# Can Cleansing Regimens Effectively Eliminate Saliva Contamination from Lithium Disilicate Ceramic Surface?


## ABSTRACT

*This study evaluated the effect of cleaning protocols on the bond strength of resin cement to glass-ceramic. Ceramic specimens (N=120, n=12 per group) were etched with hydrofluoric acid and rinsed with water. After saliva contamination, specimens were cleaned as follows: water, 37% H3PO4, cleaning-paste (Ivoclean), or isopropanol. Non-contaminated specimens acted as the control. Resin cement was bonded to the specimens, and tested either after 24 h or x5000 thermocycling. Both the cleaning method (p=0.001) and the storage conditions (p=0.005) significantly affected the bond strength results. In dry conditions, the groups PA and IV showed no significant difference, being also similar to the non-saliva contaminated control group (p>0.05). In dry conditions, no significant difference was observed between the mean DW and IS being significantly lower than those of other groups (p<0.05). Except for the group IV, thermocycling decreased the results significantly in all groups (p<0.05). Predominantly mixed failure type was observed in both dry and aged conditions. SEM micrographs of ceramic surfaces after cleaning agents showed no major differences but on the specimens from the IV group, small, rounded-zirconia particles were observed. In case of saliva contamination of acid-etched glass-ceramics, mechanical cleaning can restore adhesion to the baseline situation.*


## INTRODUCTION

The clinical success of all-ceramic restorations in dentistry is highly dictated by adhesive cementation to the tooth substance, increasing the fracture resistance[1,2] and decreasing microleakage.[3] Adhesion between enamel/dentin and all-ceramic restorations is achieved after etching the glassy matrix ceramics with hydrofluoric acid (HF) followed by the application of silane coupling agent and adhesive resin.[4]

HF selectively dissolves glass from the ceramic microstructure, promoting micromechanical retention.[5,6] In addition, this process increases the surface-free energy of the ceramic, yielding to low contact angles and thereby, better wettability.[7] Silane coupling agent then makes covalent bonds between the inorganic and organic surfaces with the bifunctional monomers in the composition.[6]

Ceramic etching with HF could be performed either at chairside by the dentist or in the dental laboratory by the dental technician.[8] When performed by the dental technician, the subsequent try-in procedure leads to surface contamination with saliva and its proteins.[9] Saliva contains organic


## Keywords

*Bond Strength*
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*Hydrofluoric Acid*
*Cleaning Protocol*



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materials such as salivary proteins, enzymatic molecules, bacteria and food debris, and inorganic compounds such as mineral ions in water.[10] Adhesion of salivary proteins to dental materials and tooth surfaces, result in formation of acquired enamel pellicle that is free of bacteria at a thickness of 10-20 nm within a few minutes.[11] With the increase in the protein transmission from saliva, the thickness of this protein layer reaches to 100-1000 nm between 30-90 minutes.[11] The resulting persistent protein contamination from saliva in particular was shown to hinder adhesion of the resin cements to glass ceramics.[12,13]

Therefore, several cleaning methods have been proposed to eliminate the contamination from the ceramic surface, one of which is re-etching with HF.[14-16] In fact, due to the hazardous potential of HF, its chairside application should be performed with caution.[17] As a result, alternative cleaning methods without the use of HF has been sought. Other cleaning protocols include rinsing with water,[18] etching with phosphoric acid, and cleaning with alcohol.[19] Despite the fact that these methods are easy to apply, they did not seem to recover the bonding capacity of ceramic restorations compared to non-contaminated surfaces.[18,19]

The objectives of this study therefore were to evaluate the effect of different cleaning methods of a lithium disilicate glass-ceramic surface contaminated with saliva on the adhesion of resin cement with and without aging conditions. The null hypotheses tested were that the cleaning method and the aging conditions would not affect adhesion of resin cement to lithium disilicate ceramic.

# MATERIALS AND METHOD

The types, brands, manufacturers, chemical compositions and batch numbers of the materials used in this study are listed in Table 1.

