Bond durability of universal adhesives to intact and ground enamel surfaces in different etching modes

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Contents

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Summary

When using self-etch systems without a selective etching procedure, a lower degree of adhesion to the intact enamel surface (IE) may accelerate enamel margin degradation. A previous study that investigated the influence of enamel griding on the immediate bond strength of self-etch adhesives found that the self-etch adhesives showed no significant differences in shear bond strength (SBS) between IE and ground enamel surface (GE) in etch-and-rinse mode, but significantly lower SBS were found for IE in self-etch mode. However, there is a possibility that a layer of the prismless structure remains at the interface between enamel and cured adhesive even after phosphoric acid-etching is performed. There is little information about long-term enamel bond durability to IE, regardless of phosphoric acid etching procedure. The purpose of the present study was to determine the enamel bond durability of self-etch adhesives to IE and GE in different etching modes based on bond strength test after thermal cycling (TC) and on shear fatigue strength (SFS).

The universal adhesives used were Clearfil Universal Bond Quick ER (CUQ) and Scotchbond Universal (SBU). A conventional two-step self-etch adhesive, Clearfil SE Bond 2 (CSB) was used as a comparison material. Ultra Etch was used as the phosphoric acid-etching agent. In this study, extracted and de-identified human lower anterior incisors were selected to obtain an intact flat enamel surface. For IE specimens, the enamel bonding surfaces were brushed with fluoride-free prophylaxis paste for 30 s, then rinsed with water spray. For GE specimens, the labial surfaces were ground with #320-grit carbide papers with accompanying water coolant. Fifteen specimens were included in each test group to determine the enamel SBS in the etch-and-rinse mode or self-etch mode. Further, experimental specimens were divided into four groups: (i) GE in etch-and-rinse mode, (ii) IE in etch-and-rinse mode, (iii) GE in self-etch mode, and (iv) IE in self-etch mode. All bonding procedures were performed in accordance with the manufacturer's instructions. Following the bonding procedures, metal rings were used

to condense the resin composite onto enamel surfaces for shear bond strength (SBS) and shear fatigue strength (SFS) tests. The resin composite was condensed in the ring and light irradiated for 30 s. The bonded specimens were stored in distilled water at 37°C for 24 h, then subjected to TC for 30,000 cycles between 5 and 55°C. After TC, a metal rod with a chisel-shaped end was used to apply a load to the metal ring immediately adjacent to the flat tooth surface. The SBS of baseline groups, which was stored in distilled water at 37°C for 24 h, were measured. For SFS testing, bonding assemblies were constructed following the same procedures described above for the SBS test. Twenty-five specimens were used for each test condition to determine the SFS. The bonded assemblies were stored for 24 h in distilled water at 37°C before testing. The staircase method of fatigue testing was used. The load was applied at a frequency rate of 10 Hz with an electric dynamic test machine (ElectroPuls E1000) using a sine wave for 50,000 cycles or until failure occurred. Representative adhesive treated enamel surfaces, restorative/enamel interfaces, and de-bonded fracture sites after SFS tests were observed by scanning electron microscopy (SEM).

Regression analysis showed that the SBS value at baseline for SBU bonded to GE using etch-and-rinse was 44.8 MPa [95% CI for this mean value = (43.4, 46.2) MPa]. Among the predictors of SBS considered, the etching mode exerted the greatest main effect by far, as self-etching resulted in SBS values that were on average 16.4 [95% CI = (14.7, 18.1)] MPa lower than those for specimens treated with etch-and-rinse. TC resulted in SBS value that was 4.6 MPa (95% CI = 2.8, 6.4) lower than at baseline, and SBS value for adhesive CSB was on average 2.3 MPa higher than that for SBU, while adhesive CUQ had SBS value 2.6 MPa lower than that for SBU. The main effect for the enamel griding was a 2.6 MPa lower SBS for IE. Regarding SFS, the mean SFS values in etch-and-rinse mode ranged from 18.1 to 20.0 MPa in GE and from 14.4 to 17.6 MPa in IE. For the self-etching mode, the mean SFS values ranged from 10.0 to 16.5 MPa in GE and from 7.8 to 8.4 MPa in IE.

