Original Article

Effect of salivary contamination during different bonding stages on shear dentin bond strength of one-step self-etch and total etch adhesive

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Abstract:

Objective: This study evaluated the effect of saliva contamination during bonding procedures without removing saliva on shear dentin bond strength of three adhesive generations when rubber dam isolation is not feasible.

Materials and Methods: Flat superficial dentin surfaces of seventy-two extracted human molars were randomly divided into three groups (A: Scotch Bond MP Plus (SBMP), B: Single Bond (SB), C: Prompt L-Pop) according to the applied adhesives and twelve subgroups (n=6) according to the following saliva contamination applied in different bonding steps. The specimens were contaminated with saliva after etching (A1 and B1), after primer application (A2), after adhesive application before polymerization (A3, B2 and C1), and after adhesive polymerization (A4, B3 and C2). Three subgroups were not contaminated as controls (A5, B4 and C3). Resin composite was placed on dentin subsequently followed by thermocycling. Shear test was performed by Universal testing machine at 0.5 mm/min crosshead speed. The collected data were statically analyzed using one and two-way ANOVA and Tukey HSD. **Results:** In contrast to SBMP and SB, the mean shear bond strength of Promote L-Pop was not significantly different between contaminated and uncontaminated subgroups.

not significantly different between contaminated and uncontaminated subgroups. Mean shear bond strengths of SBMP subgroups contaminated after adhesive polymerization or uncontaminated were significantly higher compared to the other two groups (p<0.05). **Conclusion:** Unlike Promote L-Pop, saliva contamination could reduce shear bond strength

of the total-etch adhesives. Furthermore, the step of bonding procedures and the type of adhesive seems to be effective on the bond strength of adhesive contaminated with saliva.

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INTRODUCTION

The increasing popularity of esthetic restorations has drawn attention to long-term durability and bond success of these restorations. Adequate isolation and contamination control must be considered before bonding procedures. However, the difficulty of achieving moisture control is a potential problem encountered in clincal situations, especially when rubber dam isolation is unfeasible. Saliva contamination more probably occurs in regions near or at the gingival margin and many carious lesions are found in these areas isolated difficulty [1-5].

The effects of salivary contamination on bond strength have been investigated in several studies. Some studies have suggested that saliva contamination could reduce the bond strength of adhesive systems [6-9]. Others have reported that saliva contamination could not significantly affect modern adhesives when compared to the previous generation of bonding agents [10-14]. Moreover, effects of saliva contamination were not the same in different stages of bonding when modern adhesives were used [6,15].Reduction of bond strength as a result of saliva contamination may relate to the type of resin adhesive and the stage of bonding procedures. The bond strengths may be restored by different contaminant-removing treatments depending on the stages of bonding process contaminated such as re-conditioning, washing with water and re-application of the adhesive [3-10,12,13,16,17]. There are a few studies evaluating the effect of saliva contamination without any treatment on bond strength of different adhesive systems when moisture control means very difficult achievement [17].

Self-etch adhesives contain non-rinse acidic monomers that simultaneously condition and prime, and vinyl groups that co-polymerize with resin composite. This was followed by development of a so-called self-etching primer that can etch and prime in one step. Finally the one self-etch or so called all-in-one adhesive was introduced which conditions, primes and bonds in a single step. The self etching adhesive provides decreased clinical application time and reduces the risk of saliva contamination, especially when the carious site is near or at the gingival margin and maintaining a dry field may be impossible. In addition, the technique sensitivity of this adhesive which bonds to a dehydrated collagen matrix is eliminated as a result of its water component [14,18].

The null hypothesis of this study was that saliva contamination would not affect the dentin bond strength of self-etch adhesive.

In order to test this hypothesis, the present study evaluated the effect of saliva contamination without eliminating saliva during different bonding steps of these adhesives.

