

# Evaluation of Cleaning Methods on Lithium Disilicate Glass Ceramic Surfaces After Organic Contamination

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## Clinical Relevance

Air–water spray, 35% phosphoric acid, 70% alcohol, and Ivoclean are effective cleaning methods for removing saliva from a previously etched and silanized lithium disilicate glass ceramic. When contaminated with human blood, only Ivoclean cleaning paste was able to restore the initial bond strength.

## SUMMARY

The purposes of this study were to 1) evaluate the effectiveness of different cleaning methods from a previously etched and silanized lithium disilicate

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glass ceramic (EMX) surface after contact with organic fluids (saliva or human blood) and 2) assess the effect of applying a new silane layer after the cleaning methods on the microshear bond strength

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(mSBS) of resin cement to EMX. EMX discs were etched with 5% hydrofluoric acid (HF) and properly silanized. Three control groups were created ( $n=10$ ): control (without contamination), saliva positive, and human blood positive. Later, after new contaminations, the samples were distributed into four groups according to the cleaning method ( $n=20$ ): air–water spray (AWS), 35% phosphoric acid, 70% alcohol, or Ivoclean cleaning paste. After the cleaning methods, subgroups were submitted to a new silane layer application, or not ( $n=10$ ). All samples received a thin layer of a bonding agent and, subsequently, three light-cured resin cement cylinders were prepared on each EMX surface for the mSBS test. This test was performed on a universal testing machine at a vertical speed of 1 mm/minute until rupture. Contaminated and cleaned silanized EMX surfaces were assessed by scanning electron microscopy (SEM) ( $n=1$ ). The noncontaminated control group showed an average mSBS of 18.7 MPa, and the positive saliva and human blood control groups yielded a 34% and 42% reduction in bond strength, respectively, compared to the uncontaminated control ( $p<0.05$ ). For saliva-contaminated surfaces, all cleaning methods were effective and not different from one another or the control group ( $p>0.05$ ). However, for human blood contamination, only Ivoclean cleaning paste was effective in restoring  $\mu$ SBS to uncontaminated control group levels ( $p>0.05$ ). SEM images showed a clean surface (ie, with no contaminant residues) after the cleaning methods, regardless of the organic contaminant type. All the assessed cleaning methods were effective in removing saliva from the silanized EMX surface; however, only Ivoclean was able to restore the adhesion quality when the silanized EMX surface was contaminated with human blood.

## INTRODUCTION

Glass ceramics are widely used in dentistry as a restorative material for esthetic and morphological reconstruction due to their biocompatibility, ability to mimic optical characteristics of enamel and dentin, and adequate chemical stability.<sup>1-5</sup> The clinical success (ie, strong adhesion) of glass ceramic restorations is highly dependent on the adhesive bonding–interaction between dental tissues, resin cement, and glass ceramic.<sup>6</sup>

For proper bonding to resin cements, glass ceramics are previously etched with hydrofluoric acid (HF) that dissolves the glassy phase, thereby promoting

surface roughness. This increases the surface area and surface energy for micromechanical interlocking to resin cements.<sup>7-11</sup> Thereafter, a silane coupling agent is applied to yield chemical bonding between silica present in the glass ceramics and methacrylate groups of the resin cements.<sup>12-15</sup> HF etching followed by silane coupling agent is deemed as the most adopted intaglio surface protocol for adequate bonding to glass ceramics. This technique is essential for long-lasting glass ceramic restorations.<sup>14,16</sup>

During try-in procedures (internal and proximal fit adaptation, and esthetic assessments) of the ceramic on the prepared tooth, the intaglio glass ceramic surface treatment may become contaminated with saliva or human blood.<sup>17-21</sup> Saliva or human blood contamination may take place as a result of 1) the impossibility for rubber dam isolation, 2) marginal gingival bleeding from unsatisfactory provisional restoration finishing/polishing/adaptation, 3) marginal gingival inflammation related to gingivitis, and 4) oversight of previous tooth prophylaxis. Both saliva and human blood organic contaminants have a negative influence on the bond strength between resin cements and glass ceramics.<sup>17,18,22,23</sup>