For GE with adhesive treatment, all the adhesives in etch-and-rinse mode showed similar morphological features to baseline GE with phosphoric acid etching, and a typical etching pattern was observed. The morphological features of IE with adhesive treatment in etch-and-rinse mode were varied and adhesive dependent. The treated enamel surfaces were not uniform, showing different etching patterns and prismless enamel layers in different areas. For IE in self-etching mode, all the adhesives showed a similar morphological appearance to baseline IE without any treatment. Morphological features in the vicinity of the adhesive layer and enamel interface were different in conjunction with different etching modes and enamel griding. While etched prisms were clearly visible and interpenetrated with the adhesive in GE, the prisms were not visible in IE. For GE in self-etch mode, the smear layer remained on the enamel surface and formed a hybrid smear layer. On the other hand, a nearly flat enamel surface without a smear layer was observed in IE with self-etch mode.

In the results of this laboratory study, the universal adhesives in self-etch mode with IE showed lower initial enamel bond strength and durability than those with GE. All of the examined adhesives in etch-and-rinse mode displayed significantly higher initial enamel bond strength and durability in both IE and GE. Although further consideration about the best strategy for IE when using self-etch adhesives should be needed, selective phosphoric acid pre-etching or griding the enamel surface might be helpful to establish reliable initial and long-term bonds to enamel when using universal adhesives in self-etch mode.

Introduction

Many laboratory studies have reported that a lower enamel bond strength exists in self-etch systems relative to etch-and-rinse systems (1–3). In addition, phosphoric acid-etching prior to the application of self-etch adhesives to enamel has led to significantly higher enamel bond strength and bond durability as compared with those prepared without pre-etching (4). A 13-year randomized clinical study of a two-step self-etch adhesive showed that, although restoration retention, secondary caries occurrence, and postoperative sensitivity were not significantly affected by the presence or absence of selective etching, enamel marginal defects and marginal discolorations were more frequently observed without selective etching (5). Meta analyses of clinical studies of direct anterior and class II restorations have indicated that enamel etching with 37% phosphoric acid yielded the best results for bond durability (6,7).

Degradation at restored enamel margins is thought to result from multiple causes, including occlusal force, biofilm attack, and thermal expansion discrepancy between enamel and resin composite (8,9). When using self-etch systems without selective etching procedure, a lower degree of adhesion to the intact enamel surface (IE) may accelerate enamel margin degradation. A previous study that investigated the influence of enamel griding on the immediate bond strength of self-etch adhesives found that the self-etch adhesives showed no significant differences in shear bond strength (SBS) between IE and ground enamel surface (GE) in etch-and-rinse mode, but significantly lower SBS were found for IE in self-etch mode (10). There are some cases wherein direct resin-composite restorations are placed on an unground enamel surface, such as diastema, microdont, and fissure sealant. Therefore, pre-etching with phosphoric acid prior to the application of self-etch adhesives to IE appears to be necessary to establish adequate initial effectiveness and durability of the enamel bond.

The outmost layer of IE is known to have indistinct prism structures or no prism structures due to the odontogenesis process and is, therefore, often called the prismless layer

(11). Kuroiwa (12) reported that a prismless enamel structure tended to show stronger resistance to acidic attack relative to a prismatic enamel surface. After phosphoric acid-etching, the mechanical properties of prismless enamel become weakened (12). Conflicting data exist regarding the thickness of the prismless enamel layer, previous investigations have reported findings such as 30 µm (13), 15–20 µm (14), and 10–30 µm (12). On the other hand, the etching depth in GE was 20–25 µm when using 35% phosphoric acid-etching agent for 15 s (15). Therefore, there is a possibility that a layer of the prismless structure remains at the interface between enamel and cured adhesive even after phosphoric acid-etching is performed. However, there is little information about the long-term enamel bond durability to IE, regardless of the use of phosphoric acid-etching.

