Original Article

Influence of Blood Contamination During Multimode Adhesive Application on the Microtensile Bond Strength to Dentin

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Abstract

Objectives: The present study evaluated the effects of blood contamination performed at different steps of bonding on the microtensile bond strength (μTBS) of multimode adhesives to dentin when using the self-etch approach. **Materials and Methods:** Seventy-five molars were randomly assigned to three adhesive groups comprising 25 specimens each: two multimode adhesives [Single Bond Universal (SBU) and All-Bond Universal (ABU)] and a conventional one-step self-etch adhesive [Clearfil S3 Bond Plus (CSBP)]. Each group was subdivided as follows: (1) uncontaminated (control): bonding application/light curing as a positive control; (2) contamination-1 (cont-1): bonding application/light curing/blood contamination/dry as a negative control; (3) contamination-2 (cont-2): bonding application/light curing/blood contamination/rinse/dry; (4) contamination-3 (cont-3): bonding application/blood contamination/dry/bonding re-application/light curing; and (5) contamination-4 (cont-4): bonding application/blood contamination/rinse/dry/bonding re-application/light curing. Dentin specimens were prepared for μTBS testing after the composite resin application. Data were analyzed with two-way ANOVA and post-hoc tests ($\alpha = 0.05$). **Results:** μTBS values were similar in cont-3 groups, and ABU/cont-4 and corresponding control groups, but were significantly lower in the other groups than in their control groups ($P < 0.05$). Cont-1 groups showed the lowest μTBS values ($P < 0.05$). **Conclusions:** Neither decontamination method prevented the decrease in μTBS when contamination occurred after light curing. Drying the blood contaminants and reapplying the adhesive may regain the dentin adhesion when contamination occurs before light curing. Alternatively, rinsing and drying contaminants followed by adhesive re-application may be effective depending on adhesive type.

Keywords: *Micro-tensile, novel adhesives, resin-based composites*

Date of Acceptance:
05-Dec-2016

Introduction

Recent developments in the chemistry of dental adhesives have aimed to produce more user-friendly adhesive systems with reliable bonding performance. The development of new dentin bonding materials, focused on decreasing the number of steps. Dental adhesive systems could be categorized into two major groups considering to different bonding techniques to the dental tissues: the etch and rinse and self-etch systems.[1] After phosphoric acid application in the etch and rinse system, deep etch-pits in the hydroxyapatite-rich substrate was attained at enamel and demineralizes up to a depth of a few micrometers to expose a hydroxyapatite deprived collagen mesh at dentin.[2] Therefore, these adhesives are available for use in three or two steps. Although etch and rinse adhesives are still the gold standard for dental adhesion, the collagen fibers collapse during the

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/1119-3077.224127

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How to cite this article: Kucukyilmaz E, Celik EU, Akcay M, Yasa B. Influence of blood contamination during multimode adhesive application on the microtensile bond strength to dentin. Niger J Clin Pract 2017;20:1644-50.

process of demineralized dentine drying, which leads to a decrease in bond strength, is main disadvantage of these adhesives. To overcome this problem in dentin self-etching strategy was developed.[1]

In the self-etch strategy, there is no preparative phosphoric acid applying step therefore self-etching systems are less technique sensitive. They are including hydrophilic and hydrophobic monomers, initiators, solvents, stabilizers, and fillers.[3] Additionally, self-etch adhesives contain specific monomer molecules with carboxylate or phosphate acidic groups that allow dental superficial demineralization such as conditioner and infiltrates into the dentin such as primer agents.[3] Thus, these adhesives have an easy application procedure and are less vulnerable of differences in the practitioner's technique when compared with multistep etch and rinse adhesives.[4,5] Self-etch adhesives have acceptable clinical performance.[4] But disadvantage of the self-etch protocol is the reduction in enamel bonding effectiveness. Therefore, the mild self-etch approach is recommended with additional "selective" etching of enamel using phosphoric acid.[5] Nevertheless, selective enamel etching poses the risk of inadvertently etching the dentin. Intentional etching of the dentin by using phosphoric acid has been shown to decrease bond strengths of some self-etch adhesives.[6,7]

Recently, new commercial materials called ''universal,'' ''multipurpose,'' or ''multimode'' adhesives have been manufactured to overcome this drawback. They include acidic adhesive monomers bearing acidic groups to provide strong adhesion to dental hard tissues.[8] It is assumed that the composition of multimode adhesives does not differ markedly from that of current adhesives. However, differing acidic monomer type, ratio, pH, and some new monomers may change bond strength characteristics. For instance, Single Bond Universal contains less 10-MDP than Clearfil SE Bond but includes a polyalkenoic copolymer.[9] By this way, these adhesives allow a practitioner to apply the adhesive in a self-etch or an etch-and-rinse mode depending on the clinical case.

