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# Effect of saliva contamination and different decontamination protocols on microshear bond strength of a universal adhesive to dentin

Efeito da contaminação por saliva e de diferentes protocolos de descontaminação na resistência de união ao microcisalhamento de um adesivo universal à dentina

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## ABSTRACT

**Objective:** To evaluate the effect of saliva contamination and different decontamination protocols on the microshear bond strength of a universal adhesive to dentin. **Material and Methods:** 84 bovine teeth were divided into three groups according to bonding stage at which salivary contamination occurred; before curing of the adhesive, after curing of the adhesive, and a control group with no salivary contamination. Each group was further subdivided into four subgroups according to the decontamination protocol used (n=7): no decontamination protocol, rinsing then reapplication of the adhesive, grinding with sandpaper silicon carbide grit 600 then reapplication of the adhesive and finally ethanol application then reapplication of the adhesive. Specimens were tested in micro-shear mode. **Results:** All the decontamination protocols used in this study to reverse effect of salivary contamination before curing significantly improved the bond strength to contaminated dentin ($p<0.001$). Meanwhile, after curing, ethanol decontamination protocol recorded highest bond strength followed by rinsing and grinding compared to no decontamination ($p<0.001$). **Conclusion:** Saliva contamination led to significant deterioration in the bond strength regardless of the bonding stage at which saliva contamination occurred. All decontamination protocols improved the immediate microshear bond strength when contamination occurred before curing of the adhesive, while ethanol seemed to be the most effective both before curing and after curing.

## KEYWORDS

Bond strength; Decontamination; Ethanol; Saliva; Universal adhesives.

## RESUMO

**Objetivo:** Avaliar o efeito da contaminação por saliva e de diferentes protocolos de descontaminação na resistência de união ao microcisalhamento de um adesivo universal à dentina. **Material e Métodos:** 84 dentes bovinos foram divididos em três grupos de acordo com o passo operatório do protocolo adesivo em que ocorreu a contaminação por saliva: antes da polimerização do adesivo, ou após a polimerização do adesivo e um grupo controle sem contaminação por saliva. Cada grupo foi subdividido em quatro subgrupos de acordo com o protocolo de descontaminação utilizado (n=7): sem protocolo de descontaminação; lavagem seguida da reaplicação do adesivo; lixar a região com lixa de carbeto de silício de granulação 600 e reaplicar o adesivo; aplicar etanol e reaplicar o adesivo. Os espécimes foram testados no modo de micro-cisalhamento. **Resultados:** Todos os protocolos de descontaminação utilizados neste estudo em busca de reverter o efeito da contaminação do adesivo por saliva melhoraram significativamente a resistência de união à dentina contaminada ($p<0,001$). Enquanto isso, após a polimerização, o protocolo de descontaminação com etanol resultou na maior resistência de união, seguido pela lavagem, e depois pelo lixamento, em comparação com nenhum protocolo de descontaminação (p<0,001). **Conclusão:** A contaminação por saliva levou a uma deterioração significativa na resistência de união, independentemente do passo operatório do protocolo adesivo em que ocorreu a contaminação por saliva. Todos os protocolos de descontaminação melhoraram a resistência de união ao microcisalhamento imediato quando a contaminação ocorreu antes da polimerização do adesivo, enquanto o etanol pareceu ser o protocolo mais eficaz nos dois tipos de contaminação (antes e depois da polimerização).

## PALAVRAS-CHAVE

Adesivos universais; Descontaminação; Etanol; Saliva; Resistência ao cisalhamento.

## INTRODUCTION

Saliva contamination is one of the most serious challenges facing any dental operator during restorative procedures. In addition to its water content, saliva contains macromolecule proteins and glycoproteins, alongside particles like calcium, sodium, and aminoacids [1]. Both constituents of saliva can adversely affect bond strength. Literature has extensively reviewed the effect of saliva contamination on adhesive restorations. The adverse effects of this persistent clinical challenge includes microleakage at the tooth-restoration interface with subsequent postoperative sensitivity, discoloration and recurrent caries [2].