The purpose of the present study was to determine the enamel bond durability of self-etch adhesives to IE and GE in different etching modes based on SBS after thermal cycling (TC) and on shear fatigue strength (SFS) testing. The null hypotheses considered were (i) the enamel bond durability of self-etch adhesive would not be influenced by whether the enamel adherent surface is IE nor GE, regardless of the etching-mode, and (ii) bond durability of IE in the etch-and-rinse mode would not be influenced by the type of self-etch adhesive.

Materials and methods

Study materials

The materials used in this study are listed in Table 1. The universal adhesives used were Clearfil Universal Bond Quick ER (CUQ; Kuraray Noritake Dental) and Scotchbond Universal (SBU; 3M Oral Care). A conventional two-step self-etch adhesive, Clearfil SE Bond 2 (CSB; Kuraray Noritake Dental), was used as a comparison material. Ultra Etch (Ultradent products) was used as the phosphoric acid-etching agent. Clearfil AP-X (Kuraray Noritake Dental) was used as the resin composite for bonding to enamel. A tungsten halogen visible-light curing unit

(Optilux 501; Kerr Dental) was used, and the power density of the curing unit was checked (above 600 mW/cm^2) using a dental radiometer (model 100; Kerr Dental). In this study, SBS test and SFS tests using a stainless-steel mold-in technique were selected because of the easy fabrication of specimens and uniform stress distribution.

Specimen preparation

Extracted and de-identified human lower anterior incisors were selected to obtain an intact flat enamel surface. The teeth were extracted due to dental problems and teeth of regular sizes and shapes were selected. After extraction, residual soft tissue attached to the root was immediately removed with hand instruments and each tooth was immersed in distilled water for 6 h. Extracted teeth were stored frozen (−20°C) until the experiment. This study protocol was reviewed and approved by the Ethics Committee for Human Studies at our research institution (EP20D007).

The enamel bonding sites were prepared by removing approximately two-thirds of the apical root structure. Each tooth was then mounted in self-curing acrylic resin at an orientation offering a flat area of approximately 5 mm^2 at the center of the labial side of the tooth surface. For IE specimens, the enamel bonding surfaces were brushed with fluoride-free prophylaxis paste for 30 s, then rinsed with water spray. For GE specimens, the labial surfaces were ground with #320-grit carbide papers with running water.

TC and SBS tests

The experimental protocols for the bonding procedures are shown in Table 2. Fifteen specimens were included in each test group to determine the enamel SBS in etch-and-rinse mode or self-etch mode. Further, experimental specimens were divided into four groups: (i) GE in etch-and-rinse mode, (ii) IE in etch-and-rinse mode, (iii) GE in self-etch mode, and (iv) IE in self-etch mode. All bonding procedures were performed in accordance with the manufacturer's instructions (Table 2). Following the bonding procedures, a stainless-steel metal ring (SUS 304) was placed over the bonding site and secured by clamping with a custom fixture in a modified Ultradent Bonding Jig. Metal rings were used to condense the resin composite on enamel surfaces for SBS and SFS tests. The bonding procedure resulted in a resin composite cylinder located inside the ring of 2.36 mm in diameter and approximately 2.5 mm in height. The ring was left in place for both tests.

The resin composite was condensed in the ring and light irradiated for 30 s. The bonded specimens were stored in distilled water at 37°C for 24 h, then subjected to TC for 30,000 cycles between 5 and 55°C (16). The dwelling time in the water bath was 30 s and the transfer time was 5 s. After TC, a metal rod with a chisel-shaped end was used to apply a load to the metal ring immediately adjacent to the flat tooth surface. The specimens were loaded to failure at 1.0 mm/min with a universal testing machine (Type 5500R; Instron). In addition, SBS of a baseline group, which was stored in distilled water at 37°C for 24 h, was measured. After testing, the bonding sites on the tooth surfaces and the resin composite cylinders were observed under an optical microscope (SZH-131; Olympus) at a magnification of ×10 to determine the bond failure mode. Based on the percentage of substrate area (adhesive / resin composite / enamel) observed in the de-bonded resin composites and tooth bonding sites, bond failure was classified as showing either: (i) adhesive failure, (ii) cohesive failure in the composite, (iii) cohesive failure in the enamel, or (iv) mixed failure, defined as partially adhesive and partially cohesive.

