Efficacy of Various Surface Treatments on the Bonding Performance of Saliva-contaminated Lithium-Disilicate Ceramics

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Purpose: To investigate the efficacy of different ceramic surface cleaning methods after saliva contamination on the resin bond strength to lithium disilicate ceramics.

Materials and Methods: 300 e.max CAD blocks (Ivoclar Vivadent) were polished with 600-grit silicon carbide paper and divided into five groups with or without human saliva contamination and according to the surface treatment performed (n = 10); control: no pretreatment; MP: Monobond Plus; PA+MP: 37% phosphoric acid (PA) followed by MP; HF+MP: 5% hydrofluoric acid (HF) followed by MP; MEP: Monobond Etch & Prime. The specimens were bonded with one of three resin cements: Variolink Esthetic DC (VE), Multilink Automix (MA) and Speed CEM (SC). After 24-h water storage, tensile bond strength (TBS) was measured. The ceramic surfaces after pretreatment were analyzed using x-ray photoelectron spectroscopy (XPS).

Results: XPS analysis showed similar elemental distributions between saliva contamination vs no saliva in PA, HF, and MEP. The TBSs were significantly influenced by surface treatments (p < 0.05). HF+MP and MEP showed statistically non-significantly different bond strengths to saliva-contaminated HF+MP and MEP, but were different from MP and saliva-contaminated MP. The TBSs after 24 h were significantly higher in HF+MP and MEP groups with VE. HF+MP and MEP did not show statistically significant differences among any groups with or without saliva contamination.

Conclusion: Surface treatments with PA or HF followed by silane or by MEP alone were effective in removing saliva contamination and enhancing the resin bond strength.

Keywords: lithium disilicate ceramics, saliva-contaminated, tensile bond strength.

J Adhes Dent 2019; 21: 51–58. Submitted for publication: 01.06.18; accepted for publication: 25.11.18
doi: 10.3290/j.jad.a41918

The demands for esthetic restorations have increased substantially in recent years, and the popularity of all-ceramic materials as an alternative to metal-ceramic for indirect restorations has therefore grown. A weak point for all ceramics is considered to be the extension and growth of microscopic surface flaws, but the resistance to crack propagation of ceramics has been improved by leucite, fluor mica, or alumina reinforcement of the glass matrix. Many studies have documented the success of glass-ceramic restorations bonded with resin-based cements. However, to
optimally create a resin-ceramic bond, glass-ceramic restorations should be pretreated with hydrofluoric acid (HF) and a silane coupling agent. HF creates porosities in the glass ceramic, and the coupling agent serves the dual purpose of binding to the silica of the ceramic and to the methacrylate group of the adhesive resin. Ceramic etching with HF can be performed either at chairside by the dentist or in the dental laboratory by the dental technician. As a series of surveys on clinically used adhesive ceramic bonding methods in Northern Germany showed, there is still a considerable lack of understanding about the principles of reliable ceramic bonding among dental practitioners. When performed by the dental technician, the subsequent try-in procedure leads to surface contamination with saliva. Saliva contains organic materials such as salivary proteins, enzymatic molecules, bacteria and food debris, and inorganic compounds such as mineral ions in water. Adsorption of salivary proteins to dental materials and tooth surfaces results in a pellicle consisting of free (ie, planktonic) bacteria which develops to a thickness of 10-20 nm within a few minutes. The resulting persistent protein contamination from saliva in particular was shown to hinder adhesion of the resin cements to ceramics. Therefore, several cleaning methods have been proposed to eliminate such contamination from the ceramic surface. In fact, due to the hazardous potential of HF, its chairside application should be performed with caution. As a result, alternative cleaning methods without the use of HF have been sought, including rinsing with water, etching with phosphoric acid, and cleaning with alcohol. Ammonium fluorides, especially ammonium bifluoride in combination with other acids, have been investigated as a possible etching media for dental ceramics for many years. Recently, a single bottle ceramic self-etching silane primer Monobond Etch & Prime (MEP, Ivoclar Vivadent; Schaan, Liechtenstein) was introduced. It has been claimed that effects of MEP are comparable to those of HF and silane treatment. However, details regarding bonding efficacy, surface analysis, and detailed comparison with existing cleaning materials after human saliva contamination remain unclear.

Therefore, the purpose of this study was to investigate the efficacy of different ceramic surface cleaning methods after saliva contamination on the resin bond strength to lithium disilicate ceramics. The null hypothesis was that the resin-ceramic bond strength of the resin cements to the ceramics was not influenced by contamination and cleaning method.
MATERIALS AND METHODS

The materials used in the bonding procedure are listed in Table 1.

