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Laboratory Research

Effect of Blood Contamination with 1-step Self-etching Adhesives on Microtensile Bond Strength to Dentin

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Clinical Relevance

When using 1-step self-etching adhesive systems, blood contamination significantly decreased bond strength to dentin and should be avoided.

SUMMARY

This study evaluated the effect of blood contamination and decontamination methods on the microtensile bond strength of 1-step self-etching adhesive systems to dentin contaminated after adhesive application and light curing. Three commercially available "all-in-one" adhesives (One Up Bond F, Xeno III and Adper Prompt L-Pop) and 1 resin composite (Clearfil AP-X) were used. Third molars that had been stored in distilled water with 0.5% thymol at 4°C were ground with #600 SiC paper under running water to produce a standardized smear layer. The specimens were randomly divided into groups according to the 3 adhesive systems. The adhesive systems were used under 3 conditions: no contamination, which was the control (C); contamination of the light-cured adhesive surface with blood and reapplication of adhesive (Contamination 1) and contamination of the light-cured adhesive surface with blood, then washing, drying and reapplication of the adhesive (Contamination 2). Following light curing of the adhesive, the resin composite was placed in 3 increments up to a 5-mm-thick layer on the bonded surface. All specimens were stored in distilled water at 37°C for 24 hours. The microtensile bond strength was measured using a universal testing machine (EZ test), and data were analyzed by 1-way ANOVA followed by the Duncan test to make comparisons among the groups (p=0.05). After debonding, 5 specimens were selected from each group and examined in a scanning electron microscope to evaluate the modes of fracture. For all adhesives, contamination groups showed lower bond strength than the control (p<0.05). There was no statistically significant difference among the control groups (p>0.05). For Xeno III and Adper Prompt L-Pop, contamination group #2 showed the lowest bond strength among the groups

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($p<0.05$). For One Up Bond F, contamination group #2 showed higher bond strength than contamination group #1 but showed no statistical significance between them ($p>0.05$).

INTRODUCTION

The increasing demand for esthetic restorations has generated intensive research of adhesive materials. Successful adhesion to dental hard tissues is a fundamental requirement prior to the insertion of resin-based composites.[1-3] In order to obtain successful and durable adhesion between resin composite and tooth structure, it is necessary to avoid any contamination of the preparation with oral fluids, such as saliva, blood or gingival crevicular fluid. Achieving good moisture control is a common problem encountered in restorative dentistry, especially when rubber dam isolation is not feasible. Many carious lesions, which require the use of dentin bonding agents, are found in areas for which it is difficult to obtain appropriate isolation, especially when the site is near or at the gingival margin, where blood contamination is more likely to occur.

Several recent studies have suggested that modern hydrophilic bonding systems are less sensitive to contamination with saliva than earlier bonding agents.[4-9] However, the effect of blood contamination on the adhesive properties of 1-step self-etching adhesives has not been fully understood.[8,10] Although most studies indicate that blood contamination causes a significant decrease in bond strengths, Kaneshima and others[11] reported that the effect of blood contamination varies greatly, according to the surface condition of the adherent.

Self-etching adhesive systems have become very popular with clinicians due to the "no-rinse" concept and reduced post-operative sensitivity. Acid etching is not required; therefore, the problem of over-drying or over-wetting the dentin is eliminated. This reduces the potential for post-operative sensitivity and bonding problems associated with movement of dentinal fluid through patent dentinal tubules.[12-13] The technique-sensitivity associated with bonding to a dehydrated collagen matrix is also eliminated.[13] Self-etching adhesive systems provide a faster application due to a reduced number of components and application steps. Although short application time reduces the risk of blood contamination in the field of operation, it may sometimes be impossible to maintain a dry operative field. Limited studies have investigated the effect of blood contamination on bond strengths of self-etching adhesive systems to dentin.

This study evaluated the effect of blood contamination and decontamination methods on the microtensile bond strength of 1-step self-etching adhesive systems to dentin contaminated after the adhesive had been light-cured and prior to application of the resin composite. The null hypothesis of this study was that blood contamination would not adversely affect the microtensile bond strength of 1-step self-etching adhesive systems to dentin.

