Dental Research Journal

Original Article

Comparative evaluation of different methods of saliva decontamination on microshear bond strength of composite to composite: An *in vitro* study

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ABSTRACT

Background: During the incremental technique of composite restorations, it is possible to be exposed to contamination of the oral environment. This study aimed to compare the effect of saliva contamination and different methods of saliva decontamination on microshear bond strength within composite surfaces.

Materials and Methods: This *in vitro* study created 24 rectangular XT composite blocks using a silicone mold (24 mm × 10 mm × 10 mm). The blocks were divided into eight groups as follows: Group 1: Control group (without contamination); Group 2: Contaminated with saliva; Group 3: saliva + dried; Group 4: saliva + rinsed + dried; Group 5: saliva + rinsed + dried + Clearfil Self Etch Bond; Group 6: saliva + 96% ethanol alcohol; Group 7: saliva + chlorhexidine + rinsed + dried; and Group 8: saliva + rinseddried + All-Bond Universal bond. Fifteen tubes (0.7 mm × 1 mm) were attached to the prepared surfaces in each group, with five tubes allocated to each block. They were filled with composite and light-cured and stored in distilled water for 24 h. They were tested under a microshear test. The data were analyzed using one-way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test ($P < 0.05$).

Results: One-way ANOVA test demonstrated significant differences among the groups concerning the mean bond strength ($P < 0.001$). Tukey's HSD test indicated that the bond strengths in groups 6 and 8 were similar to the control group, showing no significant difference. The lowest bond strength was observed in group 2, which measured 6 MPa.

Conclusion: Decontamination with rinsed + dried + All-Bond Universal bond and 96% ethanol alcohol were the most effective methods for improving the contaminated composites' microshear bond strength.

Key Words: Bond strength, composite resins, contamination, saliva

Received: 15-Dec-2023
Revised: 26-Oct-2024
Accepted: 15-Mar-2025
Published: 27-Jun-2025

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INTRODUCTION

Composite resins are now widely used in dentistry for tooth restoration because of their esthetic appeal, strong adhesive properties with tooth structure, minimal preparation requirements, and the possibility for repair, which aligns with minimally invasive practices.[1,2]

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Website: www.drj.ir
https://journals.lww.com/DERJ
www.ncbi.nlm.nih.gov/pmc/journals/1480
DOI: 10.4103/drj.drj_736_23

How to cite this article: Javan SO, Movahedian R, Hosseini Tabatabaei S. Comparative evaluation of different methods of saliva decontamination on microshear bond strength of composite to composite: An *in vitro* study. Dent Res J 2025;22:27.

There is an increasing focus on these restorations' durability, marginal integrity, and long-term adhesion success.[2]

The clinical use of these materials is limited due to several challenges, particularly the requirement for an isolated environment during procedures when a rubber dam cannot be used. Various factors can hinder the effective bonding of composites to dental tissues, including moisture, leakage of gingival crevicular fluid, handpiece oil, blood, lack of patient cooperation, malocclusion, and dental cervical lesions.[3-5]

Replacing a restoration can lead to less desirable outcomes, such as increased cavity preparation size, tooth damage, and the potential need for endodontic treatment or extractions. This cycle is commonly referred to as the "death spiral."[6]

Saliva contamination and other substances are known to reduce the bond strength of enamel and dentin.[7-10] Some researchers found that saliva contamination affects microshear bond strength differently depending on the type of bonding used.[3,11-13]

The incremental technique is used to reduce polymerization shrinkage and enhance the quality of composite restorations. It is helpful when additional increments of the composite are needed to refine the contour of the restorations after the rubber dam is removed. However, this technique can be time-consuming and may increase the risk of contamination.[14,15]

Furuse *et al*. found that contamination of resin–resin interfaces with saliva significantly reduced shear strength, especially after prolonged storage. They observed that bond strength values similar to the originals could be achieved by abrasion followed by adhesive application or through etching, silane treatment, and adhesive application.[15]