SFS tests

For SFS testing, bonding assemblies were constructed following the procedures described above as SBS test. Twenty-five specimens were used for each test condition to determine the SFS. The bonded assemblies were stored for 24 h in distilled water at 37°C before testing. For SFS testing, the staircase method of fatigue testing was used (17). The load was applied at a frequency rate of 10 Hz with an electric dynamic test machine (ElectroPuls E1000; Instron) using a sine wave for 50,000 cycles or until failure occurred. The load was incrementally increased or decreased (depending upon survival or failure) by approximately 10% of the initial load. After testing, the bonding sites of the tooth surface and resin composite cylinders of failed specimens were observed to determine the bond failure mode using the same procedures as described above.

Scanning electron microscopy (SEM) observations

Representative adhesive treated enamel surfaces, restorative/enamel interfaces, and de-bonded fracture sites after SFS tests were observed by SEM (ERA-8800FE; Elionix). For the observations of adhesive treated enamel surfaces, surfaces were treated in accordance with the application protocol (Table 2), and then rinsed three times with alternating acetone and water. In addition, GE and IE with or without phosphoric acid pre-etching were also observed as a baseline. For ultrastructure observation of the restorative/enamel interface, bonded samples that had been stored in 37°C distilled water for 24 h were embedded in epoxy resin (Epon 812; Nisshin EM) and then longitudinally sectioned. The sectioned surfaces were polished to a high gloss with abrasive discs followed by diamond paste down to 0.25-μm particle size. After ultrasonic cleaning for 3 min, the treated enamel specimens and resin/enamel interface specimens were dehydrated in ascending grades of *tert*-butyl alcohol (50% for 20 min, 75% for 20 min, 95% for 20 min, and 100% for 2 h) and then transferred from the final 100% bath to a freeze-drying device (Model ID-3; Elionix) for 30 min. Resin/enamel interface specimens were then subjected to argon ion-beam etching (EIS-200ER; Elionix) for 40 s with the ion beam (accelerating voltage: 1.0 kV, ion current density: 0.4 mA/cm^2) directed perpendicular to the polished surfaces. The de-bonded specimens from each condition were prepared directly for SEM. Finally, all the SEM specimens were coated in a vacuum evaporator (Quick Coater SC-701; Sanyu Electron) with a thin film of gold. Observation was carried out under SEM at an operating voltage of 10 kV.

Statistical analysis

Before comparing the data of each group, homogeneity of variance (Bartlett's test) and normal distribution (Kolmogorov—Smirnov test) were confirmed for the SBS data for each group. The SBS data were analyzed by means of multivariable linear regression analysis in which the regression coefficients estimated the effect of TC (30,000 cycles vs. baseline), etching mode (self-etch vs. etch-and-rinse), adhesive used (SBU, CSB, or CUQ), and enamel surface griding (GE or IE). All possible two-way interactions between factors were included in the initial model, but insignificant interaction terms were removed and the model re-estimated. The statistical analysis for SBS was performed with a statistical analysis software system (Stata ver. 16; StataCorp LLC). A modified *t*-test with Bonferroni correction was used for the SFS data with a custom program implemented in a spread sheet (Microsoft Excel; Microsoft).

Results

SBS

The results regarding the factors influential on SBS values are presented in Table 3. Regression analysis showed that the SBS value at baseline for SBU bonded to GE using etch-and-rinse mode was 44.8 MPa [95% CI for this mean value = (43.4, 46.2) MPa; Table 3]. Among the predictors of SBS considered, the etching mode exerted the greatest main effect by far, as self-etch mode resulted in SBS values that were on average 16.4 [95% CI = (14.7, 18.1)] MPa, and lower than those for specimens treated with etch-and-rinse. TC resulted in SBS value that was 4.6 MPa (95% CI = 2.8, 6.4) lower than at baseline, and SBS value for adhesive CSB was on average 2.3 MPa higher than that for SBU, while adhesive CUQ had SBS value 2.6 MPa lower than that for SBU. The main effect of the enamel griding was 2.6 MPa lower SBS for IE (Table 3). However, there was a considerable and statistically significant interaction between the enamel griding and the etching mode, such that IE specimens treated in self-etch mode showed much lower SBS values than GE specimens.