In light of that, clinicians use all resources available to ensure proper isolation of the dental field and simplify the restorative procedure. Rubber dam placement, as the standard protocol intended for isolation is successful. However, its placement can be at times inapplicable, or difficult like with severely fractured tooth, newly erupted crowns or with an uncooperative child or asthmatic patients [3,4].

Additionally, adhesive formulations are constantly improved to achieve clinically acceptable bond strengths and simplified procedures. For that reason, universal adhesives have become more and more popular [5,6]. Even with the inevitability of salivary contamination during multiple restorative procedures, there is little information on the adverse effects of saliva contamination on the bond strength of universal adhesives and how to handle such a clinical mishap [7-9]. Moreover, no clear recommendation exists for a clinically applicable decontamination protocol that takes into consideration when exactly contamination has occurred.

Therefore, the aim of this study was to evaluate the effect of saliva contamination at different bonding stages and different decontamination protocols on the immediate microshear bond strength of a universal adhesive to dentin. The null hypotheses to be tested was as follows: the decontamination protocols used in this study would have no effect on the bond strength to dentin after saliva contamination with no difference regarding the bonding stage at which saliva contamination occurred.

## MATERIAL AND METHODS

An adaptation of the CONSORT reporting guidelines and checklist was used relevant to the *in vitro* setting of the study [10,11].

A research study involving paired sets of subjects, focusing on a continuous response variable was conducted. Previous data [12] showed that the disparity in response within these matched pairs follows a normal distribution with a standard deviation of 4.6. In order to have a 95% probability of correctly rejecting the null hypothesis, which assumed no difference between the different decontamination protocols, given that the actual difference in mean response was 10.8, sample size was determined to be 5 specimens per group. The significance level for this hypothesis test was set at 0.05. To account for a potential 30% decrease due to pretest failure, the sample size had been increased to 7 specimens per group. The power analysis was performed using IBM SPSS Statistics for Windows, Version 20.0. (Armonk, NY: IBM Corp).

A total of 84 bovine anterior teeth were used for microshear bond strength testing. In addition to the comparable histology and structural changes to human teeth, bovine teeth provide large sound surfaces [13,14]. The roots were scraped with a scaler to remove any attached soft tissue then rinsed under running water. Teeth were stored in 0.1% thymol solution (El Gomhouria Company for Trading Chemicals and Medical Appliances, Cairo, Egypt) at 4 °C for a maximum period of one month until prepared to avoid dehydration and bacterial growth. The teeth were thoroughly rinsed under running water for five minutes to remove thymol solution remnants.

The labial surfaces of the teeth were subjected to mechanical grinding with wet 180-grit silicon carbide (SiC) (Egyptian Abrasives Co., Egypt) paper to create a flat dentin surface for microshear bond strength testing. Then, teeth were inspected using a magnifying lens (5x) under good illumination to ensure the absence of enamel islands within the flat dentin surface, recognized by its distinguishable color. Finally, roots were cut off using a low-speed, diamond, abrasive disc (Superdiaflex H 365F 190 Horico Dental, Berlin, Germany) under copious air-water spray. The pulps were then pulled out using tweezers (Carl Martin, GmbH, Germany) and H-files (Mani, Inc, Tochigi, Japan) and pulp chambers were thoroughly cleaned using distilled water to remove any remaining pulp tissue. The pulp chamber of each tooth was filled with cotton to avoid penetration of the embedding material into the tooth.

Afterwards, flat dentin surfaces were placed downwards on a clean glass slab and secured using large double-faced adhesive tape. Polyvinyl chloride (PVC) rings of ¾ inches in diameter and 2 cm in height were placed around each dentin slab to serve as molds. Self-cure acrylic resin (Acrostone, Dent Product, Egypt) was poured to fill the molds completely embedding each tooth section. The glass slab was immersed in tap water to reduce the effect of the exothermic reaction of acrylic resin during setting. After complete setting, the double-faced adhesive tape was removed and each specimen was wet ground on 600-grit silicon carbide paper (SiC) (Egyptian Abrasives Co., Egypt) for 30 seconds in order to produce a clinically relevant, uniform smear layer. Each specimen was then washed with a three-way syringe for 10 seconds after which the bonding procedures were immediately carried out [15].