Eiriksson *et al*. demonstrated that saliva contamination significantly decreased the microtensile bond strength between resin composite increments. The most effective methods for decontaminating resin surfaces exposed to saliva involve adhesives.[14]

If contamination occurs, surface treatment is necessary for proper interaction between resin increments.[15] The success of repaired resin composite restorations relies on various factors, including surface characteristics, wettability, roughness, and conditioning methods.[16-19]

These treatments serve two main purposes: first, to remove the superficial layer altered by saliva, exposing a clean and higher-energy composite surface, and second, to increase the surface area by creating irregularities, which allows for better wetting by the adhesive agents.[2,18]

Despite the differences in compositions among various brands of composites, a universally applicable technique needs to be developed to address this issue.[2] Conducting clinical performance tests *in vivo* is challenging; however, evaluating their relative bond strength is possible using recognized laboratory testing methods.[11]

Additional information is needed regarding the impact of contamination between layers of composite resin and the decontamination methods used to restore the original bond strength of resin to resin.[15]

This study aims to compare the microshear bond strength of composite materials when subjected to various saliva decontamination methods.

MATERIALS AND METHODS

This *in vitro* study received approval from the Ethics Committee of Zahedan University of Medical Sciences, Iran (IR.zaums.Rec. 1395.44).

A rectangular silicone mold measuring 24 mm × 10 mm × 10 mm was created for this laboratory study. Using the incremental technique, the mold was placed on a glass slab filled with Z350XT A2 composite (3M ESPE, St. Paul, MN, USA) in 2 mm increments. To achieve a smooth surface, another glass slab was pressed onto the mold before curing the final layer, and any excess composite was removed with a spatula. Each increment was cured for 40 s at an intensity of 650 MW/cm² using a visible light-curing device (Coltolux 75, Coltene Whaledent, Inc, Switzerland). Radiation was applied from both the top and bottom of the mold. A radiometer (Coltene Whaledent Inc., Switzerland) measured the device's intensity to ensure that three composite blocks were prepared for each group.

The blocks were categorized into eight groups based on the treatments applied, as follows:

- Group 1: Control group (no saliva contamination)
- Group 2: Stored in saliva for 10 s. *Saliva was collected from a 21-year-old individual into a sterile beaker and immediately applied to the test specimens, using a volume of 100 µL for each specimen*[20]

- Group 3: Stored in saliva for 10 s, then dried using dental air spray from a distance of 10 mm for 10 s
- Group 4: Stored in saliva for 10 s, rinsed with spray water for 10 s, and dried with dental air spray from a distance of 10 mm for an additional 10 s
- Group 5: Stored in saliva for 10 s, rinsed with spray water for 10 s, dried with dental air spray from a distance of 10 mm for 10 s, and then treated with Clearfil Self Etch (SE) Bond for 10 s. A primer applicator was used, followed by mild airflow, and adhesive was applied with a microbrush and light curing for 20 s, according to the manufacturer's instructions
- Group 6: Stored in saliva for 10 s and then treated with 96% ethyl alcohol for 10 s using a cotton pellet (Ethyl Alcohol 96%, Jahan Alcohol Teb Co., Arak, Iran), followed by drying with dental air spray from a distance of 10 mm for 10 s
- Group 7: Stored in saliva for 10 s, treated with 2% chlorhexidine antibacterial solution for 10 s using a cotton pellet (Consepsis, Ultradent Products Inc., South Jordan, UT, USA), then rinsed with spray water for 10 s and dried with dental air spray from a distance of 10 mm for 10 s
- Group 8: Stored in saliva for 10 s, rinsed with spray water for 10 s, dried with dental air spray from a distance of 10 mm for 10 s, and then applied with All-Bond Universal bonding agent (Ultradent Products Inc., South Jordan, UT, USA) using a microbrush for 10 s, followed by mild airflow and light curing for 20 s, according to the manufacturer's instructions.

The bonding agents, composite compositions, batch numbers, and manufacturers are detailed in Table 1.