Several methods (air/water spray, ethanol, phosphoric acid, and plasma) have been suggested to clean the contaminated ceramic surface prior to bonding procedures with certain degrees of success.<sup>18,19,21,23,24</sup> Recently, a commercial product was designed to effectively clean ceramic surfaces after saliva contamination and has since been confirmed.<sup>20,22,25</sup> Most of the laboratory studies evaluated the proposed cleaning methods before silane application,<sup>18,22,23,25</sup> however, higher contact angles were reported after silane application on glass ceramics as a result of a hydrophobic surface.<sup>26,27</sup> As such, it can be assumed that the cleaning methods for organic contaminants would perform better after silane application, and thereby properly restore the bonding strength to glass ceramics.

Therefore, the purpose of this laboratory study was to evaluate the efficacy of several cleaning methods on previously etched and silanized lithium disilicate glass ceramic after saliva or human blood contamination on the microshear bond strength ( $\mu$ SBS) to resin cement. The effect of a new silane layer application after the cleaning methods was also assessed. The tested hypotheses were: (1) the cleaning methods will restore the bond strength; (2) the cleaning methods will remove organic contaminants from silanized ceramic surfaces; and (3) silane reapplication after the cleaning methods will improve the bond strength.

## METHODS AND MATERIALS

### Ceramic Specimens

Two hundred and one discs (10-mm diameter x 3-mm thick) of a lithium disilicate reinforced glass ceramic (IPS e.max Press - shade LTA2, Ivoclar Vivadent, Schaan, Liechtenstein) (EMX) were fabricated according to the manufacturer's instructions.<sup>7</sup> The EMX samples were placed in a horizontal position and embedded in acrylic resin using polyvinyl siloxane (PVS) molds (20-mm diameter x 20-mm height). To obtain a flat, polished, and homogeneous surface, the samples were submitted to sequential polishing using silicon carbide abrasive papers (#400 and #800, Norton SA, São Paulo, SP, Brazil) in a water-cooled automatic polisher (Metaserv 250, Buehler, Lake Buff, IL, USA). Thereafter, all EMX specimens were cleaned in an ultrasonic bath for 10 minutes and dried using oil-free compressed air. The materials used in this study are described in Table 1.

The EMX surfaces were etched with 5% HF (Condac Porcelain, FGM, Joinville, SC, Brazil) for 20 seconds, rinsed using oil-free air–water spray (AWS) for 30 seconds, and air dried for 30 seconds. A silane coupling agent (Monobond N, Ivoclar Vivadent) was actively applied to the etched EMX surface with a disposable microbrush for 15 seconds, left to react for 60 seconds, and air-dried until all solvents were eliminated.

### Ceramic Surface Contamination

Thirty etched/silanized EMX samples were randomly assigned into three control groups (n=10): no contamination (control), saliva positive control (SPC), and human blood positive control (BPC) (Figure 1). Organic components in SPC and BPC conditions were not removed prior to bond strength testing. One hundred and sixty etched/silanized EMX samples were randomly distributed into two groups according to the organic contaminant: saliva (SA) or human blood (HB). Subgroups were created according to the adopted cleaning method (n=20): AWS, 35% phosphoric acid (PPA) (UltraEtch, Ultradent Inc, South Jordan, UT, USA), 70% liquid alcohol (70A) (Prolink, Guapiaçu, SP, Brazil), and a commercial cleaning paste (Ivoclean, Ivoclar Vivadent) (IVO). Following the cleaning methods, half of the specimens (n=10) were subjected to a new silane layer re-application, as previously described (Figure 2).

### Control Groups

For the control group (no contamination), a thin layer of a bonding agent (Scotchbond MultiPurpose Bond - “Step-3”, 3M Oral Care, St Paul, MN, USA) was applied onto the etched/silanized EMX surface and light cured for 20 seconds using a polywave LED light curing unit (Bluephase N, Ivoclar Vivadent) at 1200 mW/cm<sup>2</sup>,