## SPECIMEN PREPARATION

The glass-based ceramic blocks (e.max CAD, Ivoclar Vivadent, Schaan, Liechtenstein) were cut into plates (13 mm × 13 mm × 2 mm) (N=120, n=12 per group) in a precision cutting machine (Isomet, Buehler, Lake Bluff, IL, USA). The specimens were crystallized in the corresponding furnace (Sintramat High Temperature Furnace, Ivoclar Vivadent) according to the manufacturer's recommendation (20-25 min; 840-850°C). Specimens were then embedded in acrylic resin with their bonding surfaces exposed and wet-ground finished with silicon carbide papers (#600 to 1200). After etching with 5% HF gel (IPS Ceramic Etching Gel, Ivoclar Vivadent) for 1 min, the ceramic specimens were ultrasonically cleaned (L&R 2014 Ultrasonic Cleaning System, L&R Manufacturing Company, Keamy, NJ, USA) in distilled water for 5 min and air-dried.

The etched specimens were randomly divided into five groups according to the cleaning methods (n=12, per group):

Group C: In this group, the specimens were not contaminated with saliva and acted as control.

Group DW: In this group, the specimens were immersed in human saliva for 1 min (IRB #0304-58) and only rinsed with distilled water for 15 s.

Group PA: Saliva was collected from one person who refrained from eating and drinking 2 h before collection and rubbed on the ceramic discs and left undisturbed for 60 s.9,[20] After saliva contamination as in group DW, specimens were etched with 37% $H^3PO^4$ (Total Etch, Ivoclar Vivadent) for 60 s and rinsed with distilled water for 15 s.

Group IV: After saliva contamination as in group DW, the specimens were cleaned using cleaning paste (Ivoclean, Ivoclar Vivadent) according to the manufacturer's instructions for 20 s, rinsed with water for 15 s, and dried for 10 s. This cleaning paste containing zirconium oxide, water, polyethylene glycol, sodium hydroxide, pigments, additives. Adsorbed proteins are detached by coagulation or desorption from ceramic surface onto the cleaning particles.[21,22] Subsequent water rinsing can then remove the coagulated or desorbed proteins.

**Table 1.** The types, brands, manufacturers and batch numbers of the materials used in this study (Ivoclar-Vivadent, Schaan, Liechtenstein)

| Material | Brand | Batch number |
|---|---|---|
| **Glass-based ceramic** | E.max CAD | R55522 |
| **HF acid gel (5%)** | IPS Ceramic Etching Gel | R53559 |
| **Cleaning paste** | Ivoclean | R53033 |
| **Phosphoric acid gel (37%)** | Total Etch | R51858 |
| **Silane coupling agent** | Monobond Plus | R50513 |
| **Resin cement** | Multilink Automix | S04093 |

Group IS: After saliva contamination as in group DW, The specimens were cleaned with 70% isopropanol for 2 min, rinsed with distilled water for 15 s and air dried.

Silane coupling agent was applied (Monobond S, Ivoclar Vivadent) on the ceramic surfaces, allowed to react with the surface for 60 s and air-dried.

A cylindrical polyethylene mold (Ø=2.38 mm; height: 2.15 mm) (Bonding Jig, Ultradent Products, Inc., South Jordan, UT, USA) was positioned on the conditioned ceramic surface. The resin cement (Multilink, Ivoclar Vivadent) was mixed according to the manufacturer's recommendations and inserted into the mould. The resin cement in the mould was photo-polymerized for 40 s (LEDemetron II, Kerr Corporation, Middleton, MI, USA) Light output (800 mW/cm$^2$) was monitored with a radiometer (Demetron, Kerr Corporation, Orange, CA, USA). Finally, the moulds were removed and the specimens were stored at 37°C for 24 h.

## AGING PROTOCOL

Half of the specimens in each group were tested 24 h after cement application (dry condition) and the other half were subjected to thermocycling (TC; x5000 cycles, 5°-55°C, dwell time: 30 s, transition time: 10 s from one bath to the other).

## BOND STRENGTH TEST AND FAILURE ANALYSIS

The shear bond strength (SBS) test was performed using a Universal Testing Machine (MTS Sintech ReNew, Test Resources, Inc., Shakopee, MN, USA) at a crosshead speed of 1 mm/min. The bond strength (MPa) was calculated using the equation: SBS=Load/A0, where A is the diameter of the bonded area, load is the force for failure in Newton.

