Effect of Saliva Contamination and Cleansing Solutions on the Bond Strengths of Self-Etch Adhesives to Dentin

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ABSTRACT

Purpose: This study determined the effect of saliva contamination and cleansing solutions on microtensile bond strengths of self-etch adhesives to dentin.

Materials and Methods: Seventy-five human molars were ground flat to expose mid-coronal dentin and randomly assigned to five groups ($N = 15$): no contamination, saliva contamination without cleansing, saliva and cleansing with water, saliva and cleansing with 2% chlorhexidine, and saliva and cleansing with 5% sodium hypochlorite. One-third of the specimens in each group of 15 were bonded with Adper Prompt L-Pop (all-in-one self-etch adhesive; 3M ESPE, St. Paul, MN, USA), one-third with Adper Easy Bond (all-in-one self-etch adhesive; 3M ESPE), and one-third with Clearfil SE Bond (self-etch primer system; Kuraray America, New York, NY, USA). Specimens were restored with composite and processed for microtensile bond strength testing (5–6 rods/tooth).

Results: Mean bond strengths ranged from 17.3 MPa for Adper Prompt L-Pop after water cleansing to 69.3 MPa for Clearfil SE Bond after water cleansing. For all three adhesives, there was no statistically significant difference in bond strengths between the saliva contaminated group, the cleansing groups, and the no contamination groups.

Conclusions: Neither saliva nor the cleansing solutions adversely affected bond strengths of the self-etch adhesive systems.

CLINICAL SIGNIFICANCE

Saliva contamination of dentin does not seem to adversely affect bonding with self-etch adhesive systems. These results should be considered preliminary and need confirmatory studies before conclusive recommendations can be made for clinical practice.

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INTRODUCTION

The effect of saliva contamination of the tooth on dentin bonding has been investigated, but the results are somewhat inconclusive. Several studies report that saliva contamination has a detrimental effect on resin bonding to dentin.[1–3] In one study, reapplication of the adhesive after drying or rinsing off the saliva reestablished bond strengths to control levels.[1] Two studies suggest that complete drying of the saliva-contaminated surface should be avoided, as this may decrease bond strengths.[4,5] Others have reported that adhesives perform remarkably well even when the saliva is not removed after acid-etching,[4,6,7] which might be explained by the high percentage of hydrophilic solvents these agents contain, such as acetone or alcohol.[8] Hydrophilic bonding agents may be attracted by the saliva moisture, promptly spreading upon moist dentin or displacing the adsorbed saliva.[9] Therefore, the outcome may be related not only to the contaminant but also to the type of adhesive used.

When saliva contamination of the preparation occurs after acid-etching in anticipation of using an etch-and-rinse adhesive, phosphoric acid can be briefly reapplied and rinsed to effectively cleanse the site.[5] However, the same strategy could be detrimental to the performance of self-etch adhesives, as it has been shown that pretreatment of dentin with phosphoric acid significantly reduces bond strengths of self-etch adhesives.[10]

Given the paucity of studies on the effect of saliva contamination and of different cleansing solutions on dentin bond strengths when contemporary self-etch adhesive systems are used, the purpose of this study was to examine the effect of saliva contamination and different cleansing solutions on the bond strengths of self-etch adhesives to dentin. Water, chlorhexidine, and sodium hypochlorite were used as cleansing agents. The null hypotheses tested were (1) that saliva contamination has no effect on dentin bond strengths of self-etch adhesive systems, and (2) that rinsing saliva-contaminated dentin with water, chlorhexidine, or sodium hypochlorite has no effect on the dentin bond strengths of self-etch adhesive systems.