Table 1: *Materials Used in This Study*

Material	Brand Name (Manufacturer)	Composition
Lithium disilicate glass ceramic	IPS e.max Press (Ivoclar Vivadent)	SiO <sub>2</sub> , Li <sub>2</sub> O, K <sub>2</sub> O, P <sub>2</sub> O <sub>5</sub> , ZrO <sub>2</sub> , ZnO, other oxides and ceramic pigments
Porcelain etchant	Condac Porcelana 5% (FGM Produtos Odontológicos)	5% hydrofluoric acid (HF)
Silane coupling agent	Monobond N (Ivoclar Vivadent)	Alcohol solution of silane methacrylate, phosphoric acid methacrylate and sulphide methacrylate
Phosphoric acid	Ultra-Etch (Ultradent Inc)	35% phosphoric acid, glycol, cobalt aluminate blue spinel
Alcohol	Álcool 70 Prolink (Prolink Indústria Química)	70% alcohol solution
Commercial cleaning paste	Ivoclean (Ivoclar Vivadent)	Sodium hydroxide, ZrO <sub>2</sub> , water, polyethylene glycol, pigments
Bonding agent (adhesive)	Scotchbond MP (3M Oral Care)	Bisphenol A diglycidyl dimethacrylate (BisGMA), 2-hydroxyethyl methacrylate (HEMA), amines, photoinitiator
Light-cured resin cement	Variolink Esthetic (Ivoclar Vivadent)	Urethane dimethacrylate (UDMA) and methacrylate monomers, ytterbium trifluoride and spheroid mixed oxide, initiators, stabilizers, pigments

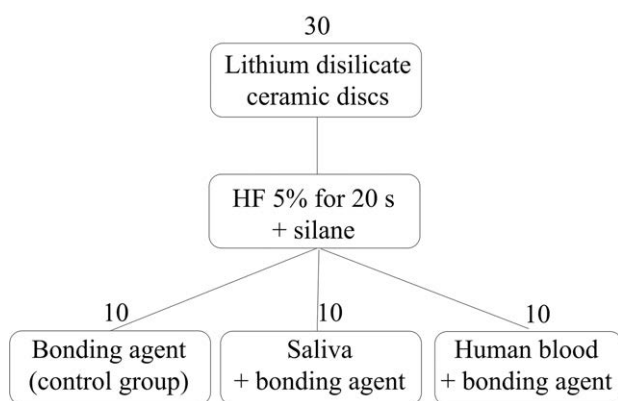


Figure 1. Distribution of the control groups.

with the curing tip positioned as close as possible to the EMX surface ( $<0.5$  mm). For the SA and HB groups, after silane application, SA or HB were dropped on the EMX surface and let to react for 60 seconds. Next, an air blast was applied to remove any excess. A thin layer of the bonding agent was applied and light cured as described above. Thereafter, the EMX samples were prepared for mSBS testing. SA and HB were collected from a healthy donor, who did not eat or drink 2 hours prior to the collection procedure. In the SA groups, 1 mL of unstimulated human saliva was applied to the EMX surface using a graduated sterile pipette and left to react for 60 seconds. For the HB groups, one drop of human blood was collected from the fingertip (previously decontaminated with 70% alcohol) with 20 gauge lancets (Roche, Mannheim, Germany). The blood was then applied to the silanized EMX surface and allowed to react for 60 seconds.

### Cleaning Methods and Silane Reapplication Groups

The following cleaning methods were applied after SA or HB ( $n=20$ ) (Figure 2): an oil-free AWS was applied on the silanized and contaminated EMX surface for 20 seconds and air dried; 35% PPA was actively applied onto the silanized and contaminated EMX surface with a disposable microbrush for 20 seconds, followed by an oil-free AWS for 20 seconds and air dried; 70A was actively applied onto the silanized and contaminated EMX surface for 20 seconds with a disposable microbrush, followed by a oil-free AWS for 20 seconds and air dried; Ivoclean (IVO) cleaning paste (Ivoclar Vivadent) was actively applied onto the silanized and contaminated EMX surface for 20 seconds with a disposable microbrush. Subsequently, an AWS was applied for 20 seconds and then air dried.