Upon comparing the different etching modes for each adhesive, all the adhesives showed significantly higher SBS values in etch-and-rinse mode than they did in self-etch mode, regardless of enamel griding. For self-etch mode, all tested adhesives showed significantly higher SBS values with GE than with IE. For etch-and-rinse mode, no significant differences in SBS values were observed between IE and GE for any adhesive.

SFS

The results for SFS are presented in Table 4. For etch-and-rinse mode, the mean SFS values ranged from 18.1 to 20.0 MPa in GE and from 14.4 to 17.6 MPa in IE. Although CSB in GE showed no significant differences in SFS values when compared to those of the universal adhesives, CSB in IE showed a significantly higher value. For self-etch mode, the mean SFS values ranged from 10.0 to 16.5 MPa in GE and from 7.8 to 8.4 MPa in IE. Although CSB showed significantly higher SFS values than the universal adhesives in GE, during SBS test, no significant differences were observed between the tested adhesives in IE. When comparing the different etching modes for each adhesive, all adhesives showed higher SFS values in etch-and-rinse mode than in self-etch mode in both IE and GE. For self-etch mode, although CSB showed a significantly higher SFS with GE than with IE, CUQ and SBU did not show any significant differences between IE and GE. For etch-and-rinse mode, no significant differences were observed between IE and GE for CSB. However, CUQ and SBU showed significantly higher SFS values with GE than with IE.

The ratios of SFS/SBS were calculated and are also presented in Table 4. For all the adhesives, the ratios of SFS/SBS with IE in etch-and-rinse mode were lower than in GE.

Failure mode analysis of de-bonded specimens

The frequencies of failure modes are presented in Tables 5 and 6. For the IE in self-etch mode, all the de-bonded specimens showed adhesive failure across these three groups. For GE in self-etch mode, although the universal adhesives presented the same pattern as seen in IE, mixed failure and cohesive failure in enamel were observed in CSB in all test groups. On the other hand, although the predominant mode of failure was adhesive failure for all adhesives in etch-and-rinse mode, some mixed and cohesive failures in enamel were observed in all tested group.

SEM observations

Representative SEM images of baseline and adhesive treated enamel surfaces are shown in Fig. 1. For baseline groups, although GE with phosphoric acid etching showed a typical etching pattern (Fig. 1A), IE with phosphoric acid etching showed different etching patterns from GE, that is, the surface prismless enamel layer was not completely removed (Fig. 1B). GE without phosphoric acid etching showed scratch marks from the carbide polishing paper, and the smear layer and some fragments on the smear layer were observed (Fig. 1C). IE without any treatments showed a smooth and flat surface (Fig. 1D).

For GE with universal adhesive treatment, all the adhesives in etch-and-rinse mode showed similar morphological features to baseline of GE with phosphoric acid etching, and a typical etching pattern was observed (Figs. 1E, I). The morphological features of IE with adhesive treatment in etch-and-rinse mode were varied and adhesive dependent. The treated enamel surfaces were not uniform, showing different etching patterns and prismless enamel layers in different areas (Figs. 1F, J). For GE with CUQ in self-etch mode (Fig. 1G), there were similarities in morphological appearance to baseline GE. The scratch marks remained, and no typical etching pattern was observed. However, the smear layer was dissolved in some areas.

For GE with CSB in self-etch mode (Fig. 1K), although the typical etching pattern was not observed, the smear layer was completely removed. For IE groups in self-etching mode, all the adhesives showed a similar morphological appearance to baseline IE (Figs. 1H, L).