Despite these advances in the development of adhesive systems, contamination (from saliva, blood, astringents, water, or hand piece lubricant) during the restorative procedure remains a major problem in daily clinical practice, which may negatively affect the bonding performance of adhesives.[10-14] A rubber dam is considered the best way to create the proper operative field during composite restorations,[15] but this procedure is not always feasible in clinical conditions. Only approximately 17% of dentists routinely use the rubber dam, and thus, contamination of the operating field is still common.[16,17] In particular, preventing blood contamination during restoration is more critical due to the proximity of blood to the operating field.

Several previous studies have investigated the effects of blood contamination on bond strength between resin composites and tooth surfaces.[12,14,17-22] Certain variables such as the substrate type, bonding agent, the time of contamination, and the specific decontamination method may play an important role in the resulting bond.[23] However, there is limited to no knowledge on the effect of blood contamination on multimode adhesives.

The present *in vitro* study evaluated the effects of blood contamination performed at different steps of bonding on the microtensile bond strength (μTBS) of multimode adhesives to dentin when using the self-etch approach. The null hypothesis of this study was that different blood contamination at various bonding steps did not affect the μTBS to dentin.

Materials and Methods

In this study, two multimode adhesives, the Single Bond Universal (SBU) (3M ESPE) and All-Bond Universal (ABU) (Bisco), and a conventional one-step self-etch adhesive, the Clearfil S3 Bond Plus (CSBP) (Kuraray), were used. The multimode adhesives were used in self-etch mode. The adhesive system compositions, batch numbers, manufacturers, and application procedures are listed in [Table 1].

After approval from the institutional ethics review board (2014/118), 75 caries-free human molars were stored in 0.5% thymol solution at 4°C and used within 1 month of extraction. The tooth roots were 2 mm below the cemento-gingival junction, and all of the occlusal enamel was removed using a water-cooled low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, Illinois, USA). Enamel removal was checked using a stereomicroscope. The exposed dentin was ground with 600-grit SiC paper for 60 s under running water to provide a standard experimental condition.[24] The 75 molars were randomly assigned to the three adhesive groups comprising 25 molars each; the groups were further divided into five subgroups as described below [Figure 1].

Uncontaminated groups (positive control)

These dentin specimens were not contaminated with blood. The adhesive systems were applied to the dentin specimens according to the manufacturers' instructions and light-cured with an LED-curing unit (Elipar S10, 3M ESPE, Seefeld, Germany) set at 1200 mW/cm^2.

Table 1: The composition, batch number, and application procedures of tested adhesive systems

Adhesive System	Composition	Application Procedure
Clearfil S^3 Bond Plus (Kuraray, Tokyo, Japan) **Batch: 041112/00010A**	MDP, HEMA, Bis-GMA, silanated colloidal silica, sodium fluoride, photo-initiators, CQ, ethanol, water	Apply bond for 10 s. Dry with mild pressure air flow for 5 s Light cure for 10 s.
All-Bond Universal (Bisco Inc., Schaumburg, IL, USA) **Batch: 1200004839**	MDP, Bis-GMA, HEMA, ethanol, water, initiators	Apply first application with scrubbing motion for 15 s. Apply second application with scrubbing motion for 15 s. Do not light cure between coats. Evaporate solvent by thoroughly air-drying for at least 10 s. Light cure for 10 s.
Single Bond Universal Adhesive (also known as Scotchbond Universal Adhesive) (3M ESPE, Neuss, Germany) **Batch: 472584**	MDP, dimethacrylate resins, HEMA, Vitrebond copolymer, filler, ethanol, water, initiator, silane	Apply adhesive and rub it for 20 s. Dry with gently air for 5 s. Light cure for 10 s.

