

# **Effect of Cleansing Solutions on the Bond Strengths of Self-Etch Adhesives to Saliva-Contaminated Dentin**

By

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## **ABSTRACT**

**HUMA SHEIKH: Effect of Cleansing Solutions on the Bond Strengths of Self-Etch Adhesives to Saliva-Contaminated Dentin**  
(Under the direction of Dr. André V. Ritter)

This study determined the effect of cleansing solutions on microtensile bond strengths of self-etch adhesives to saliva-contaminated dentin. Seventy five human molars were ground flat to expose mid-depth dentin, and randomly assigned to 5 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 (n=5) were bonded with Adper Prompt L-Pop (all-in-one self-etch adhesive), one third with Adper Easy Bond (all-in-one self-etch adhesive), and one third with Clearfil SE Bond (self-etch primer system). Specimens were restored with composite, and processed for microtensile bond strength testing (5-6 rods/tooth). The cleansing solutions were able to cleanse saliva-contaminated dentin without adversely affecting the bond strengths of the self-etch adhesive systems. Our study showed that self-etch adhesive systems are not negatively affected by saliva contamination.

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## LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of variance
CHX	Chlorhexidine
MMPs	Matrix metalloproteinases
MPa	Mega Pascal
NaOCl	Sodium Hypochlorite
SD	Standard deviation
$\mu$ TBS	Microtensile bond strength



# **Effect of Cleansing Solutions on the Bond Strengths of Self-Etch Adhesives to Saliva-Contaminated Dentin**

## **Introduction**

Self-etch adhesive systems, also known as simplified adhesives or all-in-one adhesives, are currently popular in restorative dentistry. In a self-etch adhesive, etching enamel and dentin with phosphoric acid prior to bonding is not required because the adhesive solution itself is acidic, hence the term self-etch adhesive. Despite the popularity of self-etch adhesive systems, the “gold standard” of adhesive dentistry is still the three-step total-etch, or etch-and-rinse adhesive technique.<sup>1</sup>

Although total-etch systems are reliable in their performance, they can also be technique-sensitive. Dentin over drying is a potential problem that occurs with etch-and-rinse protocols. Kanca found significantly higher bond strengths when dentin specimens were ‘wet bonded,’ compared to those that were bonded to dentin dried with compressed air from an air syringe.<sup>2,3</sup> Also, Manso and colleagues carried out an *in vitro* study in which the prepared dentin surface was etched and then rinsed with water. Half of the specimen surface was blot dried while the other half of the specimen was blasted with air for 30 seconds. The specimen was bonded using either an ethanol/water-based or an acetone-based adhesive. At both 24-hour and 3-month testing intervals, the dry-bonding

technique resulted in significantly lower microtensile bond strengths as compared to the wet-bonding technique.<sup>4</sup>

With the total-etch technique, there can also be problems with over-etching the prepared dentin surface. Hashimoto and colleagues noted that etching dentin for more than 15 seconds leads to significant decreases in bond strengths of OptiBond Solo.<sup>5</sup> This occurred because of fracture within the demineralized dentin zone, and means that over-etching may cause excessive demineralization of both intertubular and peritubular dentin which were not completely infiltrated by resin monomers.<sup>5</sup>

Self-etch adhesive systems are an alternative option that may address these technique sensitivity issues. Fewer steps are involved in the process of restoring the teeth, thereby having less potential for error.

Self-etch adhesives vary in their compositions and pH. 'Strong' self-etch adhesives have a pH < 1. They exhibit a bonding mechanism and interfacial ultra-morphology in dentin resembling that of etch-and-rinse adhesives.<sup>1</sup> An example of this type of adhesive is Adper Prompt L-Pop. 'Mild' self-etch adhesives have a pH around 2. They dissolve the dentin surface only partially, so that a substantial number of hydroxyapatite crystals remain within the hybrid layer.<sup>1</sup> An example of a mild self-etch adhesive is the two-bottle system, Clearfil SE Bond.

There are four types of adhesion. *Mechanical adhesion* is the penetration of resin and formation of resin tags within the tooth surface. *Adsorption* is the chemical bonding to the inorganic component (hydroxyapatite) or organic

components (mainly type I collagen) of tooth structure. *Diffusion* is the precipitation of substances on the tooth surface to which resin monomers can bond mechanically or chemically. Finally a combination of all three can serve as another form of adhesion.<sup>6</sup> Pashley has stated that adhesive resins may have the potential to infiltrate through the entire thickness of the smear layer and either bond to the underlying dentin matrix or penetrate into the tubules without prior etching of the prepared tooth surface.<sup>7</sup> The adhesives were observed to have maintained adequate bond strengths when compared to glass ionomers.<sup>7</sup> This may be particularly true for self-etch adhesives given their inherent acidity.

The chemistry of a self-etch adhesive can be easily disrupted by the introduction of a contaminant. Many studies have reported that saliva contamination has a detrimental effect on the bond strengths of a composite resin restoration to dentin. The results vary, with some studies stating that saliva contamination of the dentin surface resulted in lower bond strengths as compared to the controls. However, re-application of the adhesive after drying or rinsing off the saliva can re-establish the bond strengths to the control levels.<sup>8</sup> Other studies stress the point that complete drying of the saliva contaminated surface should be avoided, as this may be a possible cause for decreased bond strength.<sup>9,10</sup> Others observe that bonding agents performed remarkably well even when the saliva was not removed after acid etching.<sup>11-14</sup> This may be explained by the high percentage of hydrophilic solvents, such as acetone, or alcohol.<sup>15</sup> Hydrophilic bonding agents may be attracted by the saliva moisture, promptly spreading upon moist dentin or displacing the adsorbed saliva.<sup>16</sup>

Therefore the outcome may be related not only to the contaminant, but also to the type of adhesive used.

In a clinical situation where saliva contamination of the preparation occurs after acid-etching in anticipation of using a three-step total-etch adhesive, phosphoric acid can be briefly re-applied and rinsed to effectively cleanse the site.<sup>10</sup> However, the same strategy could be detrimental to the performance of self-etch adhesives, as studies show that pre-treatment of dentin with phosphoric acid significantly reduces bonds strengths.<sup>17</sup>

Many different solutions have been studied as decontaminants prior to bonding. Water can be a logical choice as a cleansing solution because it is readily available for the clinician and may be the first thing they reach for after contamination of the tooth surface.