After debonding, the failure modes were evaluated in a stereomicroscope (x20) (Discovery V-20, Carl Zeiss, Jena, Germany) and classified as follows: a) Adhesive: cement totally debonded from the ceramic surface with no remnants, b) Mixed: More than 60% of the cement detached from the ceramic, c) Cohesive: More than 60% of the cement/ceramic cohesively fractured. Additionally, one specimen from each group immediately after cleaning protocols was gold-sputtered and evaluated under a Scanning Electron Microscope (SEM, Inspect S50, FEI, Brno, Moravia, Czech Republic) in order to examine the topographical changes after cleaning (x5000).

## STATISTICAL ANALYSIS

Statistical analysis was performed using Statistica 8.0 software for Windows (StatSoft, Inc., Tulsa, OK, USA). Logarithmic transformation of the data (MPa) was performed and data were submitted to two-way analysis of variance (2-way ANOVA) with the bond strength as the dependent variable and aging methods (2 levels: Dry, Thermocycling) and cleaning methods (5 levels: C, DW, PA, IV, IS) as independent variables. Multiple comparisons were made using Tukey`s post-hoc test (α=0.05). P values less than 0.05 were considered to be as statistically significant in all tests.

# RESULTS

Both the cleaning method (p=0.001) and the storage conditions (p=0.005) significantly affected the bond strength (MPa) results. The interaction terms were not significant (p=0.125).

In dry conditions, the groups PA (20.5±7.4) and IV (21.1±5.8) showed no significant difference, being also similar to the non-saliva contaminated control group (p>0.05) (21.9±6.7 MPa) (*Table 2*). In dry conditions, no significant difference was observed between the mean DW (16.8±5.9) and IS (17.7±6) (p>0.05) being significantly lower than those of other groups (p<0.05).

**Table 2.** Mean (± SD) shear bond strength values (MPa) for the experimental groups. *Same lowercase superscript letters in each row, and same uppercase letters in each column indicates no significant difference (Tukey`s test, α=0.05). C: Control, DW: Distilled water, PA: 37% H3PO4, IV: Ivoclean cleaning paste, IS: 70% isopropanol.

| | Aging | | |
|---|---|---|---|
| **Cleaning Methods** | 24 h storage (Dry) (Mean ± SD) | Thermocycling (x5000) (Mean ± SD) | Total |
| **C** | 21.9 ± 6.7a | 18.9 ± 7.2a | 20.4 ± 7AB |
| **DW** | 16.8 ± 5.9b | 16.3 ± 5.5a | 16.6 ± 5.6BC |
| **PA** | 20.5 ± 7.4a | 16.5 ± 6.3a | 18.5 ± 7ABC |
| **IV** | 21.1 ± 5.8a | 21.8 ± 6.2b | 21.5 ± 5.9A |
| **IS** | 17.7 ± 6b | 10.7 ± 2.8c | 14.2 ± 5.8C |
| **Total** | 19.6 ± 6.5a | 16.8 ± 6.7b | |

**Table 3.** Distribution of the frequencies of failure types for experimental groups. Adhesive: cement totally debonded from the ceramic surface with no remnants, Mixed: More than 60% of the cement detached from the ceramic, Cohesive: More than 60% of the cement/ceramic cohesively fractured. See Table 2 for group descriptions.

| | Type of Failure n (%)* | | | | | |
|---|---|---|---|---|---|---|
| | 24 h storage (Dry) | | | Thermocycling (x5000) | | |
| | Adhesive | Mixed | Cohesive | Adhesive | Mixed | Cohesive |
| **C** | 2 (16.7) | 7 (58.3) | 3 (25) | 2 (16.7) | 3 (25) | 7 (58.3) |
| **DW** | 5 (41.7) | 5 (41.7) | 2 (16.7) | 0 | 6 (50) | 6 (50) |
| **PA** | 0 | 10 (83.3) | 2 (16.7) | 1 (8.3) | 2 (16.7) | 9 (75) |
| **IV** | 0 | 9 (75) | 3 (25) | 2 (16.7) | 5 (41.7) | 5 (41.7) |
| **IS** | 0 | 9 (75) | 3 (25) | 4 (33.3) | 5 (41.7) | 3 (25) |

Except for the group IV (21.8±6.2), thermocycling decreased the results significantly (10.7±2.8 - 18.9±7.2) in all groups ($p<0.05$).