MATERIALS AND METHODS

In this experimental interventional study, seventy-two extracted human first molars were cleaned, stored in 0.5% chloramines-T solution for 7 days, and then immersed in 0-4°C stilled water for a maximum of 6 months until further processing in the laboratory. Teeth were mounted in cylindrical molds using selfcuring acrylic resin up to their cervical areas. Buccal enamel of mounted teeth was then eliminated by diamond disc (D&Z, Diamant, Germany) and the superficial dentin was exposed in a depth of 1 mm. These surfaces were polished using 600-grit silicon carbide paper in order to prepare a uniform surface and a smear layer. The specimens were randomly divided into three groups according to the materials, and for each group the adhesive was placed on the prepared surfaces according to the manufacturer's recommendations (Table 1). A thin saliva layer collected from a single individual was applied on the surfaces with brush during the different steps of bonding mentioned in the following subgroups and left undistributed for 5 seconds. The three adhesives were applied on prepared surfaces according to the manufacturer's instructions (Table 1) as follows:

Group A, Scotch Bond Multi Purpose Plus (SBMP)

Subgroup A₁: Specimens were contaminated with saliva after etching.

Subgroup A₂: Specimens were contaminated with saliva after primer application.

SubgroupA₃: Specimens were contaminated with saliva after adhesive application before its polymerization.

Subgroup A₄: Specimens were contaminated with saliva after polymerization of adhesive.

Subgroup A_5 : Uncontaminated group as control.

Group B, Single Bond (SB)

Subgroup B_1 : Specimens were contaminated with saliva after etching.

Subgroup B_2 : Specimens were contaminated with saliva after the adhesive application be-

fore its polymerization.

Subgroup B_3 : Specimens in this group were contaminated with saliva after polymerization of the adhesive.

Subgroup B₄: Uncontaminated group as control.

Group C, Prompt L-Pop

Subgroup C_1 : Specimens were contaminated with saliva after adhesive application before its polymerization.

Subgroup C_2 : Specimens were contaminated with saliva after polymerization of the adhesive.

Subgroup C_3 : Uncontaminated group as control.

After the bonding procedure, the resin composite (Z-100, 3M Dental products, St.

Paul, MN, USA) was built up in two increments using plastic mold (Inner diameter: 3mm and height: 3mm) and individually lightcured for 40 seconds (Coltolux 75, Coltene/

Whaledent, Mahwah, NJ, USA, 500 mw/cm² measured by Digital Radiometers). All prepared specimens were thermocycled for 500 cycles between $5^{\circ}-55^{\circ}C$ with a 30 second dwell time. After storage of specimens in distilled water at 37 °C for 24 hours, shear test

was performed by Universal testing machine (Zwick/RoellZ020, Zwick GmbH & Co, KG, Germany) at the crosshead speed of 0.5 mm/min. The mechanical loading was applied to the interface of composite and dentin until debonding of the composite and the data were registered in MPa. Finally, the mode of failures which occurred during debonding were determined by stereomicroscopic (SMZ 1500, Nikon, Kanagawa, Japan)(×20).

The collected data were statistically analyzed using two-way ANOVA for three adhesives in order to compare among the subgroups which were not contaminated (A_5 , B_4 , C_3), contaminated before (A_3 , B_2 , C_1) and after (A_4 , B_3 , C_2) adhesive polymerization.

Consequently, because the interaction of saliva contamination and the adhesive type was significant, one-way ANOVA and Duncan's post hoc test were conducted for each adhesive in different contaminated bonding steps and for each contaminated bonding step with different adhesives.

The level of significance was adjusted using the Bonferroni method.

Material	Composition	Procedures
Scotch Bond Multipurpose Plus (3M, St. Paul, MN, USA)	Scotch Etch, 37% H ₃ po ₄ Silica thickened Primer: HEMA, polyalkenoic acid copolymer, water Adhesive: Bis-GMA,HEMA	15s acid etch, rinse with water, blot dry, ap- ply primer and wait for 30s, gently air flow and repeat step until glossy appearance, ap- ply resin adhesive, gently air flow, light cured for 20s.
Single Bond (3M ESPE, St. Paul, MN, USA)	Etchant : %37 H ₃ po ₄ Adhesive: Polyalkenoic acid copolymer, Bis -GMA, HEMA, Dimethacrylates, Water, Ethanol, Photoinitiator.	15s acid etch, rinse with water, blot dry, ap- ply adhesive 2 coats, mild air flow, light cured for 10 s.
Prompt L-Pop (3M ESPE, St. Paul, MN, USA)	Water, methacrylated phos- phoric acid esters, fluoride complex w/ zinc, parabenes	Apply the activated liquid mixture for 15 s with agitation, gently air thin, apply a second coat, gently air thin, light cure for 10 s

Table 1.	The utilized	materials

HEMA: Hydroxyethyl Methacrylate; Bis-GMA: Bisphenol A-Glycidyl-Methacrylate

RESULTS

Table 2 summarizes the mean shear bond strengths and standard deviations of different groups and subgroups.