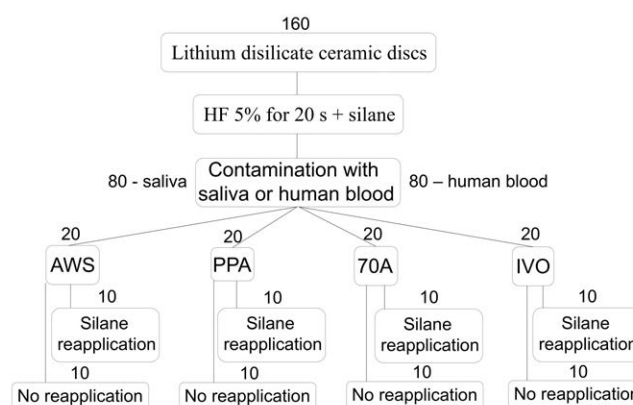


Figure 2. Distribution of the cleaning methods and silane reapplication groups.

Half of the silanized EMX surfaces received a fresh silane layer after the cleaning methods ( $n=10$ ) (Figure 2). Next, a thin layer of the bonding agent was applied onto all EMX surfaces and light cured for 20 seconds using the polywave LED light curing unit, with the curing tip positioned as close as possible to the EMX surface ( $<0.5$  mm).

### Microshear Bond Strength Test ( $\mu$ SBS)

The microshear bond strength ( $\mu$ SBS) methodology has been previously described.<sup>7,10</sup> Round, 1-mm thick elastomer molds (Oranwash L, Zhermack, Italy) containing three cylinder-shaped orifices ( $\varnothing=1$  mm) were made and positioned onto the EMX ceramic surfaces for the bonding area. The orifices were filled with a light-cured resin cement (Variolink Esthetic-Shade Neutral, Ivoclar Vivadent), and Mylar strip and glass slab were placed over the top. A vertical load of 250 g was applied for 2 minutes to standardize the height of the resin cement cylinders. Next, the load and glass slab were removed, and the resin cement was light-cured for 40 seconds using the polywave LED light curing unit with the curing tip in close contact with the Mylar strip. All specimens were stored in deionized water at 37°C for 24 hours. After storage time, the elastomer mold was carefully sectioned with a #11 scalpel blade and removed. Cylinders that presented any flaws or defects were discarded. Three cylinders were fabricated on each ceramic disc (30 cylinders for each group).

A thin steel wire with a diameter of 0.2 mm was looped around each cylinder and aligned with the bonding interface for  $\mu$ SBS assessment. The  $\mu$ SBS test was performed using a universal testing machine (EMIC DL 500; Emic, São José dos Pinhais, PR, Brazil) with a 100 N load cell at a crosshead speed of 1.0 mm/minute until failure. The bond failure areas were

classified into four modes: adhesive (mode 1); cohesive within resin cement (mode 2); cohesive within ceramic (mode 3); and mixed, involving resin cement, adhesive and/or cohesive within the ceramic (mode 4).

### Scanning Electron Microscopy (SEM) Evaluation

To observe the surface morphology of the silanized and contaminated surfaces before and after the cleaning methods, one specimen of each evaluated group ( $n=1$ ) was prepared. After EMX surface etching with 5% HF, silanization, contamination with SA or HB, and cleaning protocols, the EMX samples were mounted on aluminum stubs and sputter coated with gold (Balzers - SCD 050, Balzers Union AG, Fürstentum, Liechtenstein) for 120 seconds at 40 mA. EMX surfaces were then examined by the same operator using SEM (JSM 5600 LV, JEOL, Tokyo, Japan) with 2000 $\times$  magnification at 15 kV.

### Statistical Analysis

Ten EMX samples were tested for each group, and the mean value of the three resin cement cylinders was considered the mean  $\mu$ SBS (MPa) value for each sample. Shapiro–Wilk analysis was performed to verify data normality. The  $\mu$ SBS data from control groups were subjected to one-way ANOVA (surface contaminants) and Tukey post-hoc test ( $\alpha=0.05$ ). The comparison among the different cleaning methods was submitted to a one-way ANOVA and multiple comparisons were performed using Tukey post-hoc test ( $\alpha=0.05$ ). Evaluation of the effect of silane reapplication was performed using an independent  $t$ -test ( $\alpha=0.05$ ).

## RESULTS

### Microshear Bond Strength Test ( $\mu$ SBS)

According to Table 2, when organic contaminants (saliva or human blood) were left on the silanized EMX surface,  $\mu$ SBS values decreased compared to the uncontaminated control group ( $p<0.05$ ). Considering the saliva contamination, the cleaning methods were not different from one another when considering  $\mu$ SBS, all of which were effective in restoring the bond strength provided by the control group that was not contaminated ( $p>0.05$ ) (Table 2).

