Microshear Bond Strength of Resin Cements to Lithium Disilicate Substrates as a Function of Surface Preparation

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Clinical Relevance

There is an urgent need to establish a reliable protocol to treat the intaglio surface of lithium disilicate glass-ceramic, as several cementation protocols are currently used, including self-adhesive cements.

SUMMARY

Objectives: To investigate the effect of hydrofluoric acid (HF) etching, silane solution, and adhesive system application on the microshear bond strength (μSBS) of lithium disilicate glass-ceramic (LD) to three resin cements.

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Materials and Methods: Circular bonding areas were delimited on the lithium disilicate surfaces using a perforated adhesive tape. Specimens were assigned to 18 subgroups (n=12) according to surface treatment: NT = no treatment: HF = 4.8% HF for 20 seconds: silane solution: (1) no silane; (2) Monobond Plus, a silane/10-methacryloyloxydecyl dihydrogen phosphate solution for 60 seconds; (3) Monobond Plus+ExciTE F DSC, a dual-cure adhesive; and resin cement: (1) Variolink II, a bisphenol A diglycidyl ether dimethacrylate (bis-GMA)-based, hand-mixed, dual-cure resin cement; (2) Multilink Automix, a bis-GMAbased, auto-mixed, dual-cure resin cement; (3) RelyX Unicem 2, a self-adhesive, auto-mixed, dual-cure resin cement. Tygon tubes (Ø=0.8 mm) were used as cylinder matrices for resin cement application. After 24 hours of water storage, the specimens were submitted to the µSBS test. Mode of failure was evaluated under an optical microscope and classified as adhesive, mixed, cohesive in resin cement, or cohesive in ceramic. Data were statistically

analyzed with three-way analysis of variance and Dunnett test (p < 0.05).

Results: When means were pooled for the factor surface treatment, HF resulted in a significantly higher µSBS than did NT (p < 0.0001). Regarding the use of a silane solution, the mean µSBS values obtained with Monobond Plus and Monobond Plus+ExciTE F DSC were not significantly different but were higher than those obtained with no silane (p < 0.001). Considering the factor resin cement. Variolink II resulted in a significantly higher mean μ SBS than did RelvX Unicem 2 (p<0.03). The mean µSBS for Multilink Automix was not significantly different from those of Variolink II and RelvX Unicem 2. According to Dunnett post hoc test (p < 0.05), there was no significant difference in µSBS between the different resin cements for HF-etched and silanized (with or without adhesive application) LD surfaces.

Conclusion: LD may benefit from pretreatment of the inner surface with HF and silanization, regardless of the resin cement used.

INTRODUCTION

Following the demand for tooth-colored, highstrength restorations, ceramic systems have been developed with different proportions of glassy and crystalline phases to improve their mechanical properties while maintaining good esthetic properties. Among the options currently available is lithium disilicate glass-ceramic (LD), which was commercially marketed in 1998 by Ivoclar Vivadent as IPS Empress 2 and fabricated using a lost-wax and heat-pressed technique. The material was later reformulated and relaunched as ingots that can be press-fit (IPS e.max Press, Ivoclar Vivadent, Schaan, Liechtenstein) or as CAD/CAM blocks (IPS e.max CAD, Ivoclar Vivadent) that can be milled to fabricate monolithic restorations (anterior or posterior crowns, implant crowns, inlays, onlays, or $veneers).^2$

Unsupported glass ceramics—even those with high flexural strength—are prone to fracture under chewing loads, which justifies the use of adhesive cementation.^{3,4} Hydrofluoric acid (HF) etching is commonly indicated to partially dissolve the glassy phase and to create microporosities. The HF exposure time depends on the ceramic microstructure and on the HF concentration, which usually ranges between 2.5% and 10%.⁵ The manufacturer of LD specifically recommends etching the intaglio surface

with 4.8% HF for 20 seconds. The crystals are exposed and serve as retentive surfaces for resin cement interlocking, which enhances adhesion. Furthermore, the application of a coupling agent (silane) onto the pre-etched intaglio surface is required. This bifunctional adhesion enhancer creates a chemical interaction between the silica in the glassy phase of the ceramic and the methacrylate groups of the resin through siloxane bonds. After etching and silanization, there is an increase in surface energy and wettability, decreasing the contact angle between the ceramic and resin cement. However, HF is a hazardous substance, and thus a simplified, safe, and faster conditioning process would be desirable.