METHODS AND MATERIALS

Three commercially available 1-step self-etching adhesives and 1 resin composite were used. The bonding agents, composition and manufacturers are listed in Table 1. All bonding agents were restored with Clearfil AP-X (Kuraray Medical Inc, Okayama, Japan) resin.

Twenty-seven freshly extracted, caries-free third molars, stored in distilled water with 0.5% thymol, were used in this study. The enamel was removed and the dentin was ground with 600-grit SiC paper under running water. This process also created a standardized smear layer before bonding with adhesives.

Fresh capillary blood was collected from a single individual at the same site and time as the specimens were made. The specimens were randomly divided into

Table 1: *Adhesives in Used in This Study*

Adhesive	Manufacturer (Lot #)	Composition
One Up Bond F	Tokuyama America, Inc San Mateo, CA, USA (U481183)	Bonding agent A: Mac-10, methacryloyloxyalkyl acid phosphate, multifunctional methacrylic monomers Bonding agent B: HEMA, water, fluoroaluminosilicate glass filler
Xeno III	Dentsply Caulk Milford, DE, USA (0312000292)	Liquid A: HEMA, highly dispersed silicone dioxide, BHT(stabilizer), ethanol, water Liquid B: phosphoric acid modified polymethacrylate resin, mono-fluoro phosphazene modified methacrylate resin, UDMA, BHT, CQ, ethyl-4-dimethyl aminobenzoate
Adper Prompt L-Pop	3M ESPE St Paul, MN, USA (174701)	Water, methacrylated phosphoric acid esters, fluoride complex with zinc, parabenes, stabilizer, photoinitiator

Abbreviations:
Mac-10: 11-methacryloxy-1,1-undecanedicarboxylic acid, HEMA: 2-hydroxyethyl methacrylate, BHT: butylated hydroxy toluene, UDMA: urethane dimethacrylate
CQ: camphorquinone

groups according to the 3 adhesive systems. The adhesive systems were used under 3 conditions:

Control: Self-etching adhesive was applied to dentin according to the manufacturers' instructions and was light-cured for 10 seconds using a visible light-curing unit (VLC, Astralis 5, Vivadent, Liechtenstein, Austria).

Contamination 1: Self-etching adhesive was applied to dentin according to the manufacturers' instructions and light-cured with a VLC for 10 seconds. The bonded surface was contaminated with fresh blood for 15 seconds using a microbrush (Kerr Corporation, Orange, CA, USA). The self-etching adhesive was re-applied to the dentin surface until no blood was visualized, and the adhesive was light-cured with a VLC for 10 seconds.

Contamination 2: Self-etching adhesive was applied to dentin according to the manufacturers' instruction and was light-cured with a VLC for 10 seconds. The bonded surface was contaminated with fresh blood as previously described. The blood was then rinsed for 10 seconds with a water stream from an air-water syringe and dried by a gentle blast of air for 1-2 seconds, being careful not to desiccate the surface. The self-etching adhesive was re-applied to the surface and light-cured with a VLC for 10 seconds. Details of the bonding procedure are presented in Figure 1.