*MDP: 10-Methacryloyloxydecyl dihydrogen phosphate, HEMA = hydroxyethyl methacrylate, Bis-GMA = bisphenol A glycidyl methacrylate, CQ = camphorquinone,

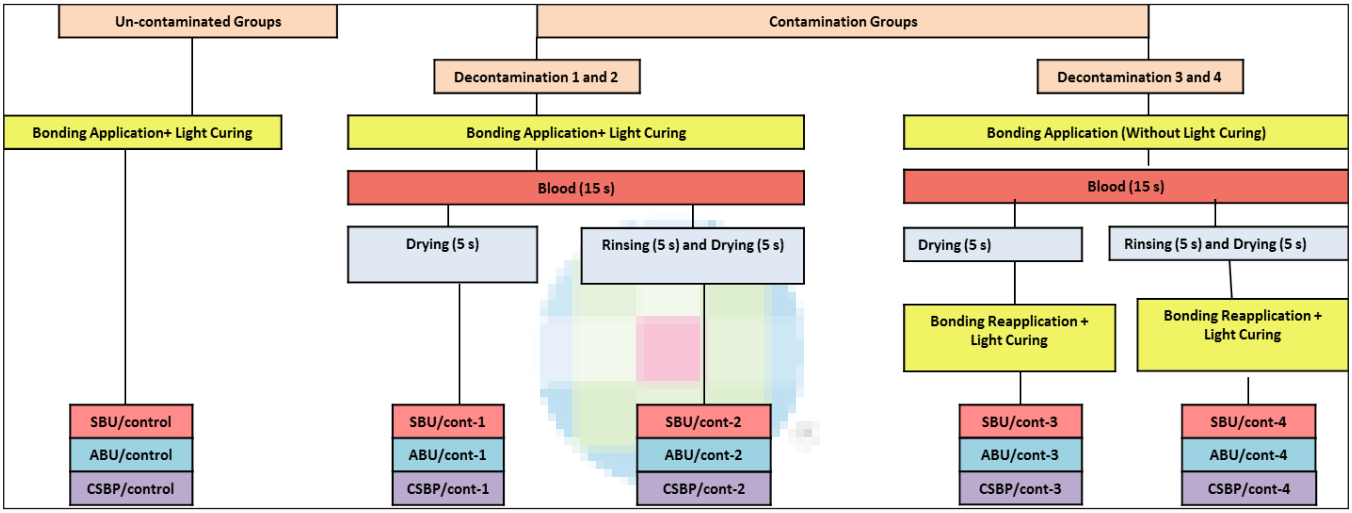

Figure 1: Schematic representation of the experimental design. The steps at which blood contamination occurred during the bonding procedure and the methods of decontamination are described.

Contamination-1 groups (negative control)

The adhesive systems were applied to the dentin specimens according to the manufacturers' instructions and light-cured. The bonded surface was contaminated with fresh blood for 15 s using a microbrush. Fresh human capillary blood was collected from a single participant simultaneously during specimen preparation. The surface was gently dried with oil-free compressed air for 20 s from a distance of 10 cm.

Contamination-2 groups

The adhesive systems were applied to the dentin specimens according to the manufacturers' instructions and light-cured. Then, the bonded surface was contaminated with fresh blood as described earlier. The blood was rinsed with water administered from an air-water syringe for 10 s and gently air-dried for few seconds.

Contamination-3 groups

The adhesive systems were applied to the dentin specimens according to the manufacturers' instructions without light curing, and the bonded surface was contaminated with fresh blood as described earlier. The blood was then gently air-dried for 20 s from a distance of 10 cm. The adhesives were re-applied to the surface and light-cured.

Contamination-4 groups

The adhesives were applied to the dentin specimens according to the manufacturers' instructions without light curing. The bonded surface was contaminated with fresh blood as previously described above. The blood was rinsed with water administered from an air-water syringe for 10 s and gently air-dried for few seconds, with care taken to avoid surface desiccation. The adhesives were reapplied to the surface and light-cured.

Following the adhesive application, the shade A2 nanohybrid resin composite (Filtek™ Ultimate, 3M ESPE, Minnesota, USA) was placed in three increments, in which each layer was light-cured to create a 5-mm layer on the bonded surface.

The specimens were stored in distilled water at 37°C for 24 h and vertically sectioned into 1.2 mm × 1.2 mm × 8 mm beams perpendicular to the bonded interface by using a low-speed water-cooled diamond saw. Five central nontrimmed beams from each tooth were selected for the μTBS test. Each subgroup contained 25 beams.

Microtensile bond strength test

The dimensions of each beam specimen were measured using a digital caliper (±0.01 mm). Each specimen was fixed to the microtensile test apparatus (Bisco Inc., Schaumburg, Illinois, USA) by using a cyanoacrylate adhesive (Pattex, Henkel, Dusseldorf, Germany). The μTBS test was performed at a 1.0-mm/min crosshead speed until failure occurred. The μTBS was calculated in MPa by dividing the maximum load at fracture by the bonded surface cross-sectional surface area. If any debonding occurred during specimen sectioning or mounting, the μTBS was recorded as 0 MPa.