For the groups contaminated with human blood, IVO was not different than AWS and PPA methods ( $p>0.05$ ). IVO removed more organic compounds than 70A ( $p<0.05$ ) and was the only the cleaning method able to restore the bond strength with values that did not differ from the uncontaminated control group (Table 2).

The reapplication of silane after contamination with saliva and AWS decreased bond strength values ( $p<0.05$ ) (Table 3). When human blood contamination was subjected to PPA and 70A, the reapplication of the silane also decreased the bond strength values ( $p<0.05$ ). For the other groups, silane reapplication did not result in higher bond strength values, regardless of the contaminant or cleaning method performed ( $p>0.05$ ).

There was no effect of the organic contaminants, cleaning methods, or silane reapplication on the distribution of failure patterns ( $p>0.05$ ). Bond failure occurred due to adhesive failure in 96.9%, cohesive failure in 2.6%, and mixed failure in 0.5% of cases involving resin cement. There were no cohesive failures in EMX.

### SEM Evaluation

The images resulting from SEM analysis are presented in Figures 3 through 6. The uncontaminated surface etched with 5% HF depicted the glassy matrix removal and exposure of lithium disilicate crystals (Figure 3). Organic contaminants were found on the silanized EMX surface when not submitted to any cleaning method (Figure 4). The cleaning methods were able to remove organic contaminants (Figure 5 – saliva; Figure 6 – human blood). When exposed to human blood, organic contaminant was found on the silanized ceramic surface, except when subjected to IVO.

Table 2. Means of  $\mu$ SBS (SD) of the Cleaning Methods Compared to Control Groups<sup>a</sup>

Groups	$\mu$ SBS (MPa)	
	Saliva Contamination	Human Blood Contamination
AWS	16.6 (5.7) A	15.2 (5.2) BC
PPA	15.5 (6.1) A	15.3 (4.5) BC
70A	15.8 (5.9) A	13.9 (5.9) C
IVO	16.0 (5.8) A	16.8 (5.2) AB
Contaminated control group	12.3 (4.1) B	10.8 (3.8) D
Uncontaminated control group	18.7 (4.9) A	

Abbreviations: AWS, Air–water spray; PPA, 35% Phosphoric acid; 70A, 70% Alcohol; IVO, Ivoclean.

<sup>a</sup>Letters within a column indicate statistical difference among groups ( $p<0.05$ ).

Table 3: Group Mean  $\mu$ SBS (SD) of Silane Reapplication Following Different Cleaning Methods for Human Saliva/Blood Removal from EMX Surfaces<sup>a</sup>

Groups/ Cleaning Methods	$\mu$ SBS (MPa)			
	Saliva Contamination		Human Blood Contamination	
	+	–	+	–
AWS	15.0 (5.5) B	18.1 (5.6) A	15.3 (5.0) A	15.2 (5.5) A
PPA	16.4 (6.5) A	14.6 (5.7) A	14.0 (4.5) B	16.6 (4.2) A
70A	16.1 (5.4) A	15.4 (6.4) A	12.5 (5.6) B	15.3 (4.6) A
IVO	16.8 (5.9) A	15.2 (5.6) A	17.4 (6.2) A	16.2 (3.9) A

Abbreviations: AWS, Air–water spray; PPA, 35% phosphoric acid; 70A, 70% Alcohol; and IVO, Ivoclean.

<sup>a</sup> Letters within a column indicate statistical difference among groups ( $p < 0.05$ ).

## DISCUSSION

This laboratory study aimed to evaluate the efficacy of different cleaning methods to remove saliva or blood from previously etched and silanized lithium disilicate reinforced glass ceramic and the influence on bond strength. The first two tested hypotheses were accepted, since the cleaning methods restored the bond strength and the cleaning methods removed the organic contaminants from the silanized ceramic surface; however, the third was rejected, since silane reapplication after cleaning methods did not improve the bond strength.