Another possible option is chlorhexidine. Evidence shows that there is no difference in bond strengths between a standard total-etch protocol and the technique in which 2% chlorhexidine solution was applied after etching. Chlorhexidine was actually noted to inhibit degradation of the adhesive.<sup>18</sup> There is evidence that chlorhexidine preserves dentin-resin bonds by inhibiting the matrix metalloproteinases (MMPs). MMPs have been said to be partially responsible for hybrid layer degradation.<sup>19,20</sup> In addition to its action on MMPs, chlorhexidine is also well known to affect the metabolic activity of bacteria because, in low concentrations, it is bacteriostatic and prompts both changes to the functioning of the cellular membrane as well as leakage of intracellular

constituents, while in high concentrations it acts as a bactericide, prompting irreversible precipitation of the cellular content.<sup>21,22</sup>

In addition to water and chlorhexidine another alternative cleansing agent is sodium hypochlorite. Sodium hypochlorite is a well-known nonspecific proteolytic agent capable of removing organic material.<sup>23</sup> It might have the potential to deproteinate the smear layer-covered dentin surface of a prepared tooth. Mountouris and colleagues studied the effect of 5% sodium hypochlorite treatment on the molecular composition and morphology of human coronal dentin. After treating the coronal dentin surfaces with 5% sodium hypochlorite for times ranging from 5 to 120 seconds, there was a reduction in organic matrix, but no effect on carbonates and phosphates. This implied that deproteination of mineralized dentin surfaces within a clinically relevant time frame may provide methods for bonding to dentin alternative to conventional technique-sensitive dentin hybridization.<sup>24</sup> However, exposure time to sodium hypochlorite is a critical factor in determining strength. When an extended period of exposure is carried out, such as in a study by Fuentes and colleagues, 5% sodium hypochlorite actually reduced tensile strength and microhardness.<sup>25</sup> This might not be an immediate clinical concern, as the dentin specimens were stored in 5% sodium hypochlorite for 2 days, as compared to other studies in which exposure to sodium hypochlorite was only seconds to minutes.<sup>24,26,27</sup> A study by Arias showed that 10% sodium hypochlorite gel did not affect bond strengths. A 10% sodium hypochlorite solution significantly increased Gluma One Bond dentin bond strengths.<sup>28</sup> A clinical pilot study done by Saboia and colleagues to assess

the retention rates of adhesives after 10% sodium hypochlorite treatment revealed retention rates of the adhesives approximately 20% higher than the teeth that were not treated with sodium hypochlorite before bonding.<sup>29</sup> In another study, Vargas and colleagues applied 5% sodium hypochlorite to the dentin surface following acid conditioning with no significant effect on the dentin shear bond strength for Scotchbond Multi-Purpose, and a significantly positive effect on the bond strength of All-Bond 2 specimens.<sup>14</sup>

Conversely, these cleansing agents carry the potential of being dentin contaminants themselves. A study done by Roberts and colleagues examined the effect of antimicrobial agents present in the waterlines effect on dentin bond strength. They tested a 0.12% chlorhexidine gluconate, 12% ethyl alcohol solution and determined that the mean shear dentin bond strength was  $12.96 \pm 4.01$  MPa compared to  $22.59 \pm 8.93$  MPa for the control group in which distilled water was used. Similarly, a 3-ppm mixture of sodium hypochlorite and water was applied to the dentin surface, and the mean shear dentin bond strength was  $18.13 \pm 6.65$  MPa. This suggests that these antimicrobial agents may have a detrimental effect on the dentin bond strengths.<sup>30</sup> The preparation protocol involved the prepared tooth surface to be rinsed with each antimicrobial agent for 15 seconds and then the surface was blotted dry. There was no mention of rinsing the surface with distilled water afterwards. This may have led to the observed reduced bond strengths. In our current protocol we were sure to rinse the prepared tooth surface with water after it was cleansed with either chlorhexidine or sodium hypochlorite.

Given the lack of a comprehensive investigation on the effect of different cleansing solutions on dentin bond strengths when contemporary self-etch adhesive systems are used on saliva-contaminated dentin, **the purpose of this study was to examine the effect of different cleansing solutions on the bond strengths of self-etch adhesives to saliva-contaminated dentin.**

Water, chlorhexidine, and sodium hypochlorite were used as cleansing solutions. The null hypothesis tested was that *rinsing saliva-contaminated dentin with water, chlorhexidine, or sodium hypochlorite has no effect on the bond strengths of self-etch adhesive systems.*

From the current self-etch adhesive systems in the market we chose Adper Prompt L-Pop (3M ESPE, St Paul, MN), Adper Easy Bond (3M ESPE, St Paul, MN), and Clearfil SE Bond (Kuraray, Kurashiki, Japan) to be tested in this study. Adper Prompt L-Pop was chosen as it is a two-component, one-step self-etch adhesive system. It has a pH of 0.7, which is quite acidic in the realm of self-etch adhesive systems. It was important in our study to observe how a comparably more acidic adhesive system would respond to contamination with saliva and cleansing afterwards. Adper Easy Bond was tested as an all-in-one self-etch adhesive system with a less acidic pH of 2.7. It was important in this study to observe the response of a comparably less acidic adhesive system to contamination and cleansing. Finally we chose Clearfil SE Bond, which is a two-bottle self-etch adhesive system. This adhesive has been used commonly in previous studies and serves as a standard for self-etch adhesive systems. It has a pH of 2.0 which is in between Adper Prompt L-Pop and Adper Easy Bond.