The effect of each contaminated bonding step on different adhesives' shear bond strength is revealed as follows: a) In the uncontaminated condition, shear bond strength of Prompt L-Pop was significantly lower than SBMP's (p<0.05); however, this value was not significant between SB and the other two groups. b) When saliva contamination occurred before adhesive polymerization, shear bond strengths of the three adhesives were not significantly different (p=0.43), c) There was significant difference in the contamination applied after adhesive polymerization (SBMP>SB> Prompt L-Pop) (p<0.05).

The effect of each adhesive in different contaminated bonding steps on the shear bond strength is as follows. There was significant difference between the shear bond strength of contaminated and uncontaminated specimens in groups A and B (p<0.05); whereas, in group C there was no such difference (p=0.411). In addition, there was no significant difference between subgroups A_2 and A_3 (p=0.714), subgroups A_1 , A_2 and A_4 (p=0.054), and subgroups B_1 , B_2 and B_3 . (p=0.16).

Stereomicroscope observation showed that the specimens' fracture types contained I) adhesive failure II) cohesive failure and III) mixed failure (Table 2).

DISCUSSION

This study evaluated the effect of saliva contamination during bonding steps without removing saliva on the shear dentin bond strength of three adhesives. The present results showed that saliva contamination and the type of contaminated adhesive could be effective on the bond strength. In contrast to SBMP and SB, saliva contamination did not affect the bond strength of Prompt L-Pop. In self-etching adhesive systems, all three basic steps (etching, applying primer and adhesive) occur simultaneously. Thus, at the same time these adhesives demineralize dentin while infiltrating it with monomers to the same depth, and then polymerization in situ is disclosed. Therefore, no gaps would be left between the resin surface and the demineralized dentin surface.

	No Contamination	After Etching	After Primer Application	Before Adhesive Polymerization	After Adhesive Polym- erization
Scotch Bond Multipurpose Plus	[A5] 29.05±9.88 1A, 1C, 4M	[A1] 21.32±5.33 2A, 1C, 3M	[A2] 13.77±4.93 3A, 2C, 1M	[A3] 12.45±4.45 3A, 1C, 2M	[A4] 20.56±4.66 2A, 2C, 2M
Single Bond	[B4] 23.20±6.28 2A, 2C, 2M	[B1] 14.99±1.92 3A, 1C, 2M	_	[B2] 11.69±3.53 4A, 1C, 1M	[B3] 15.47±4.06 3A, 1C, 2M
Prompt L-Pop	[C3] 13.67±4.47 4A, 1C, 1M	_	_	[C1] 11.03±2.62 4A, 0C, 2M	[C2] 10.94±4.35 5A, 1C, 0M

Table 2. The mean shear bond strength in MPa and types of fracture in the studied groups

A: Adhesive failure; C: Cohesive failure; M: Mixed failure

Simplicity, time saving and fewer time points for probable contamination during bonding procedures are the advantages of self-etching adhesive, especially in the saliva contamination condition [14,17,18]. In the present study, Prompt L-Pop was not affected by saliva contamination which may result from its water componant and simultaneous bonding steps. Thus, the hydrophilicity of this adhesive may allow its diffusion through the salivary film.

For the polymerized self-etch adhesive which was not significantly affected by saliva, it may be speculated that the chemical property of its poorly polymerized oxygen-inhibited surface may be responsible.