Specimens were randomly divided out into three groups according to bonding stage at which salivary contamination occurred:

**Group Cb:** saliva contamination before curing of the adhesive;

**Group Ca:** saliva contamination after curing of the adhesive; and

**Group $C_0$**: control group with no salivary contamination.

Each group was further subdivided into four subgroups according to the decontamination protocol used (n=7):

**Group $D_0$**: no decontamination protocol applied;

**Group Dr**: rinsing for 10s, drying for 5s then reapplication of the adhesive;

**Group Dg**: grinding with sandpaper SiC grit 600 for 10s, then rinsing for 5s and drying for 5s followed by reapplication of the adhesive [16,17]; and

**Group De**: application of ethanol with a microbrush for 15 seconds then rinsing for 5s and drying for 5s followed by reapplication of the adhesive, as shown in Figure 1.

To best simulate natural salivary composition, fresh unstimulated saliva was collected in a plastic cup from the principal investigator. The principal investigator was medically free and did not take any medication throughout the study. All the procedures were done in the morning. Oral hygiene measures (tooth brushing with 1450 ppm fluoridated toothpaste) were done twice per day (one time in the morning and the other before sleeping). The procedures were done one hour after cessation of any salivary stimulation (no intake of any food, beverage, smoking or chewing gum, tooth brushing) [18] to ensure less variation in pH, electrolyte, enzyme and protein levels. One coat of saliva was applied on the flat dentin surface with a microbrush and left undisturbed for 10 seconds. Salivary contamination was performed according to the stage of bonding whether before curing of the adhesive or after curing of the adhesive. Each of these groups was then subjected to one of the decontamination protocols investigated.

For microshear testing, each specimen received four polyethylene tubes (0.8 mm diameter and 1 mm length) positioned over the uncured adhesive [19] and the All-Bond Universal adhesive (Bisco, Inc., Schaumburg,IL,USA) was light cured for 10 seconds according to the manufacturer's instructions. Light curing was performed using LED light curing unit 3M™ *Elipar*™ *DeepCure*-S with an output of 1200 mW/cm². The flowable resin composite material *Aeliteflo* (Bisco, Inc., Schaumburg,IL,USA) was injected into