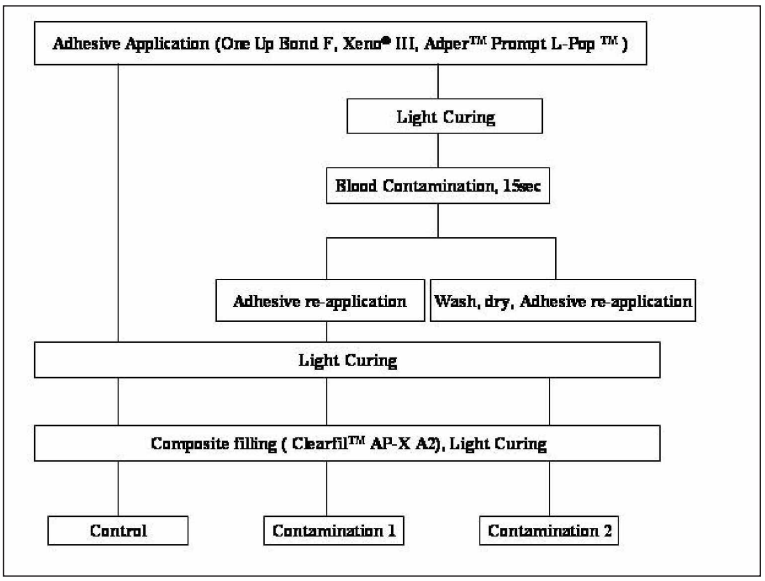

Figure 1. *Schematic representation of the bonding procedures.*

Following adhesive application, resin composite (Clearfil AP-X) was placed in 3 increments up to a 5-mm-thick layer on the bonded surface, and each increment was light-cured with a VLC for 40 seconds. The light intensity was measured periodically during the restorative procedures using a radiometer (Model 100, Demetron/Kerr, Danbury, CT, USA) that ranged from 520 to 560mW/cm^2. After completing the restorative procedure, the roots were sectioned approximately 2 mm below the cementoenamel junction using a high speed bur under copious water spray. The pulp was removed and the pulp chamber was filled with Clearfil AP-X.

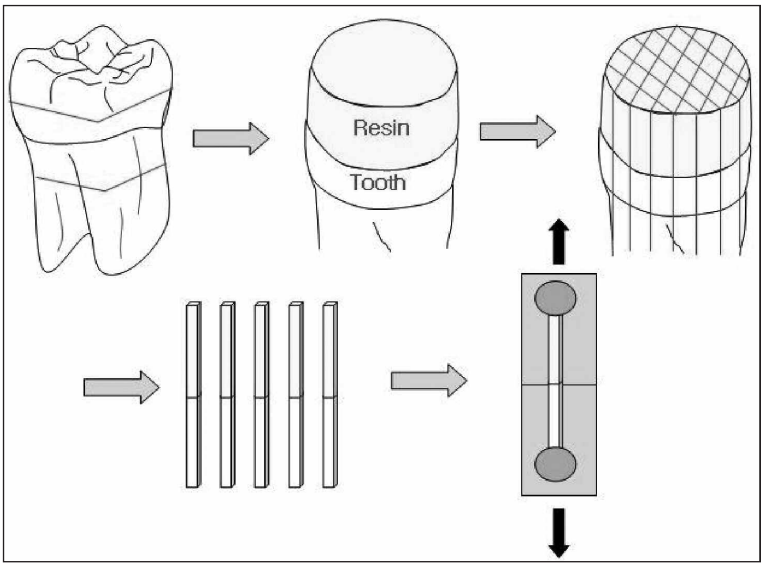

Figure 2. *Illustration of specimen preparation and the test design of microtensile bond strength test.*

The specimens were stored in distilled water at 37°C for 24 hours, then vertically sectioned into 1.0 mm x 1.0 mm x 5 mm beams perpendicular to the bonded interface using a low-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling. Three to 6 beams from the central portion of each tooth were selected for the microtensile bond strength test. Each subgroup contained n=15 beams.

Microtensile Bond Strength Test

Figure 2 shows the specimen preparation and test design of the microtensile bond strength test. The beams were attached to a Ciucchi's jig with a cyanoacrylate adhesive (Zapit, DVA, Corona, CA, USA), which, in turn, was mounted in a universal testing machine (EZ test, Shimadzu Co, Kyoto, Japan) for tensile testing. A tensile force was applied to each specimen at a crosshead speed of 1.0 mm/minute until failure occurred. Microtensile bond strength was calculated by dividing the maximum load at fracture by the cross-sectional surface area of the bonded surface. If spontaneous debonding occurred before the bond strength testing, the bond strength was recorded as 0 MPa.

The data were subjected to 1-way ANOVA, followed by the Duncan test, to make comparisons among the groups ($p<0.05$). The statistical analyses were carried

out with SPSS 10.0 for Windows software (SPSS Inc, Chicago, IL, USA).