## Literature Review

The current literature for saliva contamination and self-etch adhesives has results that are controversial. Previous studies have stated that if there has been saliva contamination of the prepared tooth surface, and bonding takes place, there will be a decrease in bond strengths.<sup>8,31</sup> Other studies have concluded that even after saliva contamination, there appears to be no detrimental effect on bond strengths.<sup>11,32,33</sup> An *in vitro* study done by Yoo and colleagues looked at the effect of saliva contamination on the microshear bond strength of one-step self-etching adhesive systems to dentin. The authors evaluated decontamination methods from drying the contamination surface with an air syringe, to rinsing the surface with a water syringe. Results from their study concluded that when contamination occurred before light curing the adhesive (One Up Bond and Adper Prompt L-Pop) bond strengths were maintained by either drying the surface and curing, or rinsing and drying and re-applying the adhesive and curing.<sup>34</sup> In a study by Park and colleagues a similar problem was analyzed. They used Clearfil SE Bond as their self-etch adhesive. They concluded that after the primer was applied, if there was saliva contamination, one way that they could maintain shear bond strengths was to rinse the contaminated tooth surface and re-apply the primer. Also they noted that there was no effect on shear bond strengths of the self-etch adhesive if saliva contamination occurred before primer application. From the results of this study it seems that the primer was able to penetrate the saliva layer and maintain bond strengths.<sup>10</sup>



Recently there have been studies assessing the microleakage of self-etch adhesive systems when contaminated with saliva. An *in vitro* study by Yazici and colleagues noted that when saliva contamination occurred, either before or after curing of the adhesive, the microleakage did not worsen.<sup>35</sup> The results were obtained after a 24 hour thermocycling period, and may change after long term thermocycling, but the results are still promising.

Townsend and colleagues made an interesting finding about self-etch adhesive systems and their reaction to saliva contamination. They tested the shear bond strengths of both enamel and dentin. Their results determined that enamel was detrimentally affected by saliva contamination, but dentin shear bond strengths were not significantly affected.<sup>33</sup>

Not only does it seem to matter if the contamination occurred in enamel or dentin, but also what type of self-etch adhesive system was used. Various studies by el-Kalla and colleagues noticed that from the four self-etch adhesive systems tested (Prime & Bond 2.1, One Step, Tenure Quik, and Syntac Single Component) all but one seemed to have similar results. There was no effect on the bond strengths or the hybrid layer formation of all the self-etch adhesive systems, except for Syntac Single Component, which showed a lowering of bond strengths in enamel and a thin hybrid layer formation after saliva contamination.<sup>11,32</sup> The discrepancy in results may be due to the pH, hydrophilic or hydrophobic nature of the adhesives, or the effects of the different solvents present in their composition.

The differences in the results of these studies may also be due to the fact that there is variation in the technique of the methods. Some studies contaminated the tooth surface with saliva, without drying<sup>31</sup>, while others dried the tooth surface after saliva contamination.<sup>8</sup>

In the previous studies of saliva contamination and self-etch adhesive system bond strengths, water was the only cleansing solution used. It was of interest in this study to observe the reaction of the self-etch adhesive systems to different cleansing agents after saliva contamination. Chlorhexidine and sodium hypochlorite were chosen based on previous knowledge of their bactericidal effect,<sup>21,22</sup> as well as their ability to maintain the carbonates and the phosphates of the dental hard tissue.<sup>8</sup>

## **Materials and Methods**

A pilot study was performed using 10 extracted intact human molars and a power analysis was run to determine the number of teeth required for the study.<sup>36</sup> Based on the pilot study, it was determined that 75 teeth would be required to power the study at 80%. Seventy-five intact human molars, with no evidence of caries or caries that did not extend into the dentin, were collected. Many of the specimens were third molars, because these are most commonly extracted without much evidence of decay. The specimens were stored in a solution of 0.5% chloramine trihydrate (an antibacterial agent that has no detrimental effect on the tooth)<sup>37, 40</sup> for approximately 48 hours, after which they were removed

from the solution and stored at 4°C. The occlusal surfaces of the teeth were sectioned in order to expose mid-level dentin and polished using 600-grit silicon carbide paper under water to create a uniform smear layer<sup>38</sup> (Figures 2,3 & 4). The specimens were randomly divided into 5 groups of 15 specimens each:

**Group A** – Dentin was not contaminated with human saliva (positive control).

The surface was dried using a compressed air syringe for 5 seconds at a 5-cm distance from the surface.

**Group B** - Dentin was contaminated with human saliva for 5 seconds (no cleansing, negative control). Surface was dried with the air syringe for 5 seconds at a 5-cm distance from the surface.

**Group C** – Dentin was contaminated with human saliva for 5 seconds and the contaminated surface was cleansed using **water** for 2 seconds with a water syringe. The surface was dried with the air syringe at a distance of 5 cm from the tooth surface, for 5 seconds.

**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) applied by lightly scrubbing with a microbrush for 5 seconds. Then the surface was rinsed with water for 2 seconds, and dried with the air syringe at a distance of 5 cm from the surface for 5 seconds.

**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) applied by lightly scrubbing with a microbrush for 5 seconds. Then the surface was rinsed with water for 2 seconds, and dried with the air syringe at a distance of 5 cm from the tooth surface for 5 seconds.

One-third of the teeth in each group ( $n=5$ ) were bonded with a two-component one-step self-etch adhesive system (Adper Prompt L-Pop, 3M ESPE). One-third of the specimens in each group ( $n=5$ ) were bonded with an 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)

The adhesives were applied and light-activated according to manufacturers' recommendations. For Adper Prompt L-Pop the operator applied the adhesive with a rubbing motion for 15 seconds, then gently but thoroughly air-dried to remove the aqueous solvent. Then applied a second coat (no waiting time for the second layer), next gently but thoroughly air-dried to remove the aqueous solvent. Finally light cured for 10 seconds. For Adper Easy Bond the operator applied the adhesive to tooth surface for a total of 20 seconds by lightly scrubbing with a microbrush, then dried the adhesive for 5 seconds at a 5 centimeter distance, finally light cured for 10 seconds. For Clearfil SE Bond the operator applied primer and left for 20 seconds, then dried with gentle air flow at a 5 centimeter distance. Next the bond was applied and dispersed with mild air

flow to evenly distribute on the surface of the tooth. Finally it was 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 unit operating at  $>800\text{mW/cm}^2$  (Kerr, Orange, CA) for 20s. The specimens were stored in distilled water for 24 hours.

The compositions of the adhesives and cleansing solutions are listed in Tables 1 and 2, respectively.