Depending on the indication, the dentist has to select resin-based cements with different bonding strategies. Resin cements are recommended for adhesive cementation because of their low solubility in the oral environment, reduced microleakage at the restoration-tooth interface, good optical properties, and low incidence of marginal staining and recurrent caries.8 Self-adhesive cements represent the newest and most simplified category of resin cements, which rapidly gained popularity to lute allceramic restorations as a result of their ease of use. In fact, the pretreatment of the substrate with etchant and/or adhesive is not recommended with self-adhesive resin cements.9 Nonetheless, most dentists are still confused regarding the bonding protocols when using resin-based cements with LD restorations. 10 Bonding effectiveness may influence the long-term clinical success of all-ceramic restorations; thus, it seems reasonable to identify the most reliable and effective luting protocol at the cement/ ceramic restoration interface. Since only scarce information is available with regard to the bond strength of simplified resin cements to LD restorations, this laboratory research aimed at investigating the effect of HF etching, silane solution, and adhesive application on the microshear bond strength (µSBS) of LD to dual-cure resin cements representing three strategies: hand-mixed bisphenol A diglycidyl ether dimethacrylate (bis-GMA)-based; auto-mixed bis-GMA-based; and auto-mixed selfadhesive. The null hypothesis tested was that the three factors used in this project would not influence mean uSBS.

METHODS AND MATERIALS

The materials and respective compositions are displayed in Table 1. Thirty-six IPS e.max CAD blocks (Ivoclar Vivadent) were cut into 72 rectangu-

Material (Manufacturer)	Composition	Batch Number
IPS e.max CAD blocks (Ivoclar Vivadent)	Lithium disilicate glass-ceramic	S17323
IPS Ceramic Etching Gel (Ivoclar Vivadent)	4.8% hydrofluoric acid	S13497
Monobond Plus (Ivoclar Vivadent)	Ethanol, 3-trimethoxysilylpropyl methacrylate, methacrylated phosphoric acid ester (10-MDP), disulfide acrylate	S14727
ExciTE F DSC (Ivoclar Vivadent)	Dimethacrylates, HEMA, phosphonic acid acrylate, catalysts, stabilizers, fluoride, ethanol, silicon dioxide	S08275
Variolink II (Ivoclar Vivadent)	bis-GMA, UDMA, TEGDMA, ytterbium trifluoride, boroaluminofluorosilicate glass, spheroidal mixed oxide, benzoylperoxide, stabilizers, pigments	R69347
Multilink Automix (Ivoclar Vivadent)	Dimethacrylates, HEMA, t-amine (J05820), silicon dioxide filler, ytterbium trifluoride, catalysts, stabilizers, pigments, dibenzoyl peroxide	S14543
RelyX Unicem 2 (3M ESPE)	Methacrylate monomers containing phosphoric acid groups; methacrylate monomers, silanated fillers, alkaline (basic) fillers; initiator components, pigments, stabilizers, rheological additives	518863

lar sections (16 mm×2 mm) using a low-speed diamond wheel saw (Model 650, South Bay Tech Inc, San Clemente, CA, USA) under water irrigation. After cleaning ultrasonically with distilled water for 15 minutes, LD specimens were fired following the crystallization program recommended by Ivoclar Vivadent. After cooling, specimens were positioned in polyvinyl chloride (PVC) plastic rings and embedded in epoxy resin (Epo-Thin Resin, Buehler Inc, Lake Buff, IL, USA) and wet polished with up to 600grit silicon carbide paper for one minute. The delimitation of the bonding area was conducted in accordance with the method of Shimaoka and others¹¹ (Figure 1). An acid-resistant, double-sided adhesive tape (Scotch Permanent Double Sided Tape, 3M, St Paul, MN, USA) was perforated with three 0.8-mm-diameter holes and positioned over the ceramic surface. Eighteen groups (n=12) were

