slightly moist dentin. Dentin sample was assembled into the jig as in the conventional resin cement group. RelyX™ Unicem was inserted in silicone tube by silicone gun. Light-curing procedure was performed the same as in the conventional resin cement group. The silicone tube was, then, gently removed.

Control of each group was performed by the same protocol as mentioned above without any contamination. All samples were re-examined to confirm whether the visible bubbles were present or not. All samples that presented visible bubble were excluded and replaced by new ones before the next experiment was performed.

Artificial aging process

All specimens were kept in sterile distilled water at 37°C for 3 months. Water was gently changed every week to avoid contamination.

Shear bond strength test

After 3 months of water storage, SBS test was performed by using a universal testing machine (EZ-S500N, Shimadzu, Kyoto, Japan). Each specimen was mounted into the metal holder, the cross-head speed was set at 1 mm per minute. A shear force was applied until failure. The bond strength was recorded and calculated by Trapezium 2 program. Mean and standard deviation of SBS values in each group were collected and analyzed.

Failure mode analysis

The fractured specimens were evaluated for mode of failure under a stereomicroscope at a magnification of 35X. Modes of failures were classified as the followings: adhesive failure, mixed failure, cohesive failure of resin composite and cohesive failure of dentin. The collected values of each mode were calculated based on the total specimens of each group and presented as a percentage of that group.

Statistical analysis

All statistical procedures were performed by SPSS statistical data editor Version 25 (IBM SPSS Statistics version 28.0.0.0 (190), Armonk, NY: IBM Corp). Normal distribution was analyzed by Shapiro-Wilk test. Then, the homogeneity of variance of collected data was analyzed by Levene test. For conventional resin cement groups (SE1, SE2, SE3, SE4, SE5), which demonstrated inequality of the homogeneity of variance, Welch's ANOVA followed by Games-Howell post hoc test was used for analyzing the data. For self-adhesive groups (SA1, SA2, SA3, SA4, SA5) which revealed both normal distribution and equal homogeneity of variance, F-test one-way ANOVA followed by Tukey HSD post hoc test was used for analyzing the data. Statistical significance was determined at p-value<0.05.

Results

Shear bond strength of resin cements

Mean SBS values of conventional resin cement combined with self-etch universal adhesive to dentin were shown in Table 2. The mean SBS values of all the test groups (SE2, SE3, SE4, SE5) demonstrated no statistically significant difference compared to the control group (SE1) (p=0.537). Despite no significant difference, non-contaminated dentin demonstrated the highest mean SBS values among all the groups.

Table 2 *Mean SBS value (MPa) and standard deviation (SD) of a self-etch resin cement (RelyX™ Ultimate) to normal dentin and contaminated-dentin*

Group	Mean SBS (SD)
SE1 (Control)	14.45 (5.55)[a]
SE2 ($AlCl_3$ + water)	12.12 (3.34)[a]
SE3 ($AlCl_3$ + EDTA)	12.68 (1.26)[a]
SE4 ($Fe_2(SO_4)_3$ + water)	10.57 (2.33)[a]
SE5 ($Fe_2(SO_4)_3$ +EDTA)	12.69 (3.06)[a]

The values with same lowercase letter superscript represent no statistically significant difference.

Mean SBS values of self-adhesive resin cement bonded to blood-contaminated dentin were shown in Table 3. The mean SBS values of self-adhesive resin cement to blood-hemostatic agent-contaminated dentin were significantly decreased irrespective of type of hemostatic agents (SA2, SA3, SA4, SA5) compared to non-contaminated dentin (SA1) (p<0.001). Both water rinsing and 17% EDTA cleansing showed no statistically significant difference in mean SBS values.