MATERIALS AND METHODS

A pilot study using 10 extracted intact human molars determined that 75 teeth would be required to power the study at 80%. Seventy-five intact human molars were collected and stored in a solution of 0.5% chloramine trihydrate at 4°C. The occlusal surfaces of the specimens were sectioned to expose mid-coronal dentin and polished using 600-grit silicon carbide paper under water to create a uniform smear layer.[11] Peripheral enamel was removed using diamond rotary instruments in water-cooled high-speed handpiece. The specimens were randomly divided into five groups of 15 specimens each:

1. Group A—Dentin was not contaminated (positive control)
2. Group B—Dentin was contaminated with human saliva for 5 seconds (no cleansing, negative control)
3. Group C—Dentin was contaminated with human saliva for 5 seconds and the contaminated surface was rinsed using distilled water for 2 seconds
4. Group D—Dentin was contaminated with human saliva for 5 seconds and the contaminated surface was cleansed using 2% chlorhexidine digluconate solution (Cavity Cleanser, Bisco, Schaumburg, IL, USA) applied by lightly scrubbing with a microbrush for 5 seconds. Then the surface was rinsed with distilled water for 2 seconds
5. Group E—Dentin was contaminated with human saliva for 5 seconds and the contaminated surface was cleaned with 5% sodium hypochlorite (ACROS Organics, Somerville, NJ, USA) applied by lightly scrubbing with a microbrush for 5 seconds. Then the surface was rinsed with distilled water for 2 seconds.

TABLE 1. TRADE NAMES, MANUFACTURERS, LOT NUMBERS, AND COMPOSITION OF THE PRODUCTS USED IN THIS STUDY.

Product	Composition
5% sodium hypochlorite solution ACROS Organics Somerville, NJ, USA LOT A0248559	5% NaOCl
Cavity cleanser Bisco, Schaumburg, IL, USA 2% Chlorhexidine Solution LOT 0700007926	2% Chlorhexidine Digluconate ($C_{22}H_{30}Cl_2N_{10} \cdot 2\ C_6H_{12}O_7$)
Adper Prompt L-Pop 3M ESPE, St. Paul, MN, USA LOT 332454	methacrylated phosphoric esters, Bis-GMA, initiators based on camphorquinone, stabilizers, water, 2-hydroxyethyl methacrylate (HEMA), polyalkenoic acid
Adper Easy Bond 3M ESPE LOT 299001	2-hydroxyethyl methacrylate, bisphenol a diglycidyl ether dimethacrylate, water, ethanol, phosphoric acid-6-methacryloxy-hexylesters, silane treated silica, 1,6-hexanediol dimethacrylate, copolymer of acrylic & itaconic acid, (dimethylamino)ethyl methacrylate, camphorquinone, 2,4,6-trimethlybenzoyldiphenylphosphine oxide
Clearfil SE Bond Kuraray Dental, Kurashiki, Japan LOT 61832	10-methacryloyloxydecyl dihydrogen phosphate, 2-hydroxyethyl methacrylate, hydrophilic dimethacrylate, dl-Camphorquinone, N,N-Diethanol-p-toluidine, water, bis-phenol A diglycidylmethacrylate, silinated colloidal silica
Filtek Supreme Plus Universal Restorative A2 Body Shade 3M ESPE LOT 20070802	silane treated ceramic, silane treated silica, bisphenol a polyethylene glycol diether dimethacrylate, diurethane dimethacrylate bisphenol a diglycidyl ether methacrylate, triethylene glycol dimethacrylate, water

The specific cleansing agents were selected as they are readily available to clinicians and would be likely choices for removing saliva contamination.

One-third of the specimens in each group ($N = 5$) were bonded with a two-component all-in-one self-etch adhesive (Adper Prompt L-Pop, 3M ESPE, St. Paul, MN, USA). One-third of the specimens in each group ($N = 5$) were bonded with another all-in-one self-etch adhesive system (Adper Easy Bond, 3M ESPE), and one-third with a two-step self-etch adhesive system (Clearfil SE Bond, Kuraray Dental, Kurashiki, Japan). The compositions of all products used are listed in Table 1, and the flowchart of the experimental procedures is depicted in Figure 1.

The adhesives were applied and light-activated according to manufacturers' recommendations, after the surfaces were dried using a compressed air syringe at a distance of 5 cm from the tooth surface for 5 seconds. (A pilot study showed no significant difference between air and blot drying the prepared tooth surface prior to bonding.) Adper Prompt L-Pop was applied with a rubbing motion for 15 seconds, then gently but thoroughly air-dried to remove the aqueous solvent. A second coat was then applied (no waiting time for the second layer) and gently but thoroughly air-dried to remove the aqueous solvent. Finally, the adhesive was light cured for 10 seconds.