Experiment 1: Tensile bond test

Specimen fabrication

Lithium disilicate ceramics blocks (e.max CAD, Ivoclar Vivadent) were used in this study. Specimens (5 mm thick x 15 mm wide x 15 mm long) were retrieved from original blocks using a low-speed diamond saw (IsoMet, Buehler; Lake Bluff, IL, USA) and sintered. One surface of each specimen was ground using 600-grit silicon carbide paper under water rinsing. The specimens were ultrasonically cleaned in deionized water for 5 min followed by ethanol for 2 min and then air dried.

Surface preparation

The specimens were left as is or were contaminated with human saliva. To contaminate, the specimens were immersed in saliva for 1 min. Saliva was collected from one of the authors who had refrained from eating and drinking 1.5 h prior to the collection procedure under a protocol approved by the Ethics Committee at Tokyo Medical and Dental University (No. D2017-053). All experiments were performed using fresh saliva collected on the same occasion.

Then excess saliva was removed by water spraying for 15 s, followed by air drying for another 15 s.

Each group was further divided into five subgroups (n = 20, with and without contamination) according to the surface treatment performed:

- **Control:** no pretreatment.
- **MP:** a thin coat of Monobond Plus (MP; Ivoclar Vivadent) was applied with a brush and the material allowed to react for 60 s. Subsequently, any remaining excess was dispersed with a strong air stream.
- **PA+MP:** surfaces were etched with 37% phosphoric acid (PA; Total Etch, Ivoclar Vivadent) for 20 s and then rinsed and dried. After etching, MP was applied as in the MP group.
- **HF+MP:** surfaces were etched with < 5% HF (IPS Ceramic Etching Gel, Ivoclar Vivadent) for 20 s and then rinsed and dried. After etching, MP was applied as in the MP group.
- **MEP:** MEP was applied, rubbed for 20 s, and left on the surface for a further 40 s for sufficient reaction. It was subsequently washed off with water and then dried with air for another 10 s.

Tensile bond strength test

After surface treatment, a 100-μm-thick piece of aluminum masking tape with a 4-mm diameter hole was placed on
each ceramic surface. A metal rod treated with a metal adhesive primer (Alloy Primer, Kuraray Noritake; Tokyo, Japan) was attached to each ceramic block using one of the 3 resin cements (Ivoclar Vivadent): Variolink Esthetic DC (VE), Multilink Automix (MA), and SpeedCEM (SC). The resin cement was light cured for 40 s with a curing unit at a light intensity of 600 mW/cm² (Optilux 501, Demetron-Kerr; Danbury, CT, USA). The cemented specimens were left to complete polymerization at room temperature for 30 min, then stored in distilled water at 37°C for 24 h. A total of 300 specimens were produced in 5 surface treatment groups with 3 resin cements and tested under two conditions: with or without saliva contamination (n = 10). Tensile bond strength (TBS) was measured in a universal testing apparatus (Autograph-J, Shimadzu; Kyoto, Japan) at a crosshead speed of 2 mm/min.¹⁶

Failure mode analysis
The fractured interfaces of the de-bonded specimens were examined using a light microscope (OCS 912042, Olympus; Tokyo, Japan) to calculate the de-bonded area and assign failure modes. The failure modes were classified into the following three types: type I: adhesive failure, no resin cement remnant on the ceramic block; type II: mixed failure, fracture comprises both the ceramic block and resin cement (some ceramic and some resin cement visible); type III: cohesive failure, fracture within resin cement, fracture surface consists of only resin cement. Representative samples were examined in a scanning electron microscope (SEM, JSM-5310LV, JEOL; Tokyo, Japan) with an acceleration voltage of 15 KV after sputtering using a conductive layer of gold to determine the pattern of debonding.

Statistical analysis
According to the Shapiro-Wilk test, the data were normally distributed (p > 0.05). Therefore, TBS data in MPa were analyzed separately for each cement by two-way ANOVA, followed by the t-test with Bonferroni correction for pairwise comparisons. The two factors analyzed were surface treatments and storage conditions. For all analyses, a confidence level of 95% was assumed. All statistical procedures were performed using SPSS software (SPSS 22, IBM; Chicago, IL, USA).

Experiment 2: Surface Analysis
The surfaces of e.max CAD disks were prepared following the same procedure as the tensile bond strength test. Each group was divided into four subgroups (n = 2, with and without contamination) according to the surface treatment performed: control, 5% HF, 37% PA, and MEP in Fig 2. X-ray photoelectron spectroscopy (XPS) was used to analyze the surfaces of the e.max CAD samples. XPS (JPC-9010MC, JEOL; Tokyo, Japan) analyses were performed using a Mg Kα x-ray source under the following conditions: operating pressure, 10⁻⁷ Pa; emission current, 10 mA; accelerating voltage, 10 kV. Wide scans were measured at pass energies of 100 eV.