Each specimen was fixed in an epoxy resin block with sticky wax. Specimens were then sectioned mesiodistally using a water-cooled low-speed Isomet 1000 diamond micro-slicing saw (Buehler, Lake Bluff, IL) to obtain sections 0.9 mm thick. 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 (Figure 2). Each specimen had a cross-sectional area of  $0.9 \pm 0.2 \text{ mm}^2$ .

Each specimen was fixed to a Ciucchi Jig (EZ-Test, Shimadzu, Kyoto, Japan) (Figure 5) using cyanoacrylate (Loctite Super Glue Gel, Hartford, CT). The setting process of the cyanoacrylate was accelerated by spraying the prepared surface with Zapit Accelerator Spray, composed of heptane, acetone, and alkyl toluidines (Dental Ventures of America Inc, Corona, CA). 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) (Figure 6) with a crosshead speed of 1mm/min.<sup>47</sup> The bond strength (MPa) of each specimen was determined as the failure load (in N) divided by the cross-sectional area of the bonded interface. Bond strengths were compared amongst all groups, within adhesives. Data were subjected to factorial ANOVA and Tukey LSD post-hoc tests, where indicated, with p=0.05 significance level.

## Results

Mean microtensile bond strength values and standard deviations are presented in Table 3. For all three adhesives, there was no statistically significant difference in bond strengths when comparing the cleansing groups to the positive control groups (Figure 3).

For Adper Prompt L-Pop, the highest microtensile bond strength was observed in the negative control group with a mean value of 23.4 MPa. Next was the positive control group with a mean value of 20.1 MPa, followed by cleansing the contaminated tooth surface with sodium hypochlorite (18.4 MPa), chlorhexidine (17.7 MPa) and water (17.3 MPa).

For Adper Easy Bond, the highest microtensile bond strength was observed after cleansing the contaminated tooth surface with sodium hypochlorite, a mean value of 61.3 MPa. This was followed by water at 59.1 MPa and the negative control group at 51.0 MPa. Cleansing with chlorhexidine was the lowest at 49.3 MPa, yet still remaining higher than the microtensile bond

strength when the tooth was bonded without any contamination and cleansing (39.4 MPa).

For Clearfil SE Bond, the highest microtensile bond strength occurred in the negative control group, a mean value of 72.0 MPa. Next was cleansing the contaminated tooth surface with water at 69.3 MPa, followed by chlorhexidine at 62.8 MPa and sodium hypochlorite at 54.8 MPa. Although there was no statistically significant difference in microtensile bond strength as compared to bonding without any contamination and cleansing, there remained a slight improvement in all groups, except the sodium hypochlorite group.

## **Discussion**

Saliva contamination of tooth surfaces and its impact on self-etch adhesive systems remains to be understood, with few and inconclusive studies addressing this question. Currently available options to manage saliva contamination of tooth surfaces remain inadequate. 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.<sup>17</sup>

Water presents one viable agent 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 tooth surface. In a study by Sattabanasuk and colleagues a similar hypothesis was tested. In one of the test

groups the prepared dentin surface was contaminated with saliva, rinsed with water, and the adhesive was re-applied. Bonding procedures were done according to manufacturers' directions and a composite resin was bonded onto the surface of the prepared tooth. Micro tensile testing of the samples was done after sectioning into 1.0mm<sup>2</sup> beams. The results concluded that rinsing the contaminated tooth surface with water re-established the bond strengths to a value similar to that of no contamination at all.<sup>16</sup> Our study also showed that for Adper Easy Bond, Clearfil SE Bond, and Adper Prompt L-Pop cleansing with water did not 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 a self-etch adhesive system. An *in vivo* study done 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. They showed that the synthetic protease inhibitor, chlorhexidine, stabilized the bond strengths of the treated dentin surfaces as compared to the untreated tooth surfaces.<sup>20</sup> 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 attractive alternative given its popular application as a bacterial reducing agent in intracanal preparations.<sup>39</sup> The fact that the pH of sodium hypochlorite is around 11, highly alkaline, is what made 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 like 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.<sup>8</sup> This reiterates the fact that there is no dissolution of the mineral content of the tooth which takes part in the chemical adhesion to the restorative material. Our study proved that sodium hypochlorite does not have any detrimental effect on bond strengths of Adper Prompt L-Pop, Adper Easy Bond, and Clearfil SE Bond. The key to success is the rinsing off sodium hypochlorite with water after a few seconds of application.

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 is considered by many clinicians an efficient adhesive, due to the fact that it is very simple to use. It was chosen as its acidic pH (0.7) would serve as an important group where we can observe the response of an acidic self-etch adhesive to a contaminating agent. Adper Easy Bond is an all-in-one self-etch adhesive system. It was chosen because it is less acidic, with a pH of 2.7, and it was of interest for this study to observe how a comparatively less acidic solution would respond to a contaminating agent. Clearfil SE Bond is a two bottle

system, and has been used in many previous studies as the standard self-etch system. It has a pH in between Adper Easy Bond and Adper Prompt L-Pop, at 2.0, and therefore it was reasonable to test the response of such a system.

The contaminating agent we chose was fresh whole human saliva. We deliberately selected the use of human saliva as the contaminating agent as opposed to an artificial saliva/salivary substitute. Fresh whole human saliva is an acceptable substance in testing saliva contamination and adsorption.<sup>40</sup> It was pertinent to this study to make sure that there was a “real” contaminating agent used, otherwise we may have run the risk of conducting a study that had little to no clinical significance.

In order to better standardize the composition of the contaminating agent the saliva was collected from only one person. This was a healthy 26-year old female. Also the saliva was 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.<sup>37</sup> Using a pH meter it was determined that the pH of this saliva was an average of 7.4.

It should be mentioned that a pilot study was done with the positive control group for all three adhesives to determine if there was a difference in bond strengths between blot drying the prepared tooth surface or drying with an air syringe for 5 seconds at a 5-cm distance. The results showed no significant difference.

Another important decision made for the methods of this study was to collect 5-10 rods from each tooth and test them in order to determine their microtensile bond strength. Then the results were averaged into one mean microtensile bond strength value. By utilizing this approach there was less variability in results that could possibly be seen when one specimen is taken from the outer surface of dentin and another taken from the more pulpal surface of dentin.