When the silanized glass ceramic was contaminated with saliva, there was a reduction of 35% in the bond strength compared to the noncontaminated control

group (Table 2). This may lead to early debonding of glass ceramic restorations. Other *in vitro* studies reported similar detrimental bond strength results.<sup>17,18,22,23,28-32</sup> Saliva is a very dilute fluid and consists mainly of water (99.4%) with a small percentage of solids (0.6%). Solids are made up of macromolecules (ie, proteins, glycoprotein sugars, enzymes, and mucins), inorganic particles (ie, calcium, sodium, and chloride), and organic particles (ie, urea, amino acids, fatty acids, and free glucose). Additionally, microorganisms, food residues, white blood, and epithelial cells are present in saliva.<sup>21,28,34,35</sup> Salivary components are able to adsorb the intaglio silanized ceramic surface (Figure 4A), creating a thin and invisible residual organic film. This film significantly hinders proper micromechanical–chemical interaction between EMX surface and resin cement, and may also impair the polymerization of said luting composite resin.

Laboratory studies have evaluated different cleaning methods on solely etched lithium disilicate glass ceramic with HF after saliva contamination,<sup>18,22,25,29,31,32,35</sup> but no consensus was reached. On the other hand, in the present study, all the proposed cleaning methods applied on a silanized lithium disilicate glass ceramic successfully restored the initial bond strength after contamination with saliva (Table 2). As seen in previous studies, higher contact angles were found in the group that had received HF conditioning followed by silane application, turning the glass ceramic surfaces from hydrophilic into hydrophobic, thereby reducing the material's surface energy.<sup>26,27</sup> As the silanized EMX repels water-based contaminants, it is easier to remove salivary film from the EMX-etched surface. Several laboratory studies<sup>17,19,31,32</sup> reported that silanization prior to saliva contamination showed a “hydrophobic protective effect” on the etched glass ceramics (ethanol

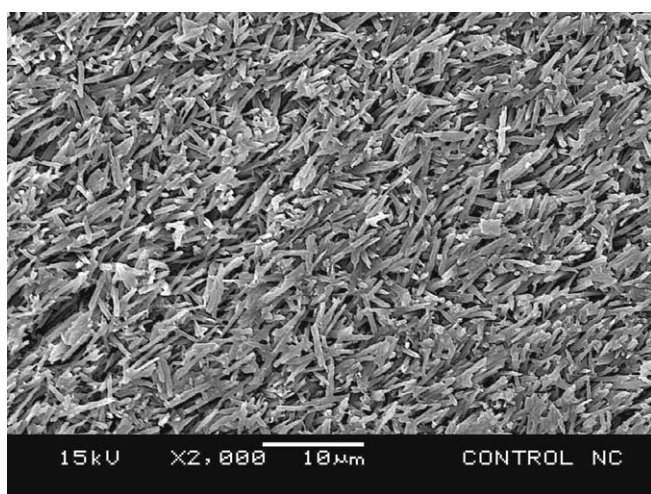


Figure 3. Representative scanning electron microscopy (SEM) image (2000 $\times$  magnification) of the control group (uncontaminated EMX surface) after etching with 5% hydrofluoric acid for 20 seconds.

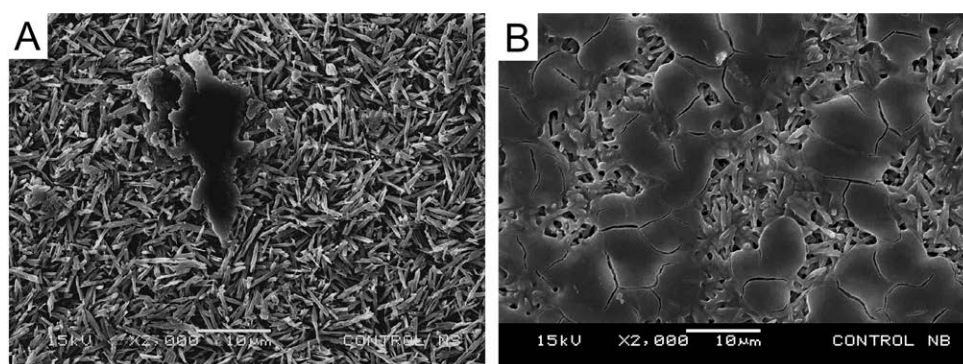


Figure 4. Representative SEM images (2000× magnification) of the positive control groups (A, saliva; B, human blood) after etching the EMX surface with 5% hydrofluoric acid (HF) for 20 seconds and further silane application.