RESULTS

Experiment 1: Tensile Bond Strength Test
Tables 2 to 4 show tensile bond strength results as well as failure modes, with or without saliva contamination. The two surface treatment factors (with or without saliva contamination and surface treatment) and their interactions were statistically significant for all the cements (p < 0.05). The typical failure modes as observed with SEM are shown in Fig 3.

Variolink esthetic DC (VE)
In both conditions, HF+MP and MEP resulted in statistically significantly higher mean TBS than did the control, MP, and PA+MP (p < 0.05) (Table 2). After saliva contamination, there were no statistically significantly differences vs groups without saliva. Regarding failure analysis, almost all
specimens showed cohesive failure, except the control group, both with and without saliva contamination.

**Multilink Automix (MA)**
The control group presented lower bond strength at baseline in both conditions (Table 3). Without contamination, MP, PA+MP, HF+MP and MEP showed the highest bond strengths. After saliva contamination, MP showed significantly lower bond strength than the other groups (p < 0.05). A higher ratio of cohesive failures was observed in groups with higher bond strengths. After saliva contamination, 100% adhesive failures were noticed after no surface treatment (control), MP, and HF+MP.

**SpeedCEM (SC)**
The control group presented the lowest bond strength at baseline with or without saliva contamination. After saliva contamination, no significant decrease was observed in PA+MP, HF+MP, and MEP (p > 0.05). However, bond strength in the MP group decreased significantly after saliva contamination (Table 4). Regarding failure mode, MEP and PA+MP resulted in 100% cohesive failure, while more adhesive failures were observed in MP and HF+MP after saliva contamination.

**Experiment 2: Surface Analysis**
The typical wide-scan spectra from XPS analysis are shown in Fig 3. Clear N-1s and C-1s peaks were identified by XPS analysis. After saliva contamination, the intensities of N-1s and C-1s peaks increased. In HF, Zn-2p peaks were detected under both conditions. In HF and PA, there were no N-1s peaks and small C-1s peaks. In MEP, C-1s and F-1s peaks were the specific peaks under both conditions, but there was no N-1s peak after saliva contamination.
DISCUSSION

Intraoral seating of pre-etched glass ceramics during a try-in procedure frequently results in salivary contamination, and if this contamination is not removed, it may result in decreased bond strength between the resin cement and the glass-ceramic surface. The null hypothesis was rejected, as statistically significant differences in the tensile bond strength of resin cement to lithium disilicate ceramic were found based on the type of surface and cleaning procedures. Etching or rinsing of the pre-etched glass-ceramic surface after contamination is necessary to dislodge the saliva and allow more effective bonding. The use of surface treatments according to the manufacturer’s instructions on an etched, saliva-contaminated lithium disilicate surface before silanization resulted in bond strengths similar to that of the uncontaminated control group. For those clinicians whose laboratories do not pre-etch the ceramics or who mill their lithium disilicate restorations chairside, rinsing the saliva-contaminated ceramic after try-in and then etching with hydrofluoric acid in the operatory is adequate, as it provides bond strengths similar to the uncontaminated control.

Saliva consists of organic materials such as salivary proteins, bacteria, and food debris in an aqueous solution. After saliva immersion, salivary protein adsorption occurs not only on the tooth surface, but also on the restorative materials. Non-covalent adsorption of salivary proteins occurred on this surface after the immersion of ceramic into saliva for 60 s. This organic coating could not be removed by rinsing with tap water for 15 s, as shown by XPS, revealing a considerable increase in C and N after saliva immersion and rinsing.

Cleaning the glass-ceramic surface with only water spray after saliva contamination resulted in significantly lower bond strength to lithium disilicate glass ceramic compared to the uncontaminated control group. All specimens showed low initial bond strengths and debonded spontaneously with 100% adhesive failure at the ceramic surface. Other cleaning procedures led to bond strength that did not differ statistically significantly from the uncontaminated groups.

The specimens bonded without silane primer in the control group had statistically significantly low TBS compared to the other groups with or without saliva contamination. The use of silane primer, alone or in combination with the etchant, is clinically essential for the adhesive bonding of lithium disilicate ceramic. The use of silane without acid etching (MP group) resulted in lower bond strengths in comparison with the PA+MP or HF+MP or MEP.

To avoid the detrimental effect of saliva contamination on resin-to-ceramic bond strength, ceramic restorations should be etched and silanized after the try-in procedure. Several studies reported that phosphoric acid cleaning was effective for saliva decontamination, whereas others reported it to be ineffective. Acid cleaning requires water rinsing, and the remaining water may inhibit bonding efficacy. In this study, phosphoric acid cleaning did not work as well as HF in VE resin cement; HF resulted in statistically significantly higher bond strength (40.6 ± 6.3 MPa) than PA (27.6 ± 4.4 MPa) in VE.