Five tubes (Tygon; Norton Performance Plastics, Cleveland, OH, USA), each measuring 0.7 mm and 1 mm in diameter, were attached to the top of the treated blocks, resulting in 15 tubes per group. The tubes were filled with Z350XT A2 composite and cured for 40 s from the top of each tube. All groups were then immersed in distilled water at 37°C for 24 h.

To determine the microshear bond strength, specimens were bonded using cyanoacrylate glue (Zapit, Corona, CA, USA) to a universal testing machine (Bisco, Livonia, MI, USA). An orthodontic ligature wire with a diameter of 0.012 inches (American Orthodontics, Sheboygan, WI, USA) was looped between the loading cell and the base of the composite cylinder. This arrangement applied shear stress by allowing the force vector to act parallel to the cylinder's cross-section.

The specimens were stressed at a cross-head speed of 0.5 mm/min. The microshear bond strength (measured in MPa) was calculated by dividing the maximum load at fracture (in N) by the cross-sectional surface area (in mm^2) of the bonded surface.

Data analysis was conducted using one-way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test to compare the microshear bond strengths among the eight groups. A $P < 0.05$ was considered statistically significant.

RESULTS

Data distribution patterns were analyzed using the Kolmogorov–Smirnov test, which confirmed that the data followed a normal distribution.

Table 2 shows a clear hierarchy of mean microshear strength. Group 1 exhibited the highest strength, averaging 23.09 ± 4.52 MPa, followed closely by Group 8 at 22.01 ± 5.29 MPa and Group 6 at 19.80 ± 2.03 MPa. In contrast, Group 2 had the lowest strength, measuring 10.91 ± 2.62 MPa.

The results from one-way ANOVA revealed a statistically significant difference among the mean micro-shear bond strength values ($P < 0.001$). In

Table 1: Materials used

Material	Manufacturer	Lot number	Composition
Two-step clearfil SE bond prime	Kuraray noritake dental Inc., Sakazu, Kurashiki, Okayama, Japan	3A0244	Bis-GMA, BPDM, HEMA, acetone MDP, HEMA, hydrophilic dimeth arylate, n, n die-thanol-p-toluidine, CQ, water
Clearfil SE bond adhesive		920343	MDP, Bis-GMA, HEMA, hydrophobicalip-hatic dimethacrylate, n, n diethanol-p-toluidine, silanated colloidal silica CQ
All-bond universal	BISCO Inc. Schaumburg, IL, USA	1500004745	MDP, bis-GMA, HEMA, ethanol, water
Filtek Z350XT	3M ESPE, St. Paul, MN, USA	N597862	bis-GMA, UDMA, TEGDMA, bis-EMA, PEGDMA, silica filler, zirconia filler, zirconia/silica (aggregated)

BisGMA: Bis-phenol-A-diglycidyl methacrylate; UDMA: Urethane dimethacrylate; bis-EMA: Bisphenol A polyetheyleneglycol diether dimethacrylate; BPDM: Biphenol dimethacrylate; TEGDMA: Triethylene glycol dimethacrylate; MDP: 10-methacryloyloxy methacryla; HEMA: Hydroxyethyl methacrylate; CQ: Camphoroquinone; SE: Self etch

Table 2: Microshear bond strength of composite to composite in groups of study

Composite surface treatment	Mean±SD (MPa)	Minimum	Maximim	*P*
1. Without saliva contamination (control)	23.10±4.52	17.5	33.5	<0.001*
2. Contaminated with artificial saliva	10.91±2.62	6	15.2	
3. Contaminated with artificial saliva + dried	11.54±3.54	4.7	16	
4. Contaminated with artificial saliva + rinsed + dried	14.21±3.13	6.8	17.5	
5. Contaminated with artificial saliva + rinsed + dried + clearfil SE bond	15.30±4.85	6.1	23.5	
6. Contaminated with artificial saliva + alcohol	19.80±2.03	16.5	24	
7. Contaminated with artificial saliva + chlorhexidine + rinsed + dried	17.91±3.60	14.4	22.2	
8. Contaminated with artificial saliva + rinsed + dried + all bond universal	22/00±5.29	17.5	27.7	

*One way ANOVA. SD: Standard deviation; SE: Self etch

addition, the Tukey HSD test showed statistically significant differences between Group 1 (the control group) and Groups 2, 3, 4, 5, and 7 ($P < 0.05$). However, the bond strengths in Groups 6 ($P = 0.159$) and 8 ($P = 0.989$) were close to that of Group 1 (the control group), and no statistically significant differences were found ($P > 0.05$).