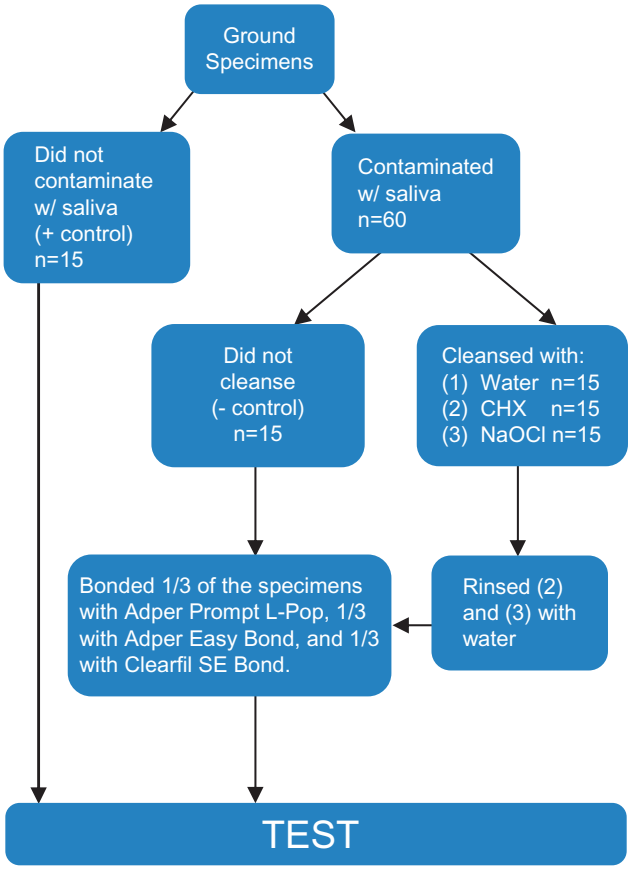

Figure 1. Flowchart of experimental procedures.

Adper Easy Bond was applied for a total of 20 seconds by lightly scrubbing with a microbrush, then dried for 5 seconds at a 5-cm distance, and light-cured for 10 seconds.

Clearfil SE Bond primer was applied and left for 20 seconds, then dried with gentle air flow at a 5-cm distance. The Clearfil SE Bond bonding agent was applied and dispersed with mild air flow to evenly distribute on the surface of the tooth. The adhesive was then light-cured for 10 seconds.

Composite resin (Filtek Supreme Plus, 3M ESPE) was used incrementally to build-up the specimen to a thickness of 4 mm. Each increment was light-activated using a high intensity L.E.Demetron II (Kerr Corporation, Orange, CA, USA) unit operating at >800 mW/cm^2 for 20 seconds. The specimens were stored in distilled water for 24 hours.

Each specimen was fixed in an epoxy resin block with sticky wax. Specimens were sectioned mesiodistally using a water-cooled low-speed Isomet 1,000 diamond micro-slicing saw (Buehler, Lake Bluff, IL, USA) to obtain 0.9-mm thick sections. The sections were further cut faciolingually to obtain 6-mm-long, 0.9-mm-thick rods, with the dentin-composite interface located at the center. Each specimen had a cross-sectional area of 0.9 ± 0.2 mm^2, measured with a digital caliper (Digimatic IP67, Mitutoyo Co., Kawasaki, Japan). Between five and nine rods were obtained for each tooth.

Each specimen was fixed to a Ciucchi Jig (EZ-Test, Shimadzu, Kyoto, Japan) using a cyanoacrylate-based adhesive (Zapit Base and Accelerator, Dental Ventures of America Inc, Corona, CA, USA). The specimens were carefully placed on the jig so that the composite-dentin interface was exactly perpendicular to the axis of the testing assembly. The microtensile bond strengths of all specimens were tested using a universal testing machine (EZ-Test, Shimadzu) with a crosshead speed of 1 mm/min. The bond strength (MPa) of each specimen was determined as the failure load (N) divided by the cross-sectional area of the bonded interface.