Interestingly, in the present study, TBS was comparable when resin cement was bonded to lithium disilicate ceramic using MEP or HF+MP with all resin cements. MEP contains trimethoxypropyl methacrylate for silanization and polyfluoride for etching. It has been indicated that the bond between silica and fluoride is extremely strong. Therefore, a possible explanation for the effective bonding of MEP to lithium disilicate ceramic may be attributed to the chemical affinity between silica in lithium disilicate ceramic and ammonium trifluoride in MEP.

The bond quality of ceramic restorations should not only be assessed by bond strength measurements. Another important quality indicator is provided by the analysis of fracture modes. The failure modes of the experimental groups were investigated using a light microscope and SEM. If adhesive failure modes occur more frequently, it can be an indication of lower bond quality. In this study, adhesive failure modes were observed in specimens which were not silanized or only silanized after contamination. Cohesive failures (fractures within the composite resin) frequently occurred in MEP. In these cases, the bond strength

**Fig 3** Representative SEM images (35X) of typical failure modes. A clean ceramic surface was indicative of adhesive failure between ceramic and cement. Mixed failure showed surfaces with both ceramic and resin cement. Cohesive failure in resin cement was indicated by resin cement particles attached to the ceramic surface. RC: resin cement; LD: lithium disilicate.
between the glass-ceramic surface and the composite resin could exceed the internal strength of the composite resin. Consequently, MEP self-etching silane primer of glass ceramic surface after saliva contamination is a simple and useful method to increase the quality of the bond.

XPS was performed to identify the contamination and the efficacy of cleaning methods. According to XPS analysis, there were no N-1s and small C-1s peaks in the control group. However, after saliva contamination, both N and C peaks derived from salivary proteins on the lithium disilicate surface were detected. In HF group, Zn-2p peaks were detected both with and without saliva contamination. HF formed microporosities as the glass matrices were dissolved, leaving ZnO exposed on that treated surface. In HF and PA groups, N-1s was not detected and the C-1s peak was small even with saliva contamination. This shows that these acids were able to remove the contaminated saliva protein on the lithium disilicate surface. MEP is partly composed of trimethoxypropyl methacrylate, which leaves a thin silane layer to chemically bond to the ceramic after water rinsing and drying of the treated surface. Although the mechanism of action of MEP is not fully clear, EL-Damamhoury and Gaintantzopoulou found fluorine residue on the treated ceramic surface using energy dispersive x-ray analysis. In this study, C-1s and F-1s were the characteristic peaks in both conditions. However, there was no N-1s peak after saliva contamination. The F-1s and C-1s peaks in the MEP group were higher than those in the other groups. A small F-1s peak might be the result of remaining ammonium polyfluoride, which mildly etched the lithium disilicate surface to achieve the etching pattern. A C-1s peak was also detected in the saliva contaminated group. However, these peaks are always accompanied by an N-1s peak. C peaks in the MEP group, however, lack corresponding N-1s peaks both with and without saliva contamination. From this point of view, the C-1s peak in the MEP group could be coming from the silane coupling agent on the lithium disilicate surface.

Although adhesion between resin cement and etched and silanized glass ceramics is well established, possible saliva contamination during the intraoral try-in procedures may impair bond strengths, demonstrating the clinical significance of this investigation particularly for minimally invasive preparations which rely on retention from adhesive bonding. Adhesive cementation protocols should consider removal of contamination media as a critical step to improve the longevity of bonded restorations. More in vitro studies are needed to obtain further information under long-term water storage and thermal cycling conditions after saliva contamination.

CONCLUSION

Saliva contamination on the bonded surface of the lithium disilicate glass ceramics before its cementation should be avoided. Treating the surface with phosphoric or hydrofluoric acid in combination with silane coupling agent or the use of Monobond Etch & Prime were effective methods for removing saliva contamination and ensuring proper bonding of the resin cement to the lithium disilicate glass-ceramic bonding surface.

ACKNOWLEDGMENTS

We would like to thank Ivoclar Vivadent for supplying lithium disilicate disks. This work was supported by the JPSP Grant-in-Aid for Scientific Research (C) 17K11701 and the Cooperative Research Program of the Institute for Catalysis, Hokkaido University (Grants #16B1004 and #17B1007).

REFERENCES


Clinical relevance: For adhesive cementation of lithium disilicate glass ceramics, the newly developed MonoBond Etch & Prime can effectively remove saliva contamination and provide bond strengths comparable to the combination of hydrofluoric acid with Monobond Plus.