Examinations of the debonded specimens revealed predominantly mixed failure type in both dry and aged conditions *(Table 3*). While SEM micrographs of ceramic surfaces after cleaning agents showed no major differences, on the specimen surfaces from the IV group, small, rounded zirconia particles were observed *(Figures 1a-e*).

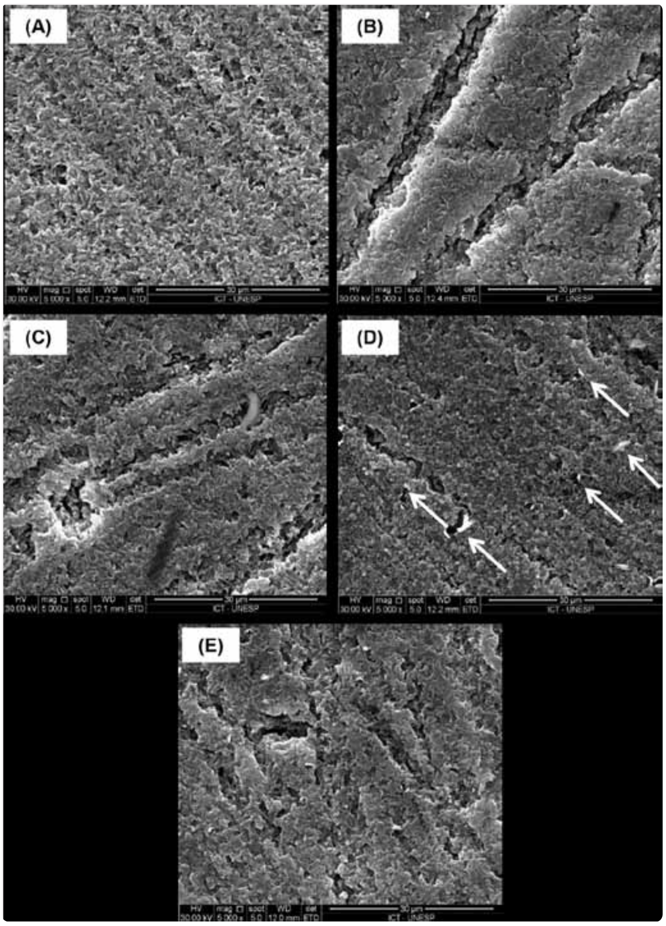


**Figure 1a-e:** SEM micrographs (x5000) of lithium disilicate ceramic surfaces from groups a) C, b) DW, c) PA, d) IV, e) IS. Note similar surface topography after cleaning methods but possible remnants of zirconia particles after cleaning with IV indicated by arrow. See Table 2 for group descriptions.

# DISCUSSION

This study was undertaken in order to propose the most effective cleaning regimen to remove saliva contamination from HF etched lithium disilicate glass ceramic without impairing the adhesion of the resin cement after aging. Based on the results obtained, since both cleaning method and aging parameters affected the results significantly, the null hypotheses were rejected.

In dry conditions, cleaning the ceramic surfaces with either PA or IV delivered bond strength results similar to that of the control group where ceramics were not contaminated with saliva. Under the same conditions however, DW and IS were less effective in terms of bond strength. In previous studies, PA etching was considered as a suitable alternative for removing saliva film from zirconia surfaces but adhesion was not improved after this acid etching.[18,20] It has to be noted that zirconia surfaces present a smoother topography than etched glass ceramics. Although IV and PA etching performed similar in dry conditions, thermocycling decreased the bond strength of the latter except for the group IV. Thus, it can be stated that mechanical cleaning method is compulsory for the glass ceramics to remove the saliva film.