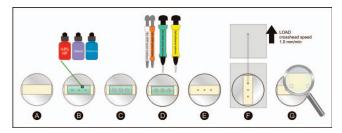


Figure 1. Schematic overview of the microshear bond strength test (μSBS) setup. (A) LD block embedded in epoxy resin; (B) Adhesive application over the double-sided tape with three perforations (\emptyset =0.8 mm); (C) Tygon tubes coinciding with the pretreated surfaces; (D) Insertion of resin cements in the tubes; (E) Tubes and tapes were removed after 24 hours of water storage; (F) μSBS at a crosshead speed of 1.0 mm/min; (G) Failure analysis.

created using the following combination of factors (a $2\times3\times3$ design):

- Surface treatment: 1) NT = no treatment; 2) HF = the ceramic surfaces were etched with 4.8% HF (IPS Ceramic Etching Gel, Ivoclar Vivadent) for 20 seconds, thoroughly rinsed with water spray, and ultrasonically cleaned in distilled water for 180 seconds.
- Silane solution: 1) No silane; 2) Monobond Plus (Ivoclar Vivadent), an ethanol-based silane coupling and 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) solution, was applied with a brush and allowed to react for 60 seconds. Subsequently, the excess was dispersed with a strong stream of air to ensure the solvent evaporation. 3) Monobond Plus+ExciTE F DSC (Ivoclar Vivadent), a dual-curing single-component adhesive, was applied for 10 seconds and air-dried for five seconds.
- Resin cement: 1) Variolink II (Ivoclar Vivadent): equal amounts of base and catalyst paste of a bis-GMA-based, hand-mixed, dual-cure resin cement were mixed carefully for 10 seconds. 2) Multilink Automix (Ivoclar Vivadent): a mixing tip was used to mix the base paste and catalyst of a bis-GMA-based, auto-mixed, dual-cure resin. 3) RelyX Unicem 2 (3M ESPE): a mixing tip was used to mix the base paste and catalyst of phosphate monomer-based, self-adhesive, auto-mixed, dual-cure resin cement.

After the surface treatment (NT or HF) and application of the silane solution (no silane, Mono-