application after rinsing with water, water rinsing only, 37% phosphoric acid or 80% ethanol, and experimental cleaning paste containing zirconium oxide and sodium hydroxide).<sup>19,32</sup> The proposed cleaning methods were also effective at restoring the bond strength, corroborating the results of the present study.<sup>32</sup>

The HB groups presented a reduction of 42% in bond strength compared to the uncontaminated control group (Table 2). This result is in agreement with other laboratory studies that have shown that human blood (consisting of several types of cells—ie, leukocytes, erythrocytes, and platelets—immersed in plasma)<sup>36,38</sup> contamination causes a large decrease in adhesive strength between resin increments during a resin restoration,<sup>34</sup> and between resin cement and dentin.<sup>39-42</sup> Phark and others<sup>38</sup> verified through X-ray photoelectron spectroscopy that contamination by saliva or blood left a complex organic and inorganic layer (thickness that did not exceed 10 nm) over

microporosities of a modified zirconia. This was also observed in the present SEM images (Figure 4). This “dirt” layer may be responsible for the reduction in the bond strength values of the group contaminated with blood (Table 2). Both SA and HB impaired adequate micromechanical interaction between resin cement–EMX and the adequate chemical interaction between silane and the adhesive–resin cement.

To the best of our knowledge, there are no laboratory studies evaluating different cleaning methods on silanized lithium disilicate glass ceramic surface contaminated with human blood. SEM images (Figure 4) depicted that blood contamination forms a film much more complex than saliva, making it almost impossible to visualize the lithium disilicate crystals. The augmented barrier associated with blood contamination is due to the difference in the type and quantity of organic and inorganic elements. Even after the application of silane, the human blood may

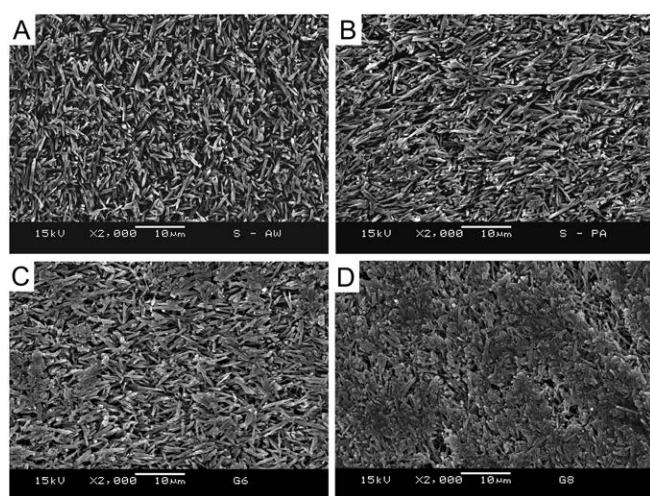


Figure 5. Representative scanning electron microscopy (SEM) images (2000× magnification) of the contaminated EMX surface with saliva and later subjected to the cleaning methods: A, air-water spray (AWS); B, 35% phosphoric acid; C, 70% liquid alcohol (70A); and D, Ivoclean.

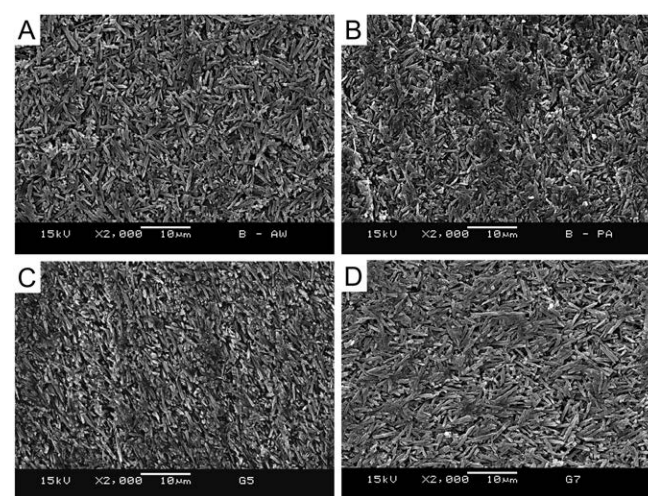


Figure 6. Representative scanning electron microscopy (SEM) images (2000× magnification) of the contaminated EMX surface with human blood and later subjected to the cleaning methods: A, air-water spray (AWS); B, 35% phosphoric acid; C, 70% liquid alcohol (70A); and D, Ivoclean.