Scanning Electron Microscopic Evaluation

After debonding, the specimens were fixed in 10% neutral buffered formalin solution for at least 8 hours for fixation of the proteins and decontamination of the specimens. From each subgroup, 5 pairs of fractured composite-dentin specimens, representative of the mean bond strength, were prepared for SEM observation. To evaluate the debonding surface and mode of fracture, both the dentin and composite sides of the fractured specimens were mounted on stubs, gold sputter coated (SEM coating unit 5100, Polaron Instruments Inc, Agawan, MN, USA) and examined under a SEM.

Table 2: *Mean Microtensile Bond Strength for Each Subgroup (MPa ± SD)*

Control Group	One Up Bond F (n=15)	51.38 ± 11.84^{A}
	Xeno III (n=15)	52.11 ± 11.51^{A}
	Promp L Pop (n=15)	45.44 ± 9.68^{A}
Contamination Group #1	One Up Bond F (n=15)	36.85 ± 12.12†
	Xeno III (n=15)	33.16 ± 13.19†
	Prompt L Pop (n=15)	21.48 ± 14.15‡
Contamination Group #2	One Up Bond F (n=15)	42.38 ± 9.25^{a}
	Xeno III (n=15)	21.26 ± 9.90^{b}
	Prompt L Pop (n=15)	9.59 ± 12.97^{c}

Same superscript indicates no statistically significant difference (Duncan test, p<0.05).

RESULTS

Microtensile Bond Strength Test

The microtensile bond strengths for the different groups are summarized in Table 2 and Figure 3. For all adhesives, the contamination groups showed lower bond strength than the controls ($p<0.05$).

There was no statistically significant difference among the control groups ($p>0.05$). However, the contamination groups showed significant differences among the materials. Adper Prompt L-Pop showed the lowest bond strength in both contamination groups ($p<0.05$). For Xeno III and Adper Prompt L-Pop, contamination group #2 (contamination and washing, drying and adhesive reapplication) showed the lowest bond strength among the groups ($p<0.05$). For Adper Prompt L-Pop contamination group #2, 9 specimens spontaneously debonded during the cutting process. For One Up Bond F, contamination group #2 showed higher bond strength than contamination group #1 (contamination and adhesive reapplication), but there was no statistical significance ($p>0.05$).

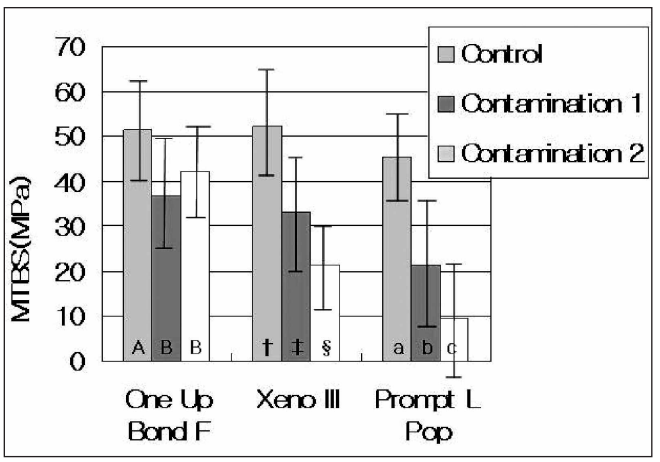

Figure 3. *Microtensile Bond Strength (MPa±SD). Same letter indicates no statistically significant difference (p>0.05).*