bond Plus, or Monobond Plus+ExciTE F DSC), translucent Tygon tubes (Tygon Medical Tubing, Saint-Gobain, Akron, OH, USA) with an internal diameter of 0.8 mm and a height of 0.5 mm were used as matrices. Each tube was positioned over the tape, ensuring that the lumen coincided with the circular ceramic area exposed by the perforation. One trained operator using magnifying loupes (Optivisor, Donegan Optical Company, Lenexa, KS, USA) positioned the matrices on the ceramic surfaces; this was followed by careful insertion of the resin cement into each matrix. A Mylar strip was positioned over the filled tube and gently pressed in place. Resin cements were cured for 40 seconds using a LED light-curing unit (Translux Power Blue, Heraeus Kulzer, Hanau, Germany) with an output of 650 mW/cm². The intensity was checked daily. After 24 hours of storage in distilled water at 37°C, matrices and tapes were carefully removed using a sharp blade to expose the resin cement cylinders. Each specimen was examined using magnifying loupes to identify specimens containing possible defects in the resin cement cylinder (bubbles, flow of resin cement beyond the limits of the bonding area, and mismatch between the cylinders and their respective delimited area). The PVC rings were attached to a shear-testing jig. A thin wire (0.2-mm diameter) was looped around the base of each resin cement cylinder, in contact with half of its circumference, keeping the setup aligned to ensure the correct orientation of the shear forces. The cement/ ceramic interface was then tested under shear mode in a universal testing machine (Instron 4444, Instron Corporation, Canton, MA, USA) at a crosshead speed of 1 mm/min until failure. The µSBS were calculated in MPa by dividing the load at failure by the surface area (mm²) of each specimen. The results were statistically analyzed (IBM SPSS 21, IBM, Chicago, IL, USA) with three-way analysis of variance (ANOVA) and Dunnett multi-comparison post hoc test (p < 0.05). The failure mode of debonded specimens was determined using 3.5× magnification and was classified as adhesive (A), mixed (M), cohesive in resin cement (CR), or ceramic (CC). Randomly selected specimens from each group were prepared for morphological examination under a field-emission scanning electron microscope (FESEM). Two extra LD specimens were prepared for morphological analysis of the HF-treated vs untreated surfaces. After drying at room temperature, the specimens were mounted on aluminum stubs with adhesive carbon tape (PELCO Carbon Conductive Tape, Ted Pella Inc, Redding, CA, USA) and colloidal quickdrying silver paint (PELCO Colloidal Silver, Ted Pella Inc). Sputter-coating was done with gold-palladium by means of a sputter-coater (Polaron E-5100 Sputter Coater, Polaron Equipment Ltd, Watford, UK) at 20 mA for 90 seconds. Specimens were observed under a FESEM (S-4700, Hitachi High Technologies America Inc, Pleasanton, CA, USA) at an accelerating voltage of 5.0 kV and a working distance of 12.0-13.0 mm.

RESULTS

The mean uSBS and fracture analyses are summarized in Table 2. No pretesting failures occurred. For the groups that did not receive HF etching or silane application (NT-no silane-Variolink II; NTno silane-RelyX Unicem 2; and NT-no silane-Multilink Automix), mean µSBSs were close to zero and presented adhesive failures. When means were pooled for surface treatment, HF resulted in a significantly higher μ SBS than did NT (p<0.0001). Regarding the silane solution, the mean µSBS values for Monobond Plus and for Monobond Plus+ExciTE F DSC were not significantly different; however, they were significantly higher than those of no silane (p < 0.001). Considering the factor resin cement, Variolink II resulted in a significantly higher mean µSBS than did RelyX Unicem 2 (p<0.03). The mean μSBS values for Multilink Automix were not significantly different from those of Variolink II and RelyX Unicem 2. According to the Dunnett post hoc test (p < 0.05), there were no significant differences in µSBS values between pairs of different resin cements for HF-etched + silanized (regardless of adhesive application) LD surfaces.

The morphology of nontreated LD after polishing (Figures 2A and 3A) showed a smooth surface without any retentive features. Etching with 4.8% HF for 20 seconds (Figures 2B and 3B) created a network of microporosities on the LD surface. The top view of etched LD in Figure 2B depicted an array of exposed LD crystals. Figure 4A shows a mixed failure for group NT+Monobond Plus+Multilink Automix, whereas Figure 4B is a higher magnification of the area of the resin cement fracture.

DISCUSSION

Although difficulties in bond strength testing and its interpretation are well-known, ¹² there is still no alternative approach with which to test bond strength with similar efficiency in terms of time and cost. When conventional "macro" bond strength tests are used, a large number of cohesive failures are observed. ¹³ When the specimen size is de-