Shear bond strenght of total etch adhesives used in the present study are affected by saliva contamination. When steps of bonding were accomplished separately, some regions of the demineralized dentin may not be penetrated by the resins. In addition, there are longer time points during placement of these adhesives when contamination can occur [17]. When surfaces are contaminated with saliva after etching, water and glycoproteins of saliva may interfere with the proper adhesion. When surfaces are contaminated with saliva after application of primer and adhesive before light curing, saliva can affect the degree of conversion and bond strength; because hydroxyethyl methacrylate (HEMA) molecules with their hydrophilic nature may retain water within the adhesive layer and they dispersed in water, thus they become unable to participate in chain growth during polymerization. When surfaces are contaminated with saliva after light curing, absorption of glycoproteins to the poorly polymerized, air-inhibited adhesive surface may cause reduction of bond strength. These glycoproteins may prevent complete infiltration of the next resin layer and sufficient copolymerization [3,12-14,17]. Saliva or blood contamination is a major clinical problem in restorative procedures, especially when the caries site is near or at the gingival margin [12]. In the preceding clinical situation, sulcular fluid and saliva contamination can not be controlled sufficiently. Therefore, in the present study unlike numerous previous reports, the specimens were not treated at all after contamination with saliva in order to evaluate the effect of contamination on bond strength when proper isolation is not possible.

Previous researchers have evaluated the effect of dried or rinsed-off saliva contamination [2-5,9,12-14,16]. This implies that the examiners were conscious of this contamination. In the present investigation, saliva-contaminated specimens were not blotted, dried or rinsed in order to study the effects of an "unobserved" saliva-contaminated surface for totaletch and self-etch ahesive.

In contrast to the results of our study, Johnson et al [9] showed that there was no significant difference in the mean shear bond strength of Scotch Bond MP Plus between control and contaminated groups, and lowest shear bond strength belongs to a group contaminated after primer application. Contrary to Johnson's [9] report in which the excess of saliva was gently shaken off and dried, in the present study saliva was not removed and the lowest shear bond belongs to the additional group contaminated with saliva after adhesive application before light curing (A3).

Abdalla *et al* [11] evaluated the effect of blood and saliva contaminations on shear dentin bond strength, and demonstrated that saliva could not affect the shear bond strength of one-bottle significantly. Unlike Abdalla *et al*'s study in which saliva contamination was removed only after etching, in the present study saliva contamination was examined during all bonding steps (after etching, after bonding, before caring, after bonding and after curing) without any treatment.

Few reports evaluated the effect of saliva contamination on shear bond strength of uncured adhesive between composite and dentin during bonding procedures. Contrary to Fritz *et al* [12] who showed that saliva contamination blot-dried before adhesive polymerization (acetone as a solvent) could not affect the shear bond strength, in our study this value was reduced may be due to the remaining saliva and different adhesive solvents (water and ethanol).

The results of our study demonstrated that the self etch adhesive with hydrophilic feature may be less sensitive to salivary contamination compared to previous generations of adhesive systems which is in agreement with the findings of previous studies for modern adhesives [6,12,17]. However, in these investigations contaminated surfaces were treated by decontaminated methods such as blot-dry or wash off [6,12]. Yoo et al [5] reported that saliva contamination could significantly affect the bond strength of all-in-one adhesive systems to dentin. Because in their study, the adhesive layer was removed during washing and drying of unpolymerized resin, and the demineralized surface remained without infiltration of monomers.

Although limited studies compared shear bond strength of different generations of adhesives, Abdalla *et al* [11] stated that the shear bond strength of the fourth generation adhesive (SBMP) was higher when compared to the fifth generation, but this difference was not significant. This report is in accordance with the result of our study which stated that there are no significant differences between shear bond strengths of the fourth and fifth generation (p=0.09), and also the fifth and seventh (p=0.366). However, significant differences are observed between the shear bond strength of the fourh and seventh generation (p<0.05).

CONCLUSION

In this in vitro investigation, Prompt L-Pop (self-etch adhesive) appears more tolerant to "unnoticed" saliva contamination compared to SBMP and SB (total etch adhesive). In contrast to the self etch adhesive, the total etch

adhesive groups displayed a significant decrease in mean shear bond strength when contaminated with saliva, but the difference between contaminated subgroups was not significant. However, mean shear bond strengths of SBMP in contaminated or uncontaminated subgroups were higher in comparison to the other two groups.

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