The failure mode was determined using an optical stereomicroscope (Stemi 2000-C; Zeiss, Oberkochen, Germany) and a digital camera at 40 × magnification. Failure mode was classified as (1) adhesive failure, (2) mixed failure, or (3) cohesive failure.

Statistical analysis

Statistical analyses were performed using the SPSS software system, version 20.0 (IBM Corporation, New York, USA). The sample size was calculated considering 80% power and a significance level of 0.05 using data (effect size = 0.90) obtained from the study by de Carvalho Mendonça *et al.*[15] Although the data in this study suggested that a total of three specimens would be sufficient for the analysis, a worst-case scenario was proposed with a 0.6 effect size. According to this scenario, a total sample size was calculated to be 75 (n = 5) considering 89% power at a significance level of 0.05.

The interaction between the adhesive type and contamination type on μTBS was determined using two-way ANOVA. Furthermore, one-way ANOVA and post-hoc tests were used to perform multiple comparisons. The Fisher's least significant difference (LSD) test was used when equal variances were assumed, and the Dunnett-C test was used when equal variances were not assumed. For all tests, the probability level for statistical significance was set at $\alpha = 0.05$.

Results

The μTBS values, the standard deviations, and the number of pre-test failures were compared between each group [Table 2], [Figure 2]; the failure modes are shown in [Figure 3]. Two-way ANOVA showed that the adhesive type, contamination type, and their interactions were significant ($P < 0.05$).

There was no significant difference between SBU/control and CSBP/control groups ($P > 0.05$) whereas the ABU/control group had the lowest μTBS value among the control groups ($P < 0.05$).

The lowest bond strength for all adhesives was observed in the cont-1 groups ($P < 0.05$). The SBU/cont-1 group showed significantly higher bond strength than the other contamination-1 groups ($P < 0.05$). Pre-test failure occurred only in contamination-1 groups; all specimens in the ABU/cont-1 and CSBP/cont-1 groups and eight specimens in the SBU/cont-1 group were affected.

The ABU/cont-2 group had the highest μTBS value of the contamination-2 groups ($P < 0.05$). No significant difference was observed between the adhesive types in both the contamination-3 and contamination-4 groups.

The all cont-3 and ABU/cont-4 groups had μTBS values similar to their corresponding positive controls, whereas the other groups showed significantly lower μTBS values than their corresponding positive controls ($P < 0.05$).

Different failure modes were observed in the specimens depending on the contamination type and adhesive tested [Figure 3]. In general, specimens with a lower bond strength failed more frequently at the resin–dentin interface (adhesive failure), whereas those with higher bond strengths more frequently experienced mixed and cohesive failures.

Discussion

Preventing blood contamination during restoration can be a clinical challenge without proper isolation, especially in the cavity walls near the gingival margins. The rationale behind this study was to establish a proper decontamination method to regain the μTBS of multimode adhesives when blood contamination was carried out at different steps of the bonding process.

The performance of the adhesives used in this study was different when the dentin specimens were not contaminated. All-Bond Universal (ABU/control) adhesives were observed to have a lower μTBS than that of the other multimode adhesive (Single Bond Universal [SBU/control]) and the control 1-step adhesive (Clearfil S3 Bond Plus [CSBP/control]) in uncontaminated groups. In the present study, multimode adhesives showed

Table 2: Microtensile bond strength values means (standard deviations) in MPa, number of pre-test failure, and the statistical comparisons

Contamination	Adhesives	Mean (SD)	*p* value
Control Groups	Single Bond Universal	27.0 (6.1)^{A} 0/25	0.009 §
	All Bond Universal	23.2 (5.8)^{B} 0/25	
	Clearfil S^3 Bond Plus	28.2 (5.4)^{A} 0/25	
Contamination-1 Groups	Single Bond Universal	8.1 (2.2)^{a} 8/25	0.000 ¶
	All Bond Universal	0 (0)^{b} 25/25	
	Clearfil S^3 Bond Plus	0 (0)^{b} 25/25	
Contamination-2 Groups	Single Bond Universal	11.3 (3.9)^{x} 0/25	0.000 ¶
	All Bond Universal	16.1 (5.7)^{y} 0/25	
	Clearfil S^3 Bond Plus	12.4 (2.5)^{x} 0/25	
Contamination-3 Groups	Single Bond Universal	24.4 (5.8)$^{\alpha}$ 0/25	0.190 ¶
	All Bond Universal	22.8 (3.8)$^{\alpha}$ 0/25	
	Clearfil S3 Bond Plus	25.5 (6.0)$^{\alpha}$ 0/25	
Contamination-4 Groups	Single Bond Universal	21.3 (6.9)$^{\Phi}$ 0/25	0.526 ¶
	All Bond Universal	20.3 (4.2)$^{\Phi}$ 0/25	
	Clearfil S3 Bond Plus	22.0 (4.3)$^{\Phi}$ 0/25	