Surface Treatment ^a	Silane Solution ^a	Resin Cement ^a	Mean μ SBS \pm SD ^b	Failure Mode, % ^c
NT	No silane	Variolink II	0.28 ± 0.30 G	A = 100; M = 0; CR = 0; CC = 0
		Multilink Automix	0.18 ± 0.25 g	A = 100; M = 0; CR = 0; CC = 0
		RelyX Unicem 2	3.07 ± 1.07 F	A = 100; M = 0; CR = 0; CC = 0
	Monobond Plus	Variolink II	28.90 ± 6.20 BCDE	A = 66; $M = 34$; $CR = 0$; $CC = 0$
		Multilink Automix	$27.70\pm6.50\;\text{CDE}$	A = 75; $M = 25$; $CR = 0$; $CC = 0$
		RelyX Unicem 2	25.96 ± 5.46 DE	A = 66; M = 44; CR = 0; CC = 0
	Monobond Plus+ExciTE DSC	Variolink II	30.16 ± 5.98 BCDE	A = 0; $M = 100$; $CR = 0$; $CC = 0$
	_	Multilink Automix	24.64 ± 5.23 DE	A = 92; M = 8; CR = 0; CC = 0
		RelyX Unicem 2	21.34 ± 5.35 E	A = 34; $M = 66$; $CR = 0$; $CC = 0$
HF	No Silane	Variolink II	42.74 ± 10.38 AB	A = 50; $M = 50$; $CR = 0$; $CC = 0$
	_	Multilink Automix	28.76 ± 5.28 BCDE	A = 66; M = 34; CR = 0; CC = 0
	_	RelyX Unicem 2	22.68 ± 6.85 E	A = 34; $M = 66$; $CR = 0$; $CC = 0$
	Monobond Plus	Variolink II	40.70 ± 8.34 AB	A = 0; $M = 100$; $CR = 0$; $CC = 0$
		Multilink Automix	45.68 ± 9.65 A	A = 0; $M = 100$; $CR = 0$; $CC = 0$
	_	RelyX Unicem 2	41.78 ± 6.45 A	A = 0; $M = 100$; $CR = 0$; $CC = 0$
	Monobond Plus+ExciTE DSC	Variolink II	42.46 ± 5.08 a	A = 0; $M = 100$; $CR = 0$; $CC = 0$
		Multilink Automix	39.87 ± 9.16 ABC	A = 0; $M = 100$; $CR = 0$; $CC = 0$
		RelyX Unicem 2	35.86 ± 8.05 ABCD	A = 0; $M = 100$; $CR = 0$; $CC = 0$

Abbreviation: SD, standard deviation; µSBS, microshear bond strength.

creased the probability of such flaws occurring is reduced, which favors the utilization of the micromechanical approach. Considering the 216 specimens tested in this study, no cohesive failures (either in resin cement or in ceramic) were observed. The circular bonding area delimitation may have induced better stress distribution around the adhesive interface and prevented the fracture from occurring beyond the limits of the cylinder. Moreover, since the experimental setup did not require any specimen cutting or trimming, pretesting failures were avoided.

Regarding the clinical relevance of testing conventional bis-GMA-based resin cements vs a self-adhesive resin cement, clinical trials are not abundant in the literature. A recent systematic review¹⁵ evaluated 12 clinical studies and indicated 97.8% as the cumulative short-term survival rate (up to five years) of 696 LD single crowns. However, the evidence for medium-term survival rate (five to 10 years) of 96.7% is still limited to just two studies. Regarding 145 LD fixed dental prostheses, five-year and 10-year cumulative survival rates are not promising (78.1% and 70.9%, respectively). All restorations were luted with self-adhesive resin cement or resin-modified glass ionomer cement. Only three clinical trials^{16,17} used LD CAD/CAM

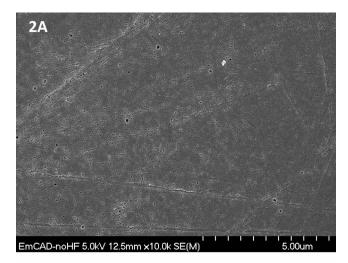
blocks (IPS e.max CAD) and followed the same surface pretreatment protocol consisting of HF etching and silanization before the application of the resin cement. Reich and Schierz¹⁶ reported a clinically satisfactory success of 96.3% after four years. The crowns were luted using a self-adhesive resin cement (Multilink Sprint, Ivoclar Vivadent). After two years, only one crown showed decementation, but it could be pretreated and recemented again. The authors attributed this failure to the short abutment of <3 mm, but unfortunately it was not possible to examine whether the tooth-resin cement or the resin cement-ceramic interface was weaker. In the clinical trial conducted by Fasbinder and others, ¹⁷ 62 chairside posterior crowns were cemented using either a bis-GMA-based, automixed, dual-cure resin cement (Multilink Automix) used with a self-etch adhesive or an experimental self-adhesive resin cement developed by the same manufacturer. Only two (5.1%) of the 39 crowns cemented with the self-adhesive resin cement debonded at two years. The authors reported that the cement was retained on the crown but not on the tooth surface.