Table 3 *Mean SBS value (MPa) and standard deviation (SD) of a self-adhesive resin cement (RelyX™ Unicem) to normal dentin and contaminated-dentin*

Group	Mean SBS (SD)
SA1 (Control)	16.16 (3.58)[a]
SA2 ($AlCl_3$ + water)	5.42 (3.56)[b]
SA3 ($AlCl_3$ + EDTA)	6.88 (3.21)[b]
SA4 ($Fe_2(SO_4)_3$ + water)	6.02 (2.12)[b]
SA5 ($Fe_2(SO_4)_3$ +EDTA)	5.36 (1.09)[b]

The values with same lowercase letter superscript represent no statistically significant difference.

Failure mode analysis

The percentages of failure mode were shown in Figure 4 and Figure 5. Both types of resin cement (conventional and self-adhesive) mainly presented adhesive failure. Cohesive failure and pretest failure were not shown in the present study.

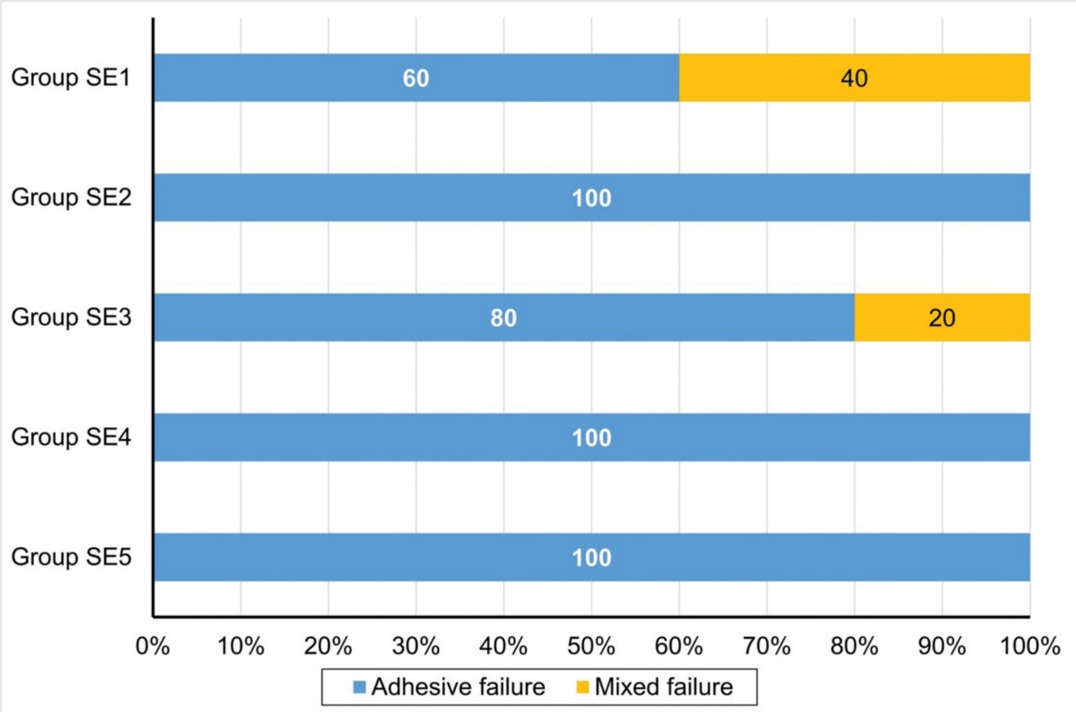

Figure 4 *Represent the failure mode of self-etch resin cement group*

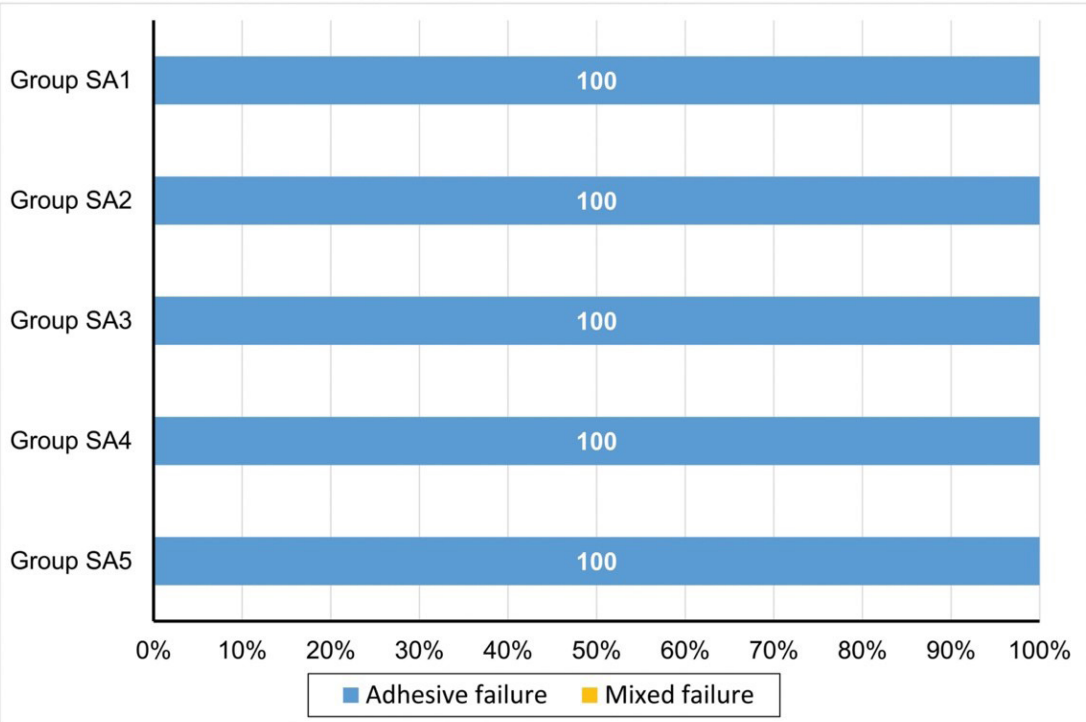

Figure 5 *Represent the failure mode of self-adhesive resin cement group*

Discussion

The present study aimed to investigate whether different cleansing methods used to decontaminate the complexion of blood-hemostatic agent on dentin surface affected the SBS values of both conventional resin cement combined with self-etch universal adhesive or self-adhesive resin cement. After cleansing the contamination of blood-hemostatic agent with either water rinsing or 17%EDTA, SBS values of conventional resin cement combined with self-etch universal adhesive to decontaminated dentin were similar to uncontaminated dentin, whereas both cleansing methods were unable to restore the SBS values back to optimal level of the self-adhesive resin cement. Therefore, the null hypothesis was rejected. Moreover, the failure mode presented in this study showed predominantly adhesive failure which represented true bond strength.[18]