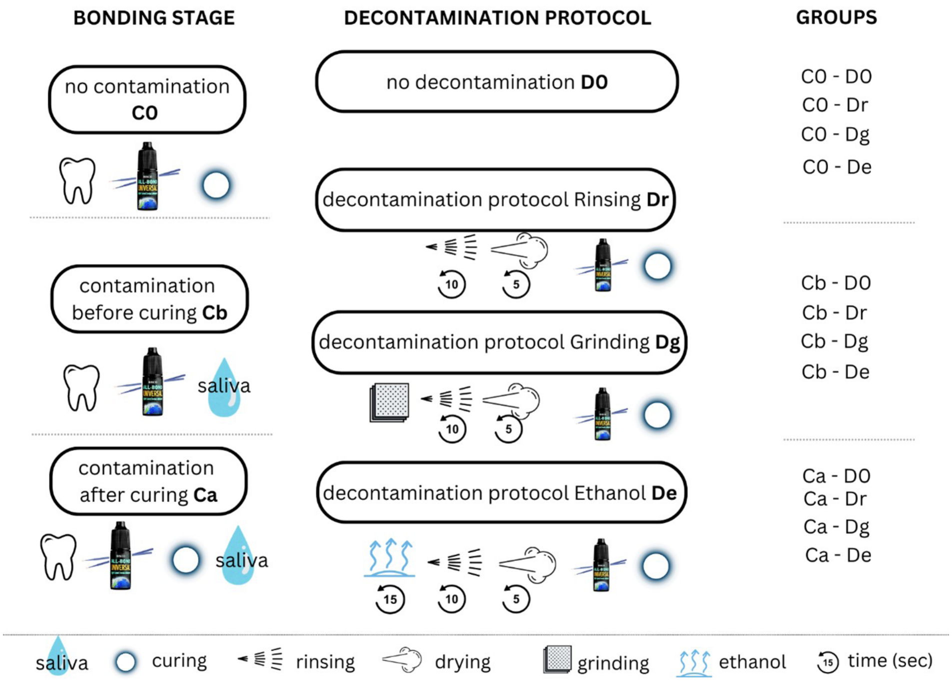


**Figure 1 -** Saliva contamination at different bonding stages and decontamination protocols.

the polyethylene tube and excess material was carefully removed. Resin composite was light cured over the polyester strip. Then, the polyethylene tube was removed using blade no.11 (Bard Parker, Xinda Surgical Blades, Wuxi Xinda Medical Device Co., Ltd., China) by placing two vertical incisions along the length of the tube and removing each half separately. Excess adhesive around each resin composite cylinder was carefully scraped using the same blade. Specimens were placed in distilled water at room temperature for 24 hours until testing. Samples were labeled to avoid any possible bias during testing and recording values by the independent investigator. It is worth noting that the use of flowable composite ensured fully compact, well-adapted composite cylinders.

Each specimen containing four resin composite specimens was tested in shear mode and the mean for each specimen was calculated. Shear mode was tested using a wire fixed to the upper jig of a universal testing machine (3365 series, Instron, IL, USA). The attachment was applied as close as possible to the resin/dentin interface while the specimen was fixed to the lower jig of the testing machine. The load was applied at a crosshead speed of 0.5mm/min until failure [20]. At the end, data was collected, tabulated and transferred to a biostatistician for analysis.

# RESULTS

Results of Two-way ANOVA showed that bonding stage during which contamination occurs was found to have a significant effect ($p = 0.004$) on immediate microshear bond strength to dentin. Also, decontamination protocols had a significant effect ($p < 0.001$), as well as the interaction between bonding stage and decontamination protocol ($p < 0.001$).

A comparison of the simple main effects presented in Table I and Figures 2 and 3, showed that saliva contamination significantly reduced the bond strength at both bonding stages compared to the control group $C_0$-$D_0$. The adhesive recorded its highest bond strength in the control group (no contamination $C_0$-$D_0$). There was no significant difference between the effect of contamination on the bond strength values when performed before curing and after curing of the adhesive ($p < 0.001$). Alone, all the decontamination protocols showed a reduction in the microshear bond strength values for the no saliva contamination groups compared to the control group $C_0$-$D_0$. Furthermore, all the decontamination protocols used in this study to reverse effect of salivary contamination before curing significantly improved the bond strength to contaminated dentin (CbDr=CbDg=CbDe>CbD$_0$) ($p<0.001$). Meanwhile, after curing, ethanol

**Table I -** Comparisons of simple main effects

| Decontamination Protocols | Microshear bond strength (MPa) (Mean±SD) | | | p-value |
|---|---|---|---|---|
| | $C_0$ | Cb | Ca | |
| D0 | 19.72±0.68$^{Aa}$ | 2.72±0.44$^{Bb}$ | 3.79±0.19$^{Bc}$ | <0.001* |
| Dr | 11.68±1.23$^{Bb}$ | 18.07±3.67$^{Aa}$ | 11.44±0.80$^{Bb}$ | <0.001* |
| Dg | 10.22±2.42$^{Bb}$ | 16.44±2.78$^{Aa}$ | 11.17±2.39$^{Bb}$ | <0.001* |
| De | 13.01±1.70$^{Bb}$ | 18.47±4.88$^{Aa}$ | 21.42±1.84$^{Aa}$ | <0.001* |
| p-value | <0.001* | <0.001* | <0.001* | |

Different superscript small letters indicate a statistically significant difference within the same vertical column; Different superscript capital letters indicate a statistically significant difference within the same horizontal row; *significant (p<0.05).