DISCUSSION

One limitation of composite resin materials used for restorations is their sensitivity to insertion techniques and environmental contamination. In such cases, a minimally invasive approach is preferred. Therefore, it is important to identify effective methods for decontaminating the affected composite rather than relying on lengthy and time-consuming processes.[7,10,14]

The results of the present study indicate that all methods utilized to eliminate saliva contamination improved bond strength to some extent. However, the least significant impact on contamination was noted in Groups 3 (contaminated with artificial saliva and left to dry) and 4 (contaminated with artificial saliva, rinsed, and dried). In fact, these groups did not significantly differ from Group 2 (contaminated with artificial saliva).

Some researchers have conducted studies on composites, and saliva contamination within resin–resin surfaces has been reported to significantly reduce the shear bond strength,[14,15,21] which is in line with the present study's findings.

It was found that saliva decontamination with air and/or water spray was insufficient to ensure adequate bonding strength between the composite increments, which is in agreement with published results. This is probably due to the nonremoval of saliva glycoproteins from the composite surface, which interferes with bonding to the composite surface.[14] Therefore, a surface treatment is recommended to improve the interaction between resin increments.

Some authors emphasize the importance of using phosphoric acid in repair procedures. It effectively removes organic contamination and debris left by mechanical treatment, which enhances the reaction between silane and inorganic particles.[2]

In contrast, this study focused on older composites, whereas our research concentrated on salivary contamination and aimed to reduce decontamination procedures.

Saliva mainly comprises water (99.4%) and solids (0.6%). The solid components include macromolecules such as proteins, enzymes, mucins, immunoglobulins, nitrogen products, and electrolytes such as calcium, sodium, and chloride. Organic substances such as urea, amino acids, fatty acids, and free glucose are also present. These components can reduce the bond strength of dental materials.[5]

The glycoproteins in saliva can be absorbed by the surface of poorly polymerized adhesives, creating a barrier that decreases the wettability of composite resins and hinders their polymerization. Furthermore, water trapped in the cured resin can interfere with the subsequent polymerization of the material.[5,15]

In addition, the lack of an oxygen-inhibited layer between the polymerized surface and the newly applied unpolymerized material can be another reason.[2]

In Group 5, the use of Clearfil SE Bond improved bond strength. However, there were no significant differences compared to Groups 3 (dried with dental air spray), 4 (sprayed with water and then dried with dental air spray), and 7 (2% Chlorhexidine). Furthermore, Group 7 showed no significant differences compared to Groups 4, 5, and 6 (96% alcohol).

It is possible that solvents like ethanol and acetone when used in specific concentrations, can denature glycoproteins and assist in the decontamination of saliva from composite surfaces. Our study supports this notion, where chlorhexidine and SE Bond, both containing these solvents, proved effective.[14] However, despite these promising results, the bond strength in groups 5 and 7 remained suboptimal. This may be due to the lower concentrations and types of solvents utilized in these groups.