In order to examine the bond efficacy after decontamination by different methods, all delicate samples in the study were additionally challenged with artificially aging process. Because water storage was supposed to directly accelerate degradation of bond interface component[19], three-month water storage before SBS test was selected in the present study.

Since contamination with only hemostatic agent hardly occurs in clinical situation, heparinized human blood was used in this study. Previous study found no significant differences on bond strength values of self-etch adhesive regarding heparinized blood or fresh blood dentin contamination.[20] Human blood (pH~7.2-7.4) was reported to reduce bond strength of self-etch universal adhesive.[21] High pH value of blood may attenuate the concentration and infiltrating ability of acidic monomer and also be neutralized by high acidity of hemostatic agents used in the study (pH≤1) and vice versa. Consequently, the effect of complexion of blood-hemostatic agent on tooth structure in the study was probably not as strong as in previous reports which used either blood or hemostatic agent alone.[21,22]

Cleansing with water seems not to completely remove all contaminated remnants which are left behind in the smear layer.[7] Chaiyabutr and Kois also showed that blood-hemostatic agent contaminated dentin harmed SBS of such cement.[14] Partial removal of contamination probably resulted in negative influence on the quality of resin-dentin interface[23] which was an important factor to dictate bond performance of self-adhesive resin cement to dentin.[24] Complex of blood-hemostatic agent may still compromise the ability of ultra-mild acidity (pH=3.78) of self-adhesive resin cement to decontaminate and approach dentin surface. The impurity from contaminated smear layer that was incorporated into the resin cement layer probably reduces its bond performance.