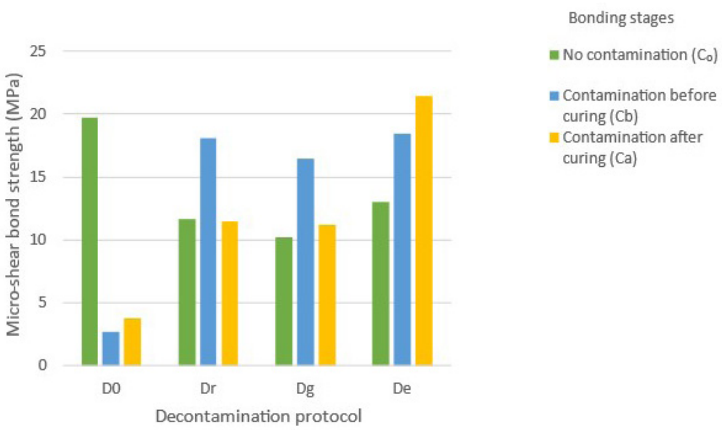


**Figure 2 -** Bar chart showing average microshear bond strength (MPa) values in different bonding stages.

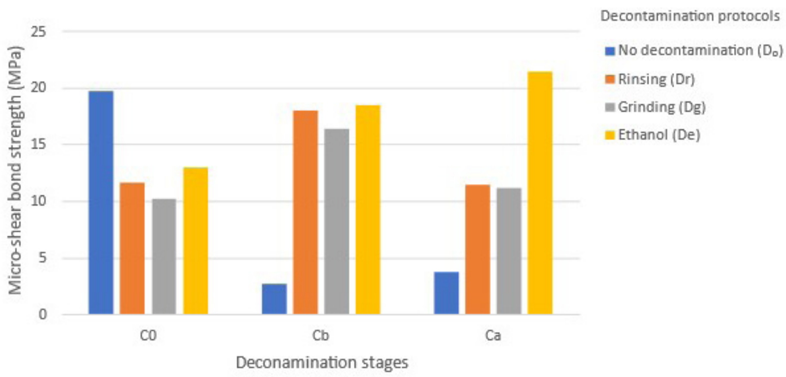


**Figure 3 -** Bar chart showing average microshear bond strength (MPa) values in different decontamination protocols.

decontamination protocol (CaDe) recorded highest bond strength followed by rinsing and grinding (CaDr and CaDg) compared to no decontamination (CaD0) (p <0.001).

Different superscript small letters indicate a statistically significant difference within the same vertical column, Different superscript capital letters indicate a statistically significant difference within the same horizontal row *significant (p<0.05).

## DISCUSSION

As a general rule for good adhesion, intimate contact between the adhesive and the adherent is required [21]. Dentin bonding is considered more difficult than that of enamel, as the water content is higher, which can prevent proper wetting by the hydrophobic dental adhesives [22]. Contaminants like saliva, blood and gingival fluid are still considered major risk factors that could further negatively affect the bonding quality to dental substrates [23].

The effect of saliva contamination to different substrates was discussed in the literature [24-26], and the need for decontamination protocols to restore bond strength was highly recommended due to its high clinical significance. Up to this point, it is hard to say we have enough information about the ideal decontamination protocol to overcome the negative effect of saliva contamination on bond strength to dentin of universal adhesives during different bonding stages.

Universal adhesives became more commonly used by dental clinicians due to their versatility and ease of use. All-Bond Universal (ABU) is a popular adhesive containing 10-MDP, crucial for durability of dentin bond strength of universal adhesives [27]. ABU was used in self-etch mode as previous studies showed no significant difference between bond strength values when used in self-etch and etch-and-rinse modes and it was also recorded that self-etch mode improved bond durability after water storage [28].