* Same superscript indicates no statistically significant difference (p<0.05). §: Fisher LSD, ¶: Dunnett-C test.

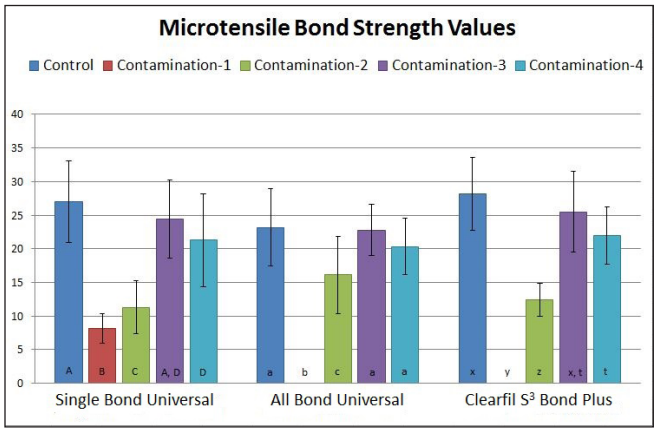

Figure 2: Microtensile bond strength values in MPa and the statistical comparisons (Dunnett-C test)

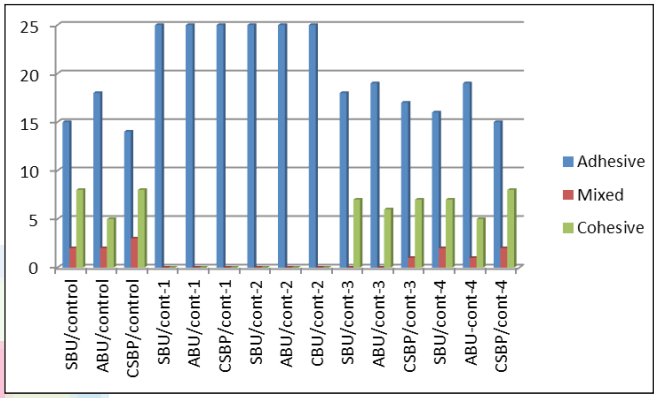

Figure 3: Failure modes

different results than those previously reported with respect to the bond strength to dentin. Muñoz *et al.*[25] and Lee *et al.*[26] observed a significantly lower μTBS to dentin in All-Bond Universal compared with Single Bond Universal and Clearfil S3 Bond Plus adhesives, whereas Wagner *et al.*[27] reported no significant differences in bond strength between Single Bond Universal and All-Bond Universal. Therefore, the compositional differences in the adhesives may play an important role in their bonding performance in the present study. One would have expected that similar performances would be observed among all the adhesives, because all of these include the 10-methacryloyloxydecyl dihydrogen phosphate (MDP) monomer, which is capable of chemically bonding to hydroxyapatite by self-assembling into nanolayers at the adhesive interface; additionally, all adhesives include hydroxyethyl methacrylate (HEMA), a water-soluble methacrylate monomer that increases the wettability and hydrophilicity of the adhesives.[28,29] However, the amount of MDP and HEMA incorporated into the adhesives markedly influences the mechanical properties and stability of the adhesive interface,[30] which may explain the different bond strengths between adhesives tested in this study. In addition, the presence of the polyalkenoic acid polymer in the composition of Single Bond Universal may explain the higher bond strength of this adhesive than that of All-Bond Universal. Although polyalkenoic acid polymer and MDP may compete for calcium bonding sites in hydroxyapatite,[29] these monomers are generally associated with improved bonding performance.[5,31]

The null hypothesis was rejected because different blood contamination procedures affected the μTBS of the tested adhesives to dentin. When the contamination occurred after light curing, two approaches were used to counteract any decrease in μTBS: air-drying alone (contamination-1) or rinsing followed by drying (contamination-2). Air-drying after light curing named negative control (cont-1 groups) resulted in the worst dentin μTBS values, which is consistent with previous reports.[15,32,33] Residual blood proteins adsorbed onto the polymerized bonding surface may have potentially

impaired dentin adhesion by eliminating an oxygen-inhibited layer that would have improved adhesion between successive layers through inherent unreacted monomers.[10,14,15,32,34]