After the bonding procedure, we stored the specimens in distilled water for 24 hours. The rationale for this was to allow the bonds to mature between the prepared dentin surface and the composite resin surface. Unfortunately because this was an *in vitro* study we were unable to reproduce the thermal changes that can occur in the oral cavity, or apply masticatory forces on the specimens.

An interesting finding was seen during our study correlating the level of acidity of the self-etch adhesive systems to the microtensile bond strengths. When comparing the one-bottle systems there were higher microtensile bond strengths seen in the less acidic adhesive, that being Adper Easy Bond. Also Clearfil SE Bond, being a less acidic self-etch adhesive in the spectrum of adhesives systems, also showed comparably high microtensile bond strengths. Based on our results, less acidic self-etch adhesive systems tend to show higher microtensile bond strengths.

During this study it was noted that for all three self-etch adhesive systems tested there was a numerical increase in microtensile bond strengths when contaminated with saliva without any rinsing. This means that self-etch

adhesives may work well in the presence of saliva contamination, and saliva may even reinforce the bond strengths. This presents the possibility that there might be no need of cleansing the tooth surface after contamination with saliva, simply dry the surface. With self-etch bonding agents, saliva may not even be considered a contaminant. This occurrence may be explained by the inherent acidity of self-etch adhesive systems. The acids present in the adhesive's composition not only modify/penetrate the smear layer but also seem to break through the mucopolysaccharides in the saliva, and maintain bond strengths.

Coming back to the initial purpose of our study, we determined that for Adper Prompt L-Pop, Adper Easy Bond, and Clearfil SE Bond there was no statistically significant difference in microtensile bond strengths when cleansing with water, 2% chlorhexidine digluconate, or 5% sodium hypochlorite. Therefore it can be concluded that cleansing with water, chlorhexidine, or sodium hypochlorite may not have any detrimental effect on the microtensile bond strengths of self-etch adhesive systems. Based on the results, we fail to reject the null hypothesis.

There were some limitations to this study. As this was an *in vitro* study, the results cannot be generalized to clinical performance. Although it may be possible that self-etch adhesive systems are not affected by saliva contamination, as long as the tooth surface is dried, clinical studies are needed to confirm the results. Also, as only three self-etch adhesive systems were tested, we cannot generalize these results for all self-etch adhesives available in the market. Additionally, only one person's saliva was used as the contaminating

agent, every person's saliva may not have the same effects as the one used. We were unable to determine the age of the teeth tested as we did not record the time of extraction, or the age of the patient from which the tooth was extracted. Although attempts were made to limit the amount of time from when the tooth was extracted until we prepared the tooth for testing there was no standardized protocol in place. Both of these factors may result in some variability in the results, as "young" dentin may respond differently to contamination, cleansing, and bonding than "mature" dentin might.

## **Conclusions**

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

1. Cleansing the contaminated tooth surface with water, chlorhexidine, or sodium hypochlorite following saliva contamination has no negative effect on the bond strengths of the self-etch adhesive systems tested.
2. The self-etch adhesives systems tested are not adversely affected by saliva contamination.

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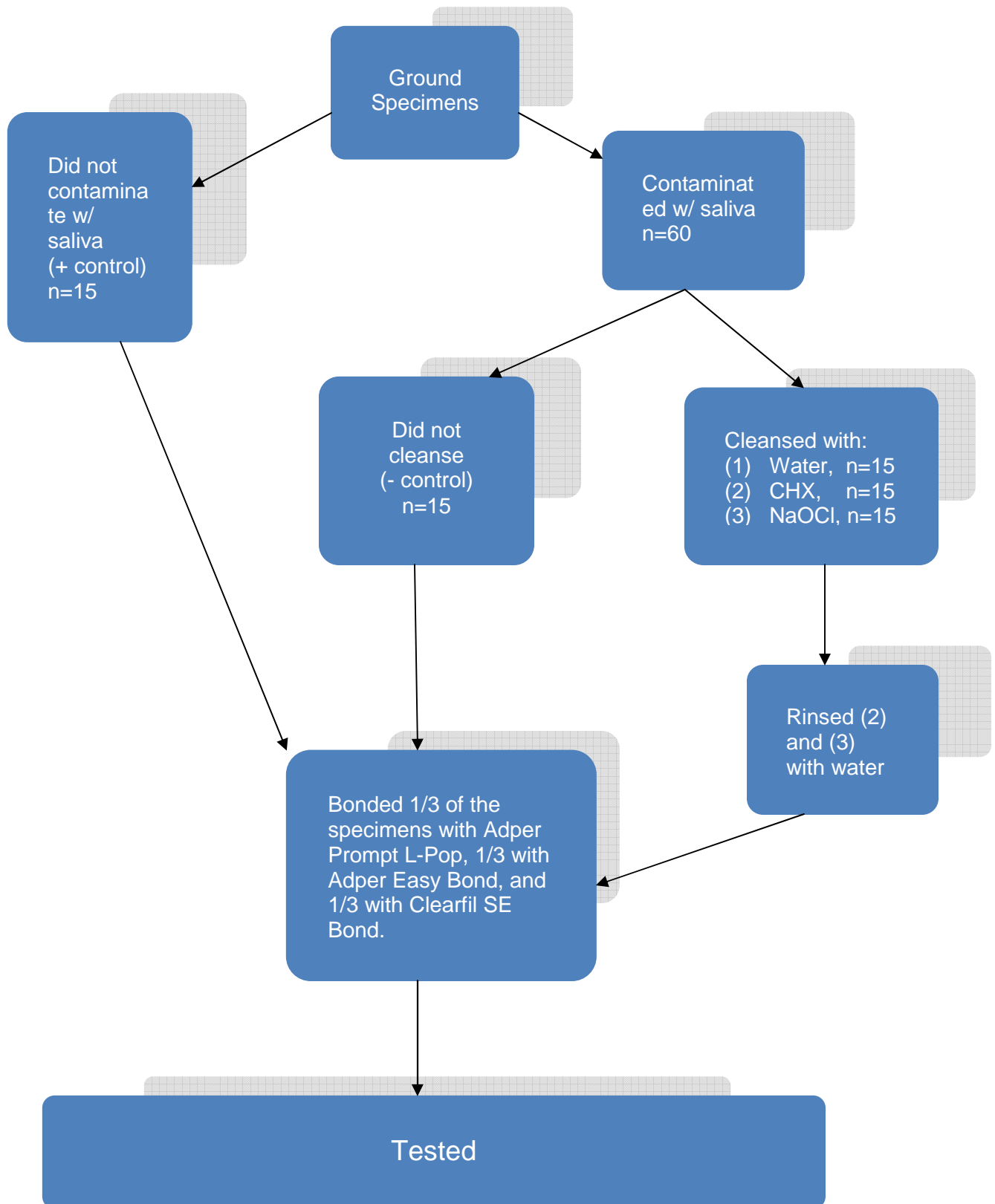
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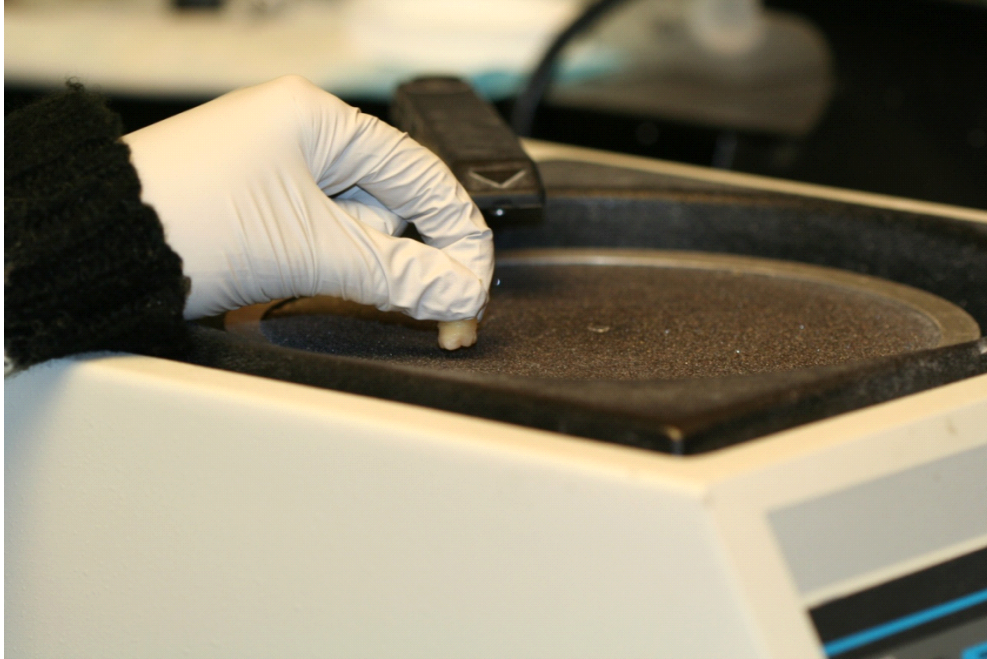
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**Figure 1: Flowchart of Experimental Procedures**