The surface treatment, the bonding agent, and the resin cement mediate the link between LD and tooth structure, playing an important role in this

^a NT, no treatment; HF, 4.8% hydrofluoric acid.

^b Means with the same small capital letter are not significantly different (Dunnett test, p<0.05).

^c A, adhesive; M, mixed; CR, cohesive in resin cement; CC, cohesive in ceramic.



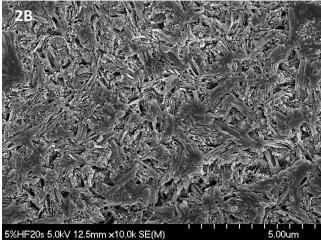
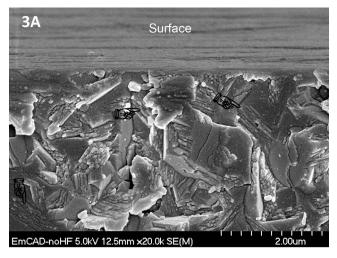


Figure 2. (A) Micrograph of a polished nontreated LD surface. Original magnification = 10,000×. (B) Polished LD surface after etching with 4.8% HF for 20 sec. Original magnification = 10,000×.

aspect. According to the above-mentioned clinical evidence, HF etching and silanization seem to improve adhesion; however, variations in chemical composition, wetting ability, viscosity, and mechanical properties of each resin cement might also be responsible for variations in the bonding strength. Despite the large number of clinical steps and, consequently, higher probability of operator mistakes, dual-cure resin cements are still considered the "gold standard" for adhesive luting as a result of their increased bond strengths, as reported in various studies. 18,19 The results obtained in our project corroborate this assertion and indicate that adhesion is improved when the complete cementation protocol (HF-Monobond Plus+ExciTE F DSC-Variolink II) is followed. Even though no significant difference was observed when neither silane (HF-



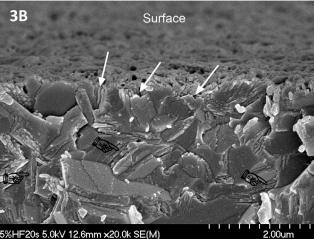
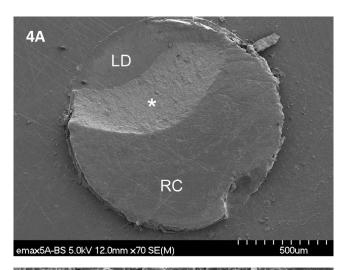


Figure 3. (A) Cross-sectional view of a polished nontreated LD surface. Original magnification = 20,000×. (B) Cross-sectional view of a polished LD surface after etching with 4.8% HF for 20 seconds. Pointers = LD crystals; Arrows = microretentive grooves from HF dissolution of the LD surface. Original magnification = 20,000×.

no silane-Variolink II) nor adhesive (HF-Monobond Plus-Variolink II) were applied on the etched surfaces, the respective mean µSBS values were not significantly different from those obtained in the nonetched but silanized groups, regardless of adhesive application (NT-Monobond Plus-Variolink II and NT-Monobond Plus+ExciTE DSC-Variolink II). Even at the nonetched LD surfaces. the functional monomers (3-trimethoxysilylpropyl, 10-methacryloyloxi-decyl-dihydrogen-phosphate) present in the silane solution (Monobond Plus) were able to achieve an adhesive strength above 28.0 MPa. In particular, 10-MDP is known for its favorable chemical bonding capabilities to diverse substrates, and it is considered a gold standard monomer for ceramic adhesion.²⁰ After silanization,