In this study, the best methods for saliva decontamination were found in Groups 6 and 8, which used the All-Bond Universal bonding agent. These groups showed no significant differences when compared to the control group. This result is consistent with findings from several other investigations.[11,14]

Universal Adhesive Agents (UAs) are one-step bonding systems that combine primer and bonding in one bottle. They utilize self-etch technologies and offer numerous advantages, including simplicity, increased user-friendliness, less sensitivity to techniques, and reduced chair time for patients, particularly in pediatric dentistry.[22]

Adhesives containing 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) demonstrated stronger bonding than those made with other acidic components. However, these results depended on several factors, including the type of mechanical testing used, the substrate type, the adhesive's acidic composition, and the specific application category of the SE system.[1,12]

Most UAs, including All Bond Universal, contain HEMA (2-Hydroxy-ethyl-methacrylate) that is utilized for its hydrophilic properties, potential to wet the surface and penetrate moist areas, and ability to inhibit phase separation between hydrophilic and hydrophobic monomers.[11]

Some adhesives, such as All Bond Universal, contain a silane coupling agent to simplify and expedite a technique-sensitive procedure, which is suggested for adhesive bonding to ceramic and repair restorations.[1,23]

Silane contains silanol groups that react with the exposed inorganic filler particles of the composite substrate. It also has organofunctional groups that react and co-polymerize with the methacrylate groups in the repair material. Moreover, silane enhances the wettability of the composite substrate surface, which facilitates the infiltration of the bonding agent into the surface's micro-retentions.[1,24]

One-step self-etch adhesives that use ethanol as a solvent or co-solvent, such as All Bond Universal, demonstrated higher shear bond strength than other self-etching bonding agents.[25]

Research by Furuse *et al*. indicates that the application of adhesive – whether used alone or in combination with silane – was the most effective method for enhancing the shear bond strength of repaired composite specimens. Bonding agents facilitate improved surface wetting and resin infiltration. Furthermore, single-bottle adhesives contain solvents that can denature glycoprotein sugars and eliminate saliva contamination,[15] as demonstrated in our study.

Due to the acidity, hydrophilicity, and ethanol solvent in All-Bond Universal adhesive, which combines the acid, primer, and adhesive into a single, thin layer, one potential explanation is that this bonding process re-etches the surface. This re-etching removes salivary proteins and enhances the adhesive's bond strength,[5] which supports our study's findings.

In addition, the 10-MDP monomer in the composition of the universal adhesive and ethanol-based solvents, as seen in groups 6 and 8, likely plays a crucial role in saliva decontamination. The presence of HEMA may significantly contribute to the effectiveness of these products, regardless of their application.

Therefore, using the universal adhesive in 10 s can prevent water absorption and significantly enhance bond strength, even after thermocycling,[14] similar to what we observed in Group 8. Some studies have indicated that saliva does not have a significant impact on the bond strength of certain adhesives,[3] which contrasts with our findings.

In most investigations where saliva contamination does not affect bond strength, self-etch adhesive systems have been utilized. The hydrophilic properties of these adhesives explain this phenomenon.[3,14]

The differences in study results can be linked to the bonding process and the type of adhesive used. The differences in study results can be linked to the bonding process and the kind of adhesive used.[1,3,14,21,26,27]

Some studies suggest that the type of composite influences the bond strength of the decontaminated composite surface.[1,17] However, we utilized only one kind of composite, FiltekTM Z350 XT. Therefore, universal adhesives that have adequate acidity and hydrophilicity, ethanol solvents, 10-MDP, silane, and solvents like 96% alcohol are effective for decontaminating saliva without the need for mechanical treatments. However, the clinical effectiveness of these substances is influenced by oral environments that cannot be replicated under *in vitro* conditions.

CONCLUSION

The results of this study indicate that saliva contamination significantly reduced microshear bond strength. All saliva decontamination methods partially improved the microshear bond strength of the specimens. However, the bond strengths observed with the rinsing, drying, and All Bond Universal bonded group, as well as the alcohol group, were similar to the control group, showing no significant differences between them.

Further studies should explore new composites and examine various materials and methods for effectively removing saliva and blood contamination, particularly in the oral cavity.

Financial support and sponsorship

This study was derived from a dental student's thesis (No: 7578) with ethics code IR.ZAUMS.Rec. 1395.44.

We thank the Research Council and the Ethics Committee of Zahedan University of Medical Sciences, Iran, for approving this plan.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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