Saliva contamination before curing of the adhesive may have led to retention of additional water molecules within the adhesive layer decreasing the degree of monomer conversion resulting in weak adhesive and reduced bond strength [29]. On the other hand, when saliva contamination occurred after curing of the adhesive, the adherence of salivary proteins to the oxygen inhibited layer of the adhesive could prevent the proper tallying and copolymerization of the following resin layer and thus similarly decreasing the bond strength [30]. Hence, for decontamination, rinsing, grinding with SiC grit 600, were used to attempt to mechanically remove the saliva contaminated surface and regain the bond strength. Alternatively, ethanol as a proven organic solvent may be able to dissolve the salivary glycoproteins from the contaminated surface, as well as excess moisture [31]. This is in correspondence to literature using ethanol for multiple reasons with regards to adhesive dentistry [32-34]. All the decontamination methods used in this study aimed to remove the salivary contaminated layer either chemically or mechanically. The variation in the decontamination protocols chosen in this study was specifically aimed at showing the potential effect of both method of removal and resultant smear layer on adhesive layer formed.

The results of our study for microshear testing after 24 hours showed that the three decontamination protocols used in this study were effective in improving the bond strength levels compared to Cb $D_0$ when saliva contamination occurred before light curing of the adhesive. Distinctively, when saliva contamination occurred after light curing of the adhesive, ethanol recorded the highest bond strength values followed by rinsing and grinding then $CaD_0$. Therefore, the null hypotheses could be rejected.

Our results for contamination occurring *before curing of the adhesive* are in agreement with the results of other studies. Brauchli et al. [35], and Tuncer et al. [36] similarly concluded that just water rinsing, simple drying and reapplication of the bonding agent was enough to achieve good bond strength after salivary contamination. They also suggested that the acidic monomer component of self-etch adhesives may be able to degrade and denature the salivary proteins thus overcoming the effect of salivary contamination and providing good bonding. The acidity of universal adhesives is determined according to the concentration of 10-MDP functional monomer [37]. Therefore, the results of our study may be attributed to the MDP- containing universal ABU that may have overcome the barrier effect of salivary glycoproteins, increasing the stability of the adhesive, reducing its hydrolytic degradation and forming strong and stable ionic bonds with hydroxyapatite crystals. All Bond Universal is also known for its low water content that contributes to more moisture resistance [38].

Therefore, the results of our study may be attributed to the MDP- containing universal ABU that may have overcome the barrier effect of salivary glycoproteins, increasing the stability of the adhesive, reducing its hydrolytic degradation, and forming strong and stable ionic bonds with hydroxyapatite crystals. All Bond Universal is also known for its low water content that contributes to more moisture resistance.

Moreover, reapplication of the adhesive could aid in recovering the bond strength. ABU contains ethanol and water as solvents. While water is effective in re-expanding collapsed collagen network if excessive drying was used, ethanol has the ability to remove the organic molecules of saliva that were attached to the bonded surface [39]. This is in harmony with Cobanoglu et al. [40] who reported that repriming of the contaminated surface recovered the bond strength in one of the adhesives tested (Optibond Solo Plus SE, ph=1.4) [30,41]. Afshar et al. [42] conducted a similar study but using a two-step total etch adhesive (Single Bond). In the same way, they reported that decontamination of uncured adhesive layer with water rinsing then reapplication of the adhesive could result in bond strength values close to that of the uncontaminated control group.

In our study, a SiC grit 600 (equivalent to the yellow-coded finishing stone) [43] was used for 10s. This simulated the mechanical action of the drill as a decontamination protocol and was able to bring the bond strength values of the contaminated group closer to those of the control group ($C_0D_0$). Grinding is believed to mechanically remove the saliva contaminated surface and regain the bond strength. On the contrary to our results, Ghavam [44] reported that decontamination with water rinsing or mechanical removal of the contaminated layer using a bur resulted in no significant difference when compared to the control uncontaminated group. However, Ghavam did not mention further details regarding bur type, pressure or time of application.