have bonded strongly to the silanized EMX surface, making it difficult to remove (blood has less water than saliva, and plasma is more viscous than water, making its removal more difficult.). Despite the fact that AWS, PPA, and 70A yielded a cleaner EMX surface (Figure 6), they improved the bond strength up to 45% compared to the silanized EMX surface HB-contaminated. However, this improvement in bond strength was not to the point of values comparable with the noncontaminated control group (Table 2). It was observed that IVO—a hypersaturated solution of zirconium oxide and sodium hydroxide particles—was the only method capable of restoring the bond strength values comparable with the noncontaminated control group. This may suggest that IVO is able to dissolve the human blood constituent proteins, and subsequent rinsing can remove it from the silanized EMX surface.

According to the manufacturer's instructions, a fresh silane layer should be applied after cleaning with Ivoclean. In the present study, reapplying silane after each cleaning method did not yield higher bond strength and in some cases decreased it (Table 3). These results are in disagreement with other laboratory studies,<sup>17,19</sup> which reported that re-silanizing after decontamination protocols positively influenced bond strength values. Despite having no deleterious effect, Nikolaus and others<sup>19</sup> state that multiple or very-thick silane layers may have a negative effect on the bond strength, as it can lead to a cohesive failure.<sup>43</sup> The negative effect of a fresh silane layer on bond strength may be due to 1) the fresh silane layer would not have new Si-OH sites to react with the ceramic surface and form siloxane bonds, since they have already reacted within the first silane layer; 2) inadequate solvent removal after application of the second layer, which may alter the properties of resin-based materials; and 3) the methacrylate groups of the fresh silane (2nd layer) may react with the methacrylate groups of the first silane layer. Thus, the chemical interaction of silane with methacrylate groups of the bonding agent–resin cement may be affected.

The cleaning methods and products of the present study were chosen because they are easily found in dental offices. It is desirable to have a contaminant-free intaglio ceramic surface prior to adhesive cementation. However, if contamination occurs, it is preferable that it occurs after it has been previously etched and silanized. Dentists should exercise caution when checking the fit of the glass ceramic on the prepared tooth, since friction might cause damage to the etched/silanized surface. To avoid any damage, the impression/scanning of the prepared tooth and the fabrication of the glass ceramics must be carried out

respecting the dental materials properties and, thus, avoiding/minimizing misadaptations. In the present study, a fresh layer of silane was not applied after contamination with saliva or human blood in positive control groups (where contaminants were not removed from the silanized EMX surface before  $\mu$ SBS testing) showing contaminants should be removed from the ceramic surface, as they impair the bonding procedure leading to reduction in bond strength. Future studies should address the effect of hydrolytic, mechanical, and thermal aging on the bond strength after cleaning methods and fresh application of silane.

## CONCLUSIONS

Within the limitations of the present study, it can be concluded that: 1) Contamination with saliva or human blood impairs adherence to silanized lithium disilicate glass ceramic; 2) all cleaning methods (AWS, 35% phosphoric acid, 70% ethanol, and Ivoclean cleaning paste) demonstrated effectiveness in removing saliva contamination of silanized lithium disilicate glass ceramic; however, when contaminated with blood, only Ivoclean cleaning paste was effective at restoring the initial bond strength to silanized lithium disilicate glass ceramic; and 3) application of a fresh silane layer after the cleaning methods of the silanized lithium disilicate glass ceramic did not yield statistically different results from groups that were not resilanized. In some groups, there was a reduction in bond strength values after the application of a new silane layer.

## Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guidelines and policies. The local Ethics Committee in Research approved the present laboratory research: approval #10271919.7.0000.5220.

## Conflict of Interest

The authors of this article certify that they 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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