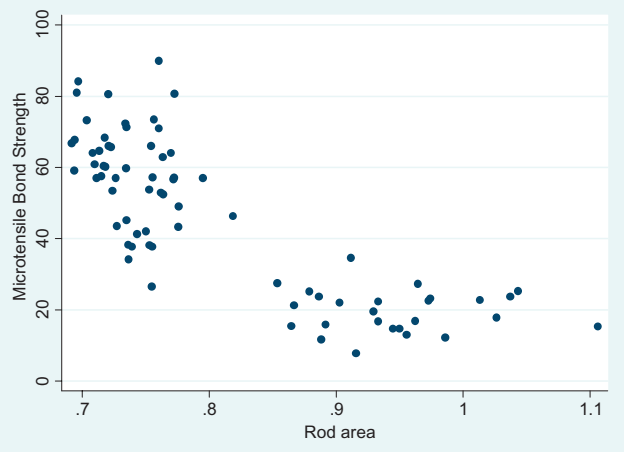

Figure 2. Relationship between rod area and microtensile bond strength ($r = –0.8094$, $p < 0.0001$).

Data were plotted using MS Excel 2007 (Microsoft, Mountain View, CA, USA) and analyzed using Stata 10 (StataCorp, College Station, TX, USA). Microtensile bond strength (MTBS) was the primary outcome (dependent variable), whereas surface treatment (saliva contamination and cleansing protocol) and adhesive were the independent variables, with tooth, rod, area, width, and thickness as independent co-variables. Means were adjusted for area and tooth to account for variations in measurements and potential differences in specimens. We examined the correlations between MTBS and rod area, width, and thickness using pairwise correlation coefficients. To explore interactions and compare means between groups, data were subjected to factorial analysis of variance (ANOVA) and Tukey's HSD post hoc tests, where indicated, with $p = 0.05$ significance level. MTBS results for rods originating from the same tooth were pooled and averaged, so that the tooth was the unit of analysis.

RESULTS

A negative linear relationship was noted between MTBS and area ($r = –0.8094$, $p < 0.0001$, Figure 2), MTBS and width ($r = –0.7658$, $p < 0.0001$), and MTBS and thickness ($r = –0.7658$, $p < 0.0001$). These negative relationships indicate that, as expected, the smaller the rod area, width, and thickness, the higher the MTBS. Using the tooth as the unit of analysis, there was no significant interaction between adhesive and treatment ($p = 0.076$). The results of the factorial ANOVA and Tukey's HSD post hoc tests (mean MTBS and standard deviations) are presented in Table 2.

For all three adhesives, there was no statistically significant difference in mean MTBS between surface treatments, indicating that neither saliva contamination nor the cleansing protocols negatively affected MTBS obtained with the positive control, uncontaminated dentin surface. When comparing adhesives within surface treatments, in general, the MTBS results were higher for Clearfil SE Bond. Clearfil SE Bond had significantly higher mean MTBS than Adper Easy Bond and Adper Prompt L-Pop for both positive and negative control treatments. For the three cleansing protocols (water, chlorhexidine, and sodium hypochlorite), MTBS obtained with Clearfil SE Bond was similar to those obtained with Adper Easy Bond, and significantly higher than the MTBS obtained with Adper Prompt L-Pop.

DISCUSSION

The purpose of this study was to examine the effect of saliva contamination and of different cleansing agents on the dentin bond strengths of self-etch adhesives to saliva-contaminated dentin. The results showed that the tested adhesives were not affected by saliva contamination and that all tested cleansing solutions, after being rinsed with water, did not negatively affect the bond strengths of the adhesives tested. We