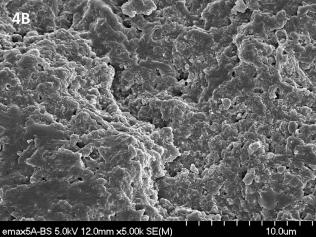


Figure 4. (A) Mixed failure of group NT+ Monobond Plus + Multilink Automix. LD = Lithium disilicate surface; RC = resin cement; asterisk = area of the RC observed in Figure 4B. Original magnification = 70×. (B) Higher magnification of fracture surface of RC (*) shown in Figure 4A. Original magnification = 5000×.

the previously hydrophilic surface turns hydrophobic and the luting material is able to optimally wet the LD surface. 21-23 The groups that received HF etching followed by Monobond Plus and Monobond Plus+ExciTE F DSC resulted in 100% mixed failures, which indicates an improved cement interlocking and possible chemical bonding, compared to the other groups that showed close to 50% adhesive failures. A rough and microretentive pattern is created after HF etching, which expands the available surface for bonding (Figures 2B and 3B). These findings are in agreement with those of other studies that found an increase in bond strength after HF etching, even though the manufacturer's recommendations were not strictly followed, specifically as they regard longer etching times and higher HF concentrations. 24-26 However, the 20-second acid-etching time and 4.8% HF concentration (as recommended by the LD manufacturer) were used in the present study because an increase of LD acid-etching time reduces the ceramic flexural strength and modifies the surface roughness. 27

When a self-adhesive resin cement was used with LD our results suggest that HF and MBP (HF-Monobond Plus-RelyX Unicem 2) should be applied on the LD intaglio surface, as demonstrated by the significant increase in mean uSBS. Beyond that, the extra step consisting of the application of the adhesive system (HF-Monobond Plus+ExciTE F DSC-RelyX Unicem 2) tended to decrease mean μSBS, even though the difference was not statistically significant. Therefore, RelyX Unicem 2 should not be applied if LD is not etched or silanized (NT-no silane-RelyX Unicem 2). However, it must also be taken into account that the chemistry of selfadhesive cements includes an acid-base reaction that is controlled through the presence of water. 10 In fact, a slightly aqueous environment is necessary to improve the ionization of the functional acidic monomers and their bonding capability to dentin. 28,29 During a clinical cementation of a crown, the cement contacts both dentin and ceramic simultaneously just after mixing the catalyst and base pastes. Therefore, it might be possible that during the setting reaction the adhesion to the ceramic surface is influenced by humidity. Further studies should address whether the bond between this resin cement and LD could be improved in the presence of moisture.

The bond between bis-GMA-based, auto-mixed, dual-cure resin cement and LD was clearly improved by silanization (HF-Monobond Plus-Multilink Automix). As with the phosphate monomer-based, self-adhesive, dual-cure resin cement (RelyX Unicem 2), the application of the adhesive system (HF-Monobond Plus+ExciTE F DSC-Multilink Automix) tended to decrease mean μSBS , albeit not significantly. Thus, silanization is mandatory when Multilink Automix is selected for cementation.

One of the limitations of this laboratory study is that the use of resin cement alone does not replicate what may occur clinically, where either tooth structure or build-up core material would be bonded to the intaglio surface. Hence, the stress patterns may be different when the resin cement is applied using a narrow transparent matrix.

CONCLUSIONS

LD benefits from pretreatment of the intaglio surface with HF and silanization, regardless of the resin cement used. Luting LD with a self-adhesive cement without previous HF etching is not recommended.

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Conflict of Interest

The author has 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.

Human Subjects Statement

This study was conducted at the University of Minnesota and the Federal University of Santa Catarina.

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