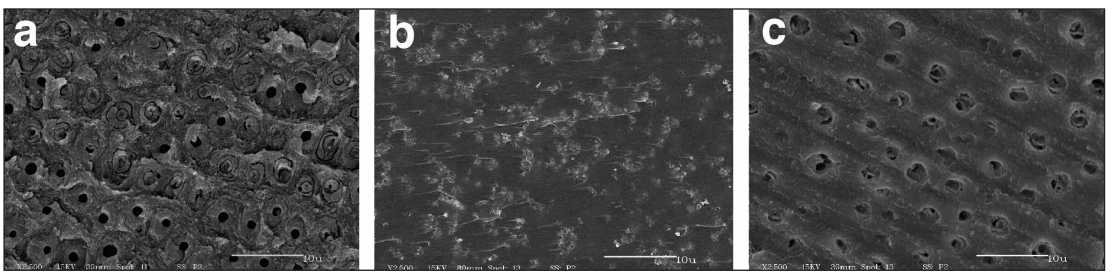

Figure 4. *SEM images of fracture modes of debonded One Up Bond F specimens (2,500x, Bar = 10 mm): (a) Control and fracture occurred at the bottom of the hybrid layer; (b) When blood contamination occurred and re-applied the adhesive (contamination 1), there was detrimental effect to the adhesion, as illustrated by cohesive failure of the adhesive; (c) When blood contamination occurred, then washed, dried and re-applied the adhesive (contamination 2), less infiltration of adhesive occurred.*

Scanning Electron Microscopic Evaluation

To illustrate the fracture modes of the different subgroups, representative images of specimens from each subgroup were selected and are shown in Figures 4-6.

For the One Up Bond F group (Figure 4), significant changes were observed in failure mode as a function of contamination. In the control group (4a), fracture occurred at the bottom of the hybrid layer, showing the typical fracture pattern for a control group. Contamination group #1 (4b) showed the cohesive failure of adhesive, which probably resulted from the detrimental effect of blood. Contamination group #2 (4c) showed less infiltration of adhesive, indicating the interference of blood.

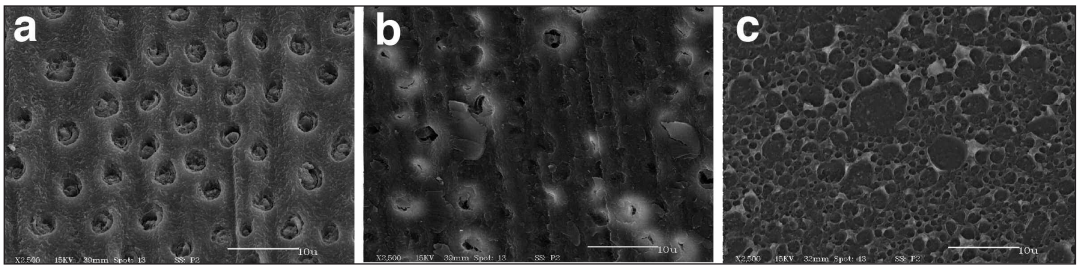

Figure 5. *SEM images of the fracture modes of debonded Xeno III specimens (2,500x, Bar = 10 µm). (a) Control, fracture occurred at the bottom of the hybrid layer. (b) When blood contamination occurred and the adhesive was re-applied (contamination 1), there was some bubbling, and fracture occurred at the top of the hybrid layer. (c) When blood contamination occurred and was washed, dried and re-applied to the adhesive (contamination 2), many voids were seen, which indicated poor adhesion.*

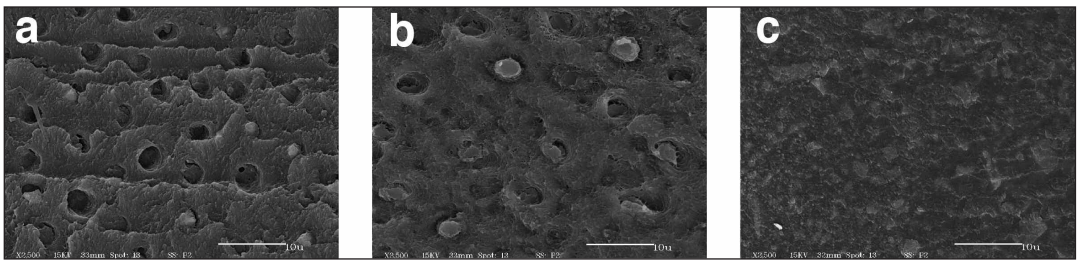

Figure 6. *SEM images of the fracture modes of debonded Adper Prompt L-Pop specimens (2,500x, Bar = 10 µm). (a) Control, fracture occurred within the hybrid layer. (b) When blood contamination occurred and the adhesive was re-applied (contamination 1), the fractured was made on an unclear surface, indicating poor adhesion. (c) When blood contamination occurred and was washed, dried and re-applied to the adhesive (contamination 2), there was a very flat surface, indicating that the fracture occurred between the 2 hybrid layers.*