Ethanol solvent in ABU has a water chasing ability allowing better removal of excess water from saliva without affecting the bonding agent [45,46]. This may suggest that ethanol component in ABU (30-60%) [47] was not autonomously enough to overcome the effect of saliva contamination. However, when used as a decontamination protocol, surplus amounts of ethanol were able to remove the residual amount of water from the contaminated surface. This may explain the observed effectiveness of ethanol as a decontamination protocol in recovering the bond strength values in our study when salivary contamination occurred before curing.

Regarding saliva contamination after curing of the adhesive, decontamination by ethanol was effective in significantly improving the bond strength values followed by rinsing and grinding compared to Ca $D_0$. It is believed that the contaminated adhesive layer should be removed in order to not interfere with surface energy, cleanliness and interfacial adaptation. The salivary glycoproteins were assumed to act as a barrier and prevent copolymerization with the following layer [36]. Ethanol as an organic solvent, may be able to better remove the salivary glycoproteins from the bonded surface as well as decreasing the surface tension of water present in saliva causing more water dispersion leading to better adhesion [29]. This result was similar to the work of Tahlan and Garg [48] where ethanol showed higher shear bond strength values than water rinsing or phosphoric acid etching when used with another ethanol-based self-etch adhesive Tetric N-Bond (Ivoclar Vivadent). Conversely, Chasqueira et al. [49] found no differences between water and ethanol as decontamination protocols after saliva contamination after curing of the adhesive.

In previous studies, grinding has not been used for decontamination except for two instances with conflicting results [44,50]. Grinding with SiC may account for embedding of the salivary glycoproteins into the dentinal tubules and interfere with proper adhesive infiltration and thus reducing the bond strength. Our results when saliva contamination occurred after curing of the adhesive were in disagreement with Furuse et al. [50] where decontamination through abrasion with finishing disks enhanced the resin-resin bonding after saliva contamination while decontamination by water rinsing and drying did not establish a good bond strength. This may be attributed to Furuse adding an etching step following grinding which may alter surface topography. Independent phosphoric acid etching may have effectively removed the salivary glycoproteins, much more effectively than ABU in self-etch mode. Meanwhile, smear layer density and thickness created during grinding may interfere with complete monomer infiltration or buffer capacity [51].

Water rinsing failed to recover bond strength values of the control group and also recorded multiple pre-test failures, not evident in uncontaminated bonding. Anjum et al. [52] condemned water rinsing as a method for salivary decontamination and considered water rinsing be worse than the salivary contamination itself. Generally, the presence of excess remaining water may negatively affect results. Conversely in our study, water rinsing yielded better microshear bond strength values than Ca-$D_0$. This is in alignment to Kim et al. [53] who reported that with ABU, simple rinsing (5s) and drying could be effective in restoring the bond strength after saliva contamination. In addition, reapplication of the adhesive could lead to further improvement of the bond strength.

# CONCLUSIONS

Using a simplified, single step, self-etch adhesive does not make it immune to salivary contamination. It is worth noting that decontamination protocols when applied without saliva contamination led to significant reduction in the bond strength values. From a clinical point of view, this may highlight that decontamination protocols could have their own compound negative side effects on bond strength, thus must be chosen carefully. Nevertheless, when decontamination is overlooked, clinical performance and durability of both adhesive and restoration are compromised. Finally, decontamination protocols have varying efficiency in restoring bond strength. All decontamination protocols were dependable before curing, and ethanol appears to be most effective both before and after curing.

## Author's Contributions

MMMES: Conceptualization, Methodology, Formal Analysis, Investigation, Resources, Data Curation, Writing – Original Draft Preparation, Writing – Review & Editing, Visualization, Supervision. KAN: Methodology, Visualization, Supervision. DSM: Methodology, Writing – Original Draft Preparation, Writing – Review & Editing, Visualization, Supervision.

## Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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## Regulatory Statement

Research proposal approved by the Research Ethics Committee at the Faculty of Dentistry Ain Shams University, Cairo, Egypt (FDASU-Rec D031822).

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