TABLE 2. MICROTENSILE BOND STRENGTH MEANS (SD) BY ADHESIVE AND SURFACE TREATMENTS (*N* = 5; RESULTS EXPRESSED IN MPa).*

Adhesive systems	Surface treatments/cleansing agents				
	No contamination	Saliva	Water	Chlorhexidine	Sodium hypochlorite
Adper Prompt L-Pop	20.1 (7.9)^{aB}	26.7 (14.1)^{aB}	17.3 (4.6)^{aB}	17.7 (4.3)^{aB}	20.6 (8.7)^{aB}
Adper Easy Bond	37.9 (4.4)^{aB}	51.0 (9.2)^{aB}	63.9 (13.8)^{aA}	49.3 (12.0)^{aA}	61.3 (11.3)^{aA}
Clearfil SE Bond	60.5 (7.0)^{aA}	72.0 (11.5)^{aA}	69.3 (10.2)^{aA}	62.5 (29.2)^{aA}	54.8 (20.3)^{aA}

*Same lowercase superscript letters indicate means that are not significantly different ($p > 0.05$) within rows (adhesives); same uppercase superscript letters indicate means that are not significantly different ($p > 0.05$) within columns (cleansing agents).

therefore failed to reject the null hypotheses tested.

Saliva contamination of tooth surfaces and its impact on the bond strengths of self-etch adhesive systems are potentially important clinical problems, with few and inconclusive studies addressing them. Currently available options to manage saliva contamination of tooth surfaces remain inadequate. The use of phosphoric acid gel to cleanse the tooth surface after saliva contamination results in over-etching of the dentin surface of the tooth, resulting in significant reduction of the microtensile bond strengths of self-etch adhesive systems.[10]

Water is an obvious and simple option to address saliva contamination of a prepared tooth surface. The water syringe may be the first device that many dentists reach for in anticipation of cleansing a contaminated tooth surface. In a study by Sattabanasuk and colleagues, a similar hypothesis was tested.[1] In one of the test groups, the prepared dentin surface was contaminated with saliva, rinsed with water, and the self-etch adhesive was reapplied. Bonding procedures were performed according to manufacturers' directions, and a composite resin was bonded onto the surface of the prepared tooth. The results showed that rinsing the contaminated tooth surface with water reestablished the bond strengths to a value similar to that of no contamination at all.[1] Our study also showed that for Adper Prompt L-Pop, Adper Easy Bond, and Clearfil SE Bond, cleansing with water did not adversely affect the bond strengths.

We selected 2% chlorhexidine digluconate solution as one of the cleansing agents for our study, given its established potential to maintain or even strengthen the microtensile bond strengths of self-etch adhesive systems.[12] An in vivo study by Carrilho and colleagues tested the hypothesis that chlorhexidine could be used to inhibit the degradation of resin-dentin bonds by blocking the action of matrix metalloproteinases.[12] These authors showed that the synthetic protease inhibitor, chlorhexidine, stabilized the bond strengths of the treated dentin surfaces as compared with the untreated tooth surfaces.[12] Our study concluded that chlorhexidine does not have any negative effect on the bond strengths of self-etch adhesives, although we were also unable to determine if chlorhexidine would improve the bond strengths of self-etch adhesives. Additional long-term studies may have to be done in order to determine the long-term effects of chlorhexidine on the bond strength of self-etch adhesive systems.

Sodium hypochlorite is another cleansing alternative, given its popular application as a bacterial reducing agent in intracanal preparations.[13] The fact that the pH of sodium hypochlorite is highly alkaline (~11) makes it a potentially acceptable alternative

as a cleansing agent, as it does not have etching potential and would have substantial benefit over the use of acidic agents such as phosphoric acid. These effects were highlighted in a study by Mountouris and colleagues where it was demonstrated that sodium hypochlorite has the potential to deproteinate the coronal dentin surface without affecting the carbonates and the phosphates.[14] This reiterates the fact that there is no dissolution of the mineral content of the tooth that takes part in the chemical adhesion to the restorative material. Our study showed that sodium hypochlorite followed by water rinsing does not have any detrimental effect on bond strengths of the adhesives tested.