When blood was rinsed with water and the specimen was air-dried after light curing (cont-2 groups), the bonding performance improved, but the μTBS was still lower than the values in the corresponding control groups. de Carvalho Mendonça *et al*.[15] also found that blood contamination impaired dentin adhesion of a two-step self-etch system when it occurred after light curing, and decontamination by water-rinsing and air-drying did not completely recover the reduced bond strength. Yoo and Pereira[35] reported similar results in a one-step self-etch adhesive, but they re-applied the adhesive after rinsing and drying the blood contaminants. This may result from the bonding resin's limited thickness, or alternatively, the disorganization of the oxygen-inhibited and nonpolymerized layers by rinsing.[33] Shiraishi[36] also suggested that blood proteins remained on the surface even after rinsing with water, presumably due to the strong surface attachment of the blood components, which may inhibit polymerization.

When the contamination occurred before light curing, two approaches were used to counter reduced μTBS values: air-drying (contamination-3) or rinsing and drying (contamination-4), each of which was followed by the re-application of the adhesive resin. The present findings indicate that adhesive re-application increased the μTBS based on the adhesive type and decontamination method applied. Drying and adhesive re-application increased the μTBS in all of the tested adhesives (cont-3 groups) compared with that of the corresponding control groups (SBU/control, ABU/control, and CSBP/cont-1 groups). Rinsing and drying followed by adhesive re-application was only effective in the All-Bond Universal adhesive group (ABU/cont-4). The superior results in the cont-3 groups may reflect the removal of the outer adhesive layer contaminated with blood during drying. Furthermore, the outer surface of the nonpolymerized adhesive layer may have been renewed following re-application of the bonding layer. However, in the SBU/cont-4 and CSBP/cont-4 groups, rinsing the contaminated adhesive layer with water may have not entirely removed the adhesive layer but instead may have degraded it. Thus, any water remaining after rinsing or the deteriorated adhesive layer may have impaired the bonding between the adhesive and composite. In contrast, the ABU/cont-4 specimens had a μTBS similar to its control; this outcome may be explained by differences in the adhesive systems' viscosity and film thickness. All-Bond Universal has a lower film thickness and viscosity, which may make it easier to remove from the entire cavity through rinsing.[37] This finding indicates that if a rinsing process is used to remove an uncured, contaminated adhesive layer, the clinician should verify that the adhesive layer is thoroughly removed, to prevent deterioration of the bonding performance due to the residual adhesive layer impaired by excessive water. In literature, alternative cleansing agents such as NaOCl, ethanol, sodium ascorbate were tested for decontamination when the dentin was contaminated with blood before etch and rinse adhesive application. NaOCl was found effective in restoring the μTBS to the same level as that of control when used with sodium ascorbate.[38] Such cleansing agents that are used to dissolve the organic tissue may be tested to remove blood contaminated adhesive layer for counteracting any decrease in bond strength of self-etch adhesives in further studies.

When the performance of each adhesive in the contamination-3 groups was compared with its counterpart in the contamination-4 groups (SBU/cont-3 vs. SBU/cont-4, ABU/cont-3 vs. ABU/cont-4, and CSBP/cont-3 vs. CSBP/cont-4), no significant differences were observed between the μTBS values. This observation indicates that the systems have similar bonding effectiveness if they are contaminated before light curing and decontaminated by the methods used in this study.

Conclusion

Within the limits of the current investigation, it can be concluded that neither of the decontamination methods used in this study counteracted any reduction in adhesive bond strength due to blood contamination occurring after light curing. Drying the blood contaminants and reapplying the adhesive may recover dentin adhesion in multimode adhesives when the contamination occurs before light curing. Alternatively, rinsing and drying the blood contaminants followed by adhesive reapplication may recover the dentin adhesion depending on the chosen adhesive. Considering that the use of multimode adhesives will become more widespread owing to their versatility and promising short-term performances, further studies are required to investigate alternative methods to counteract declines in bond strength due to blood contamination after light curing. Furthermore, alternative decontamination methods that can be used for all multimode adhesives and can enable the removal of adhesives discolored by blood contamination should be investigated when contamination occurs before light curing.

Financial support and sponsorship

Nil

Conflicts of interest

There are no conflicts of interest

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