Figure 5 shows the fracture modes of Xeno III. In the control group (5a), fracture occurred at the bottom of the hybrid layer, showing a typical fracture pattern for a control group. Contamination group #1 (5b) showed some bubbling, and fracture occurred at the top of the hybrid layer. Contamination group 2 (5c) showed many voids and had a honeycomb-like appearance, indicating poor adhesion.

Figure 6 shows the fracture modes of Adper Prompt L-Pop. The control showed failure within the hybrid layer (6a). For contamination group #1 (6b), the fracture had an unclear surface, indicating poor adhesion. Contamination group #2 (6c) showed a very flat adhesive surface, indicating that the fracture occurred between the 2 adhesive layers.

DISCUSSION

In operative dentistry, when rubber dam isolation is not used, contamination of the operating field from inadvertent contact with saliva or blood is a frequent problem. In many clinical scenarios, the rubber dam may be difficult to place and contamination may occur, requiring the use of adhesive systems that bond effectively even in the presence of contamination.

In this study, the effect of blood contamination on bond strength of three 1-step self-etching adhesive systems to dentin was evaluated. In order to better understand this effect and to provide information for interpretation of the bond strength data, the debonded interfaces were observed under SEM. To simulate gingival bleeding in a laboratory setting, blood was drawn immediately prior to restoration placement. The influence of heparinized blood vs native blood on the marginal adaptation of dentin bonded resin composite restorations has been previously reported.[14] The authors found significantly higher percentages of marginal gaps after contamination with fresh capillary blood when compared to anti-coagulated blood. Since blood coagulation might be an important factor in the effect of blood contamination on bonding, freshly drawn blood was used as the contaminant for this study.

Self-etching adhesives systems may be classified into mild, moderate and strong, based on their pH, and, consequently, their ability to dissolve the smear layer and demineralize the underlying dentin.[15-17] Using this classification, One Up Bond F can be considered a mild, Xeno III a moderate and Adper Prompt L-Pop a strong self-etching adhesive. Another criterion for classification is filler loading. Using the filler-loading classification, One Up Bond F and Xeno III are filled adhesives and Adper Prompt L-Pop is an unfilled adhesive. Despite many differences in material properties, all the adhesives used in this study showed significant low bond strength after blood contamination. SEM pictures supported these results, because all contamination groups showed different fracture patterns compared to their controls, indicating poor adhesion. In contamination group #1, the remaining protein from blood could

impair proper adhesion and copolymerization of the subsequent adhesive and resin layers. In contamination group #2, different phenomena could have occurred at the interface, resulting in reduced bond strength. Remnants of blood protein or excess water, which were not completely removed, could have impaired adhesion between the layers of adhesive and composite. In addition, rinsing could have disrupted the oxygen-inhibited and unpolymerized layer. However, the effect of disruption of the unpolymerized layer is still unclear, since it has been reported that there is no difference in bond strength when resin composite is added to and polymerized on cured adhesive with or without an unpolymerized surface layer.[18]

Further studies on the effect of blood contamination using 3-step and 2-step adhesive systems during the various "steps" are still necessary and would provide invaluable information to clinicians in the art and science of adhesive dentistry

CONCLUSIONS

Since blood contamination negatively affected the microtensile bond strength of 1-step self-etching adhesive systems to dentin, this study failed to reject the null hypothesis. In addition, neither of the decontamination methods used in this study reversed the harmful effect of blood contamination. In the clinical scenario, thorough rinsing and cleansing is important if blood contaminates the preparation, and bonding procedures should be repeated from the beginning. In addition, alternate materials should be considered if blood contamination can not be avoided. Within the limitations of this *in vitro* study, it can be concluded that blood contamination should be avoided when using all-in-one adhesives.

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