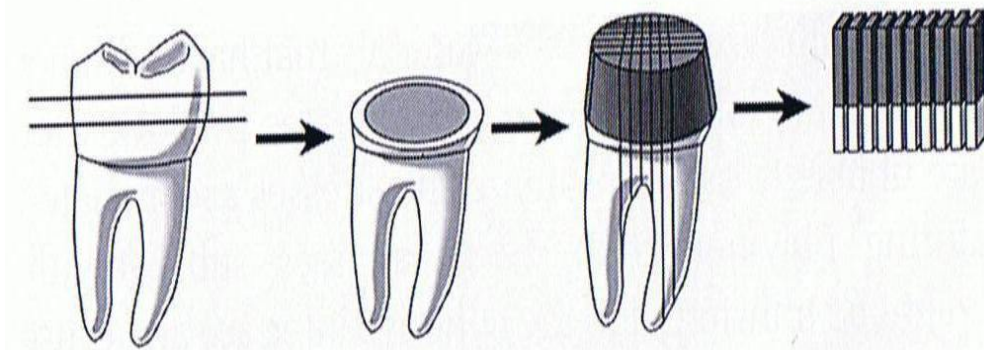


**Figure 2:** Exposing midlevel dentin with 600 grit silicon carbide paper under water to form a uniform smear layer.



**Figure 3:** Mid-level dentin exposed.

**Figure 4:** Diagram depicting the preparation of the tooth for microtensile bond strength testing.



(1.) Sectioning the tooth

(2.) Exposing mid-depth dentin, and formation of smear layer with use of 600 grit silicon carbide paper under water.

(3.) Bonding of composite to tooth with self-etch adhesives

(4.) Slicing tooth to obtain specimens.

Courtesy of Sturdevant's Art and Science of Operative Dentistry, Fifth edition, 2006



**Figure 5:** Ciucchi Jig with rod specimen in place, with the dentin composite interface located at the center.



**Figure 6:** EZ-Test Testing Machine for microtensile bond testing.

**Table 1:** Composition of the bonding agents and composite resin.

Bonding Agents & Composite Resin	Composition
Adper Prompt L-Pop LOT 332454 3M ESPE St. Paul, MN	methacrylated phosphoric esters, Bis-GMA, initiators based on camphorquinone, stabilizers, water, 2-hydroxyethyl methacrylate (HEMA), polyalkenoic acid
Adper Easy Bond, LOT 299001 3M ESPE St. Paul, MN	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-trimethylbenzoyldiphenylphosphine oxide
Clearfil SE Bond, LOT 61832 Kuraray Dental, Kurashiki, Japan	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 LOT 20070802 3M ESPE St. Paul, MN	silane treated ceramic, silane treated silica, bisphenol a polyethylene glycol diether dimethacrylate, diurethane dimethacrylate, bisphenol a diglycidyl ether methacrylate, triethylene glycol dimethacrylate, water

**Table 2:** Composition of the cleansing agents.

<b>Cleansing agents</b>	<b>Composition</b>
Distilled Water	distilled water
5% Sodium Hypochlorite Solution ACROS Organics, Somerville, NJ LOT A0248559	sodium hypochlorite
Cavity Cleanser, Bisco, Schaumburg, IL 2% Chlorhexidine Solution LOT 0700007926	chlorhexidine digluconate