Saliva proteins bound to the ceramic surface could not be completely removed from the specimens. In the present study, the use of the cleaning paste including zirconia particles proved to be an effective method to restore bond capacity of the saliva contaminated glass-based ceramic. According to the manufacturer, this solution consists of an alkaline suspension of zirconium oxide particles that react with saliva, making the ceramic surface clean. Since it contains sodium hydroxide, it is probably also capable of protein dissolution. In several other studies, cleaning pastes with zirconia particles was demonstrated to remove saliva contaminants from both glass and oxide-based ceramic surfaces but it was not effective in removing silicone residues.[18,19]

The mean bond strength obtained with phosphoric acid were not significantly different from those of DW and IS initially. However, reduction after thermocycling was more distinct for IS and PA but almost negligible for DW and IV. This is most probably due to the change in surface-free energy of the lithium disilicate glass ceramic after PA and Is cleaning methods that needs to be evaluated in future studies. In previous studies, it was reported that IS did not penetrate into the grooves on the ceramic after the try-in procedure.[7] However, when the etched ceramic surface was already silanized, cleaning with alcohol was found to be effective to restore the bond strength.[8] Thus, the results of this study need to be verified in situations where the ceramic surface was contaminated after silane application.

In previous studies water spray, alcohol and acetone seem not be effective in removing saliva residues from glass ceramics[14,21] but 35 to 37% phosphoric acid gel application presented effective cleaning.[14] The mechanism of phosphoric acid is not completely understood but it is postulated that the acid possibly penetrates the salivary film and etches the porcelain surface underneath, thereby releasing the salivary film from the surface.[14] Phosphoric acid also removes the adsorbed proteins by coagulation or desorption from ceramic surface onto the cleaning particles.[22] Subsequent water rinsing can then remove the coagulated or desorbed proteins. In fact, phosphoric acid was reported to passivate the zirconia surface when used in combination with phosphate methacrylate based primers used in adhesive cementation and decrease bond strength.[23,24] However, in this study, methacrylate based resin cement was used. Apparently, phosphoric acid did not hinder the copolymerization between silane, adhesive resin and the resin cement tested when bond strength results in dry conditions are considered.

The decrease in bond strength after thermocycling is multifactorial such as interfacial hydrolysis, cement degradation, and thermal stresses due to different thermal coefficients of the substrate and the adherend.[25,26] Nevertheless, the incidence of mixed and adhesive failures after thermocycling compared to dry conditions clearly indicates degradation of the adhesion at the bonded interface, except for IV.[8,14] Compared to adhesive failures, mixed failure type may indicate better adhesion but the area covered by cement remnants was minimal compared to adhesively debonded zones. Interestingly however, the number of cohesive failures which is a mixture of cohesive failure of the cement and/or the ceramic was also observed which indicates some further polymerization of the cement most possibly due to the temperature increase up to 55°C during thermocycling. Thus, other aging conditions such as long-term water storage above 6 months could be considered in future studies as it adds to the hydrolysis as a consequence of water sorption where temperature increase is eliminated.

SEM micrographs of ceramic surfaces after cleaning agents showed no major differences with regards to the surface topography. On the surface of the IV group, small, rounded particles possibly zirconia particles were observed. It could not be verified whether they were loose of not but obviously they did not affect the resin bond strength. Furthermore, one reason for no decrease in bond strength in the IV group after aging procedure could be attributed to the presence of particle remnants that could have contributed to micromechanical retention on the ceramic surface.

Although adhesion between resin cement and etched and silanized glass ceramics is well established, the possible saliva contamination during the intraoral try-in procedures could impair bond strength results and indicates the clinical significance of this investigation particularly for minimal invasive reconstructions. Adhesive cementation protocols should consider removal of contamination media that is a critical step to improve the longevity of bonded restorations.



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# CONCLUSIONS

From this study, the following could be concluded:

1. Bond strength results in non-aged conditions indicated that cleaning hydrofluoric acid etched, saliva contaminated lithium disilicate could be cleaned with either phosphoric acid or cleaning paste containing zirconium oxide particles, delivering similar results to non-contaminated control group.
2. Thermocycling significantly decreased the bond strength results for all cleansing groups except for the group mechanically cleaned with the paste, Ivoclean.
3. After debonding, failure types were predominantly mixed followed by adhesive failures.

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# DISCLOSURE

The authors declare that they have no conflict of interest.

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