The rationale for choosing Adper Prompt L-Pop, Adper Easy Bond, and Clearfil SE Bond as the self-etch adhesive systems is as follows. Adper Prompt L-Pop was chosen because its acidic pH (0.7) would allow us to observe the response of a strongly acidic all-in-one type self-etch adhesive to a contaminating agent. Adper Easy Bond was chosen because it is less acidic, with a pH of 2.7 (technical information, 3M ESPE), and it was of interest for this study to observe how a comparatively less acidic adhesive would respond to a contaminating agent. Clearfil SE Bond is a two-step self-etch system, has been used in many previous studies as the "standard" self-etch system, and has a relatively mild pH (2.0).

Human saliva was used as the contaminating agent rather than an artificial saliva or saliva substitute. Fresh whole human saliva is an acceptable substance in testing saliva contamination and adsorption.[15] It was pertinent to this study to make sure that there was a "real" contaminating agent used, or otherwise run the risk of conducting a study that had little or no clinical significance.

To standardize the contaminant, the saliva was collected from only one person, a healthy 26-year-old female, collected at fasting level early in the morning, before any oral hygiene regimen. Fasting-level saliva was collected in order to provide less variability in pH of the saliva, as well as altered electrolyte, enzyme, or protein content seen after consuming a food or drink.[16] Using a pH meter, it was determined that the pH of this saliva was an average of 7.4.

Typically, microtensile bond strength uses individual specimens as the unit of analysis. One potential problem with this approach is that specimens originating from the same tooth are not independent observations. Additionally, dentin from specimens obtained near the dentinoenamel junction is not the same compositionally as that of specimens obtained near the pulp. Using individual specimens as the unit of analysis can therefore result in biased data. To avoid this problem, in this study the tooth was used as the unit of analysis. MTBS values obtained from each specimen originating from the same tooth are averaged.

Although this was not our intent when planning the study and these results cannot be generalized to other adhesives, we noticed a negative correlation between the pH of the self-etch adhesive systems and the MTBS data. This observation suggests that less acidic self-etch adhesive systems tend to show higher microtensile bond strengths to dentin.

We also noted that, for all three self-etch adhesive systems tested, there was a numerical increase in microtensile bond strengths when applied to saliva-contaminated dentin without any rinsing, when compared with the positive control (no contamination). This unexpected finding may suggest that the tested self-etch adhesives may work acceptably in the presence of saliva contamination. Furthermore, if the numerical results are considered, saliva might even improve dentin bond strengths. This suggests that there might be

no need to cleanse the preparation surface after contamination with saliva prior to bonding with a self-etch adhesive. Simply drying the surface may suffice. It is possible that the inherent acidity of self-etch adhesive systems allows them to not only modify/penetrate the smear layer but also break through the mucopolysaccharides in the saliva and develop bond strengths comparable with those obtained on a noncontaminated dentin surface. This observation can possibly prove that, in a clinical situation, when saliva contamination of the prepared tooth surface occurs, it may be possible to maintain bond strengths after drying the surface. There may be no need to cleanse with any other agent.

As this was an in vitro study, the results cannot be generalized to the clinical setting. Although it may be possible that some self-etch adhesive systems are not affected by saliva contamination, as long as the tooth surface is dried, clinical studies are needed to confirm these results before broad generalizations can be made. Also, as only three self-etch adhesive systems were tested, we cannot generalize our results for all self-etch adhesives available in the market. Additionally, only one person's saliva was used as the contaminating agent, and not every person's saliva would necessarily have the same effects as the one used.

Because we rinsed the cleansing agents with water before proceeding with the bonding protocol, we are unable to determine the effect of the cleansing agents had they not have been removed but only dried before the application of the adhesives. We elected to rinse the cleansing agents because we assumed that clinicians would rinse the cleansing agent off the contaminated surface after it is applied. Nevertheless, future research could study the effect of chlorhexidine and sodium hypochlorite as surface contaminants.

CONCLUSIONS

Under the conditions of this study, the following conclusions can be made:

1. Saliva contamination of dentin did not adversely affect bonding with the three self-etch adhesive systems tested
2. Cleansing saliva-contaminated dentin surfaces with water, chlorhexidine, or sodium hypochlorite had no negative effect on the bond strengths of these self-etch adhesive systems.

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