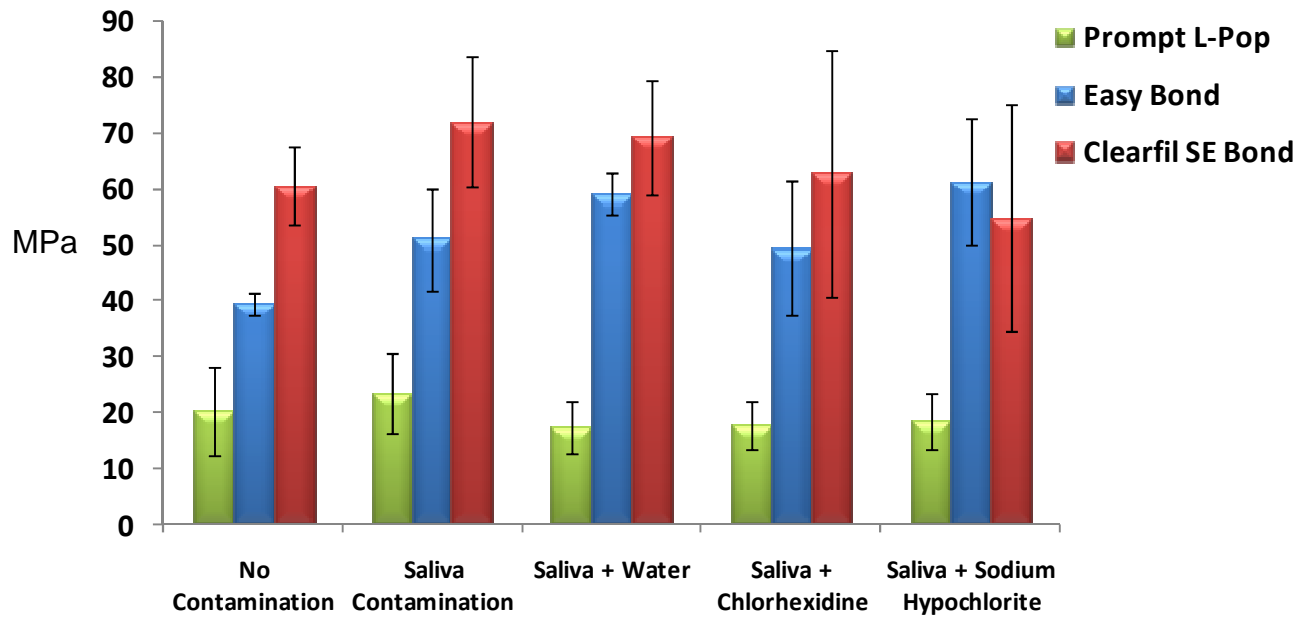
**Table 3:**  $\mu$ TBS means (SD) by adhesive and treatment (n=5).\*

	<i>Cleansing Agents</i>				
<i>Adhesive Systems</i>	No cont.	Saliva Cont.	Water	CHX	Sod. Hypo.
Adper Prompt L-Pop	20.1 (7.9) <sup>cB</sup>	23.4 (7.1) <sup>cC</sup>	17.3 (4.6) <sup>cC</sup>	17.7 (4.3) <sup>cC</sup>	18.4 (5.2) <sup>cC</sup>
Adper Easy Bond	39.4 (2.1) <sup>bB</sup>	51.0 (9.2) <sup>bA</sup>	59.1 (3.9) <sup>bA</sup>	49.3 (12.0) <sup>bA</sup>	61.3 (11.3) <sup>bA</sup>
Clearfil SE Bond	60.5 (7.0) <sup>aA</sup>	72.0 (11.5) <sup>aA</sup>	69.3 (10.2) <sup>aA</sup>	62.8 (22.1) <sup>aA</sup>	54.8 (20.3) <sup>aA</sup>

\*Same lower case superscript letters indicate non-significantly different means ( $p>0.05$ ) within adhesives (rows); same upper case superscript letters indicate non-significantly different means ( $p>0.05$ ) within cleansing agents (columns)