Previous studies showed that hemostatic agent contaminated dentin harmed the bond strength of not only self-adhesive, but also self-etch universal adhesive systems. [13,22] However, a universal adhesive in self-etch mode in the present study surprisingly presented similar bond strength to non-contaminated dentin, despite three-month artificial aging. This result may attribute to the application technique that obviously influence the bond efficacy. Scrubbing action as conducted in this study facilitated fresh acidic monomer to intimately contact with underlying dentin subsequently increased resin infiltration.[25] Such technique also decontaminated the blood-hemostatic remnant on the dentin surface through dispersing contaminated smear layer[26] resulting in comparable bond strength to non-contaminated dentin, which is consistent with the previous study[12] and showed high percentage of restorative-dentin marginal adaptation.[27]

Chemical composition of the adhesive system might attribute to the comparable result. Single Bond™ Universal adhesive uses 10-15 wt% ethanol as solvent. Ethanol-based solvent allows hydrophobic resin to infiltrate into deeper substrate than that performed by water-based solvent.[28] Moreover, extended air-blowing time may completely evaporate the ethanol-based solvent and enhance the higher degree of conversion.[29] These may promote resin adhesive to infiltrate through contaminated remnants, and ultimately increase the bond strength.

EDTA, a well-known chelating agent, was applied in different concentrations, durations, and techniques in order to modify the smear layer covering dentin surface.[30,31] 17% EDTA removed larger amount of calcium ion out from dentin compared to distilled water[32] probably resulting in attenuation of the chemical bond from acidic monomer in self-etch universal adhesive. Instead of increasing immediate bond strength of self-etch adhesive as reported in a pre-treatment on hemostatic agent-contaminated smear layer[16], EDTA cleansing in the study showed no influence on SBS values. The present result is in accordance with a recent study by Mempel CA and others which demonstrated that 17-20% EDTA did not provide a benefit for universal adhesive bonded to hemostatic agent-contaminated dentin.[33] EDTA was reported to increase nanoleakage value in the adhesive layer, albeit it did not affect the bond strength.[34] Such drawback might increase bond failure in the long term clinical service of self-etch adhesive.

In contrast to self-etch universal adhesive, setting reaction of self-adhesive resin cement partially depends on acid-base reaction which utilizes intrinsic water to ionize the methacrylate phosphoric ester. The special monomer is neutralized by alkaline filler and hydroxyapatite in dentin resulting in chemical bond which plays a major role for RelyX™ Unicem in bonding to dentin[35] despite absence of hybrid layer and resin tags.[36] Partial apatite-depleted substrate by 17% EDTA causes the reduction of SBS values as shown in the present study and in previous study[37], even though smear layer is almost completely removed by 17% EDTA pretreatment for 1 minute. Such mineral-depleted condition might delay neutralization time of RelyX™ Unicem which requires more than 24 hours to become neutral pH.[38] Remaining acidity in resinous material compromises polymerization and mechanical properties of cement[38], and probably weakens the bond efficacy of simplified cements.

Our results indicates that universal adhesive in self-etch mode has acceptable bond performance to blood-hemostatic agent contaminated dentin after water rinsing. Besides, EDTA-pre-treatment might not be beneficial for both types of cements. The optimal cleansing protocol such as changes in application time, other solutions or even techniques of application should be further investigated to fulfill the successful clinical application of bonding with resin cement.

Conclusion

Within the limitations of the present study, cleansing the dentin contaminated with complexion of blood and hemostatic agent with either water or 17%EDTA is probably suitable when self-etch universal adhesive combined with conventional resin cement is used. However, in the same situation, both cleansing techniques are not recommended when self-adhesive resin cement is used.

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