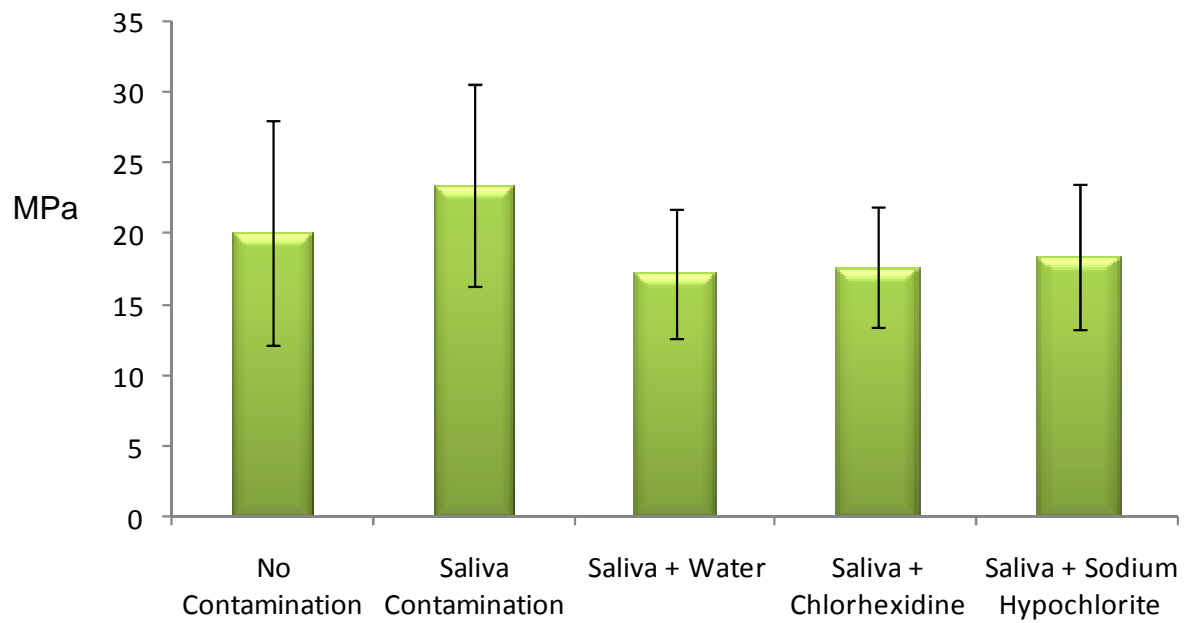


**Figure 7:** Dentin Microtensile Bond Strengths of Adper Easy Bond, Clearfil SE Bond, and Adper Prompt L-Pop in MPa.



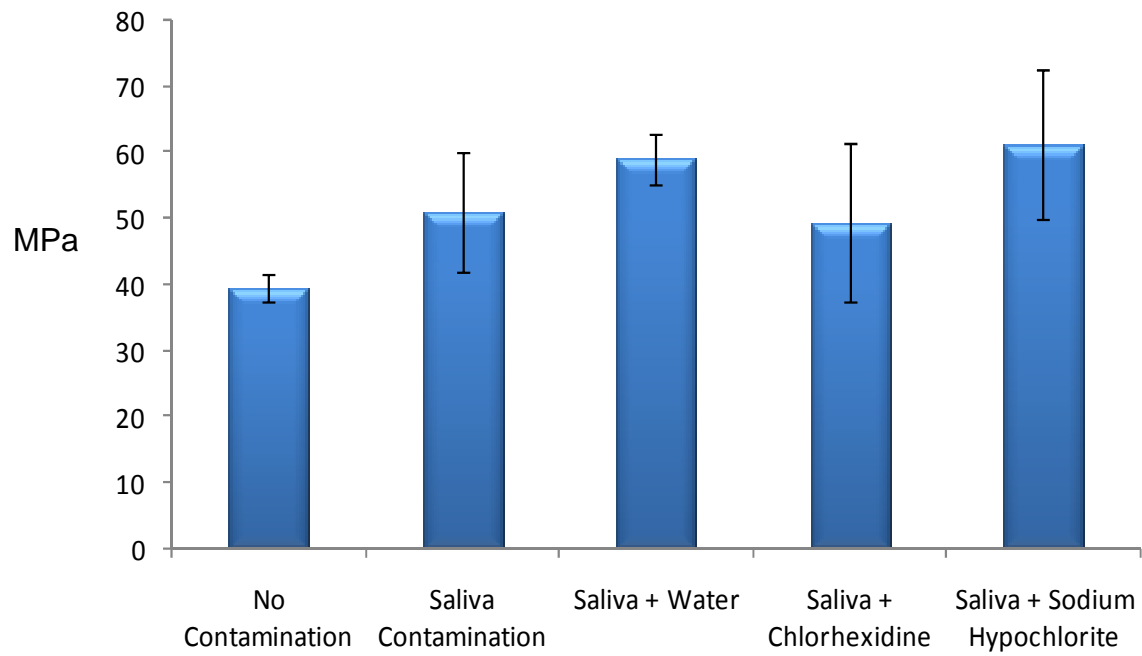
\*Mean microtensile bond strength values were not significantly different within adhesives for Adper Easy Bond, Clearfil SE Bond, and Adper Prompt L-Pop ( $p>0.05$ ).

**Figure 8:** Dentin Microtensile Bond Strengths of Adper Prompt L-Pop.\*



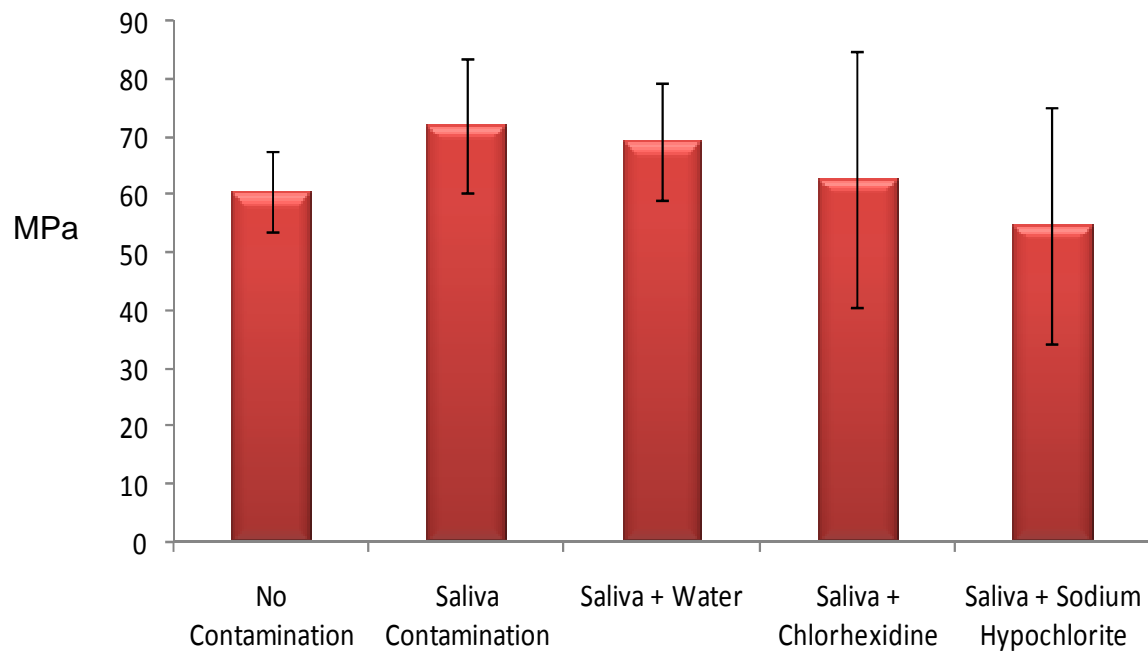
\*Mean microtensile bond strength values are not significantly different ( $p>0.05$ ).

**Figure 9:** Dentin Microtensile Bond Strengths of Adper Easy Bond.\*



\*Mean microtensile bond strength values were not significantly different ( $p>0.05$ ).

**Figure 10:** Dentin Microtensile Bond Strengths of Clearfil SE Bond.\*



\*Mean microtensile bond strength values were not significantly different ( $p>0.05$ ).