Representative SEM images of resin/enamel interfaces are shown in Figs. 2 and 3. For each adhesive, the adhesive layer thicknesses were similar regardless of enamel griding or etching mode. Although the thicknesses of the adhesive layers (indicated by yellow arrows) of the universal adhesives were around 5–10 μm (Figs. 2A, 3A), the thickness of the adhesive layer of CSB was 30–40 μm (Figs. 2B, 3B). Morphological features in the vicinity of the adhesive layer and enamel interface were different in conjunction with different etching modes and enamel griding. Although all the adhesives showed excellent adaptation between the enamel substrate and adhesive, slight detachments between the adhesive layer and enamel surface were observed among the universal adhesives for the IE in the self-etch mode (Fig. 3C). Although adhesive interpenetration with enamel in etch-and-rinse mode was obvious in both IE and GE (Figs. 2A–D), the etching patterns were different in IE and GE.

Representative SEM images of the resin side of the de-bonded specimens after SFS testing are shown in Figs. 4 and 5. The failure pattern was dependent upon the etching mode and enamel griding. However, a similar morphological appearance was observed for the universal adhesives and the two-step self-etch adhesive. For GE in etch-and-rinse mode, CUQ and CSB exhibited more cracks and cleavages in the adhesives (as indicated by white arrows in Figs. 4A, B), so that striation and attached enamel fragments were more clearly observable. Although for CSB, IE in etch-and-rinse mode (Fig. 4D) showed a similar morphological appearance to that of GE in etch-and-rinse mode (Fig. 4B), enamel fragments were not clearly observed in the universal adhesives (Fig. 4C). In self-etch mode, different morphological features were notable in different enamel griding. For GE, cracks and cleavages in the adhesives were observed as striations in both CUQ and CSB (Figs. 5A, B). However, in IE, the failure patterns were relatively flat and beach marks were not visible, regardless of the adhesive system (Figs. 5C, D).

Discussion

In the results of the SBS test at the baseline, all of the adhesives showed significantly higher SBS values in etch-and-rinse mode than in self-etch mode, regardless of the enamel surface griding. However, looking at the influence of the enamel griding on SBS in different etching modes, the trends were diverse. Although significant differences in SBS values were observed between IE and GE in self-etch mode, no significant differences in SBS were observed in etch-and-rinse mode, regardless of enamel surface griding. The results obtained here were in line with those obtained by a previous study that investigated the influence of grinding the specimen surface on the immediate enamel bond strength of universal adhesives (10). The outer surface of IE is known to have indistinct prism structures or no prism structures and is, thus, often referred to as the “aprismatic layer” or “prismless layer” (11). This enamel layer is thought to function as a defense against acidic attack thanks to its structure and uptake of fluoride ions or other trace elements (12). On the other hand, considering the mild etching capability of self-etch adhesives, it may be hard to establish strong mechanical interlocking on IE surface and the aprismatic layer may interfere in resin monomer penetration (3). It can be inferred that the chemical interactions of the functional monomers of self-etch adhesives with superficial enamel might be limited in comparison with those involving GE because the calcium/phosphate ratio in permanent teeth is lower in intact superficial enamel than it is in the subsurface enamel (12).

According to the results obtained after TC, CSB showed no significant difference in SBS between IE and GE in etch-and-rinse mode, and a significantly higher SBS value was found in conjunction with GE than IE in self-etch mode. For the universal adhesives, significantly higher SBS values were observed after TC in connection with GE than with IE, and this occurred not only in self-etch mode but also in etch-and-rinse mode. When comparing the SBS values after TC with those at the 24-h baseline time point, the universal adhesives in the IE in both etching modes showed lower SBS values after TC, although the differences were only significant in etch-and-rinse mode. Suzuki et al. (16) investigated the influence of TC and long-term water storage on the bond durability of three universal adhesives attached to GE in self-etch mode. Here, none of the universal adhesives showed significantly different enamel SBS values following either 30,000 TC or 2-yr of water storage when compared with the 24-h baseline time point or the other tested degradation conditions. Therefore, the enamel bond durability of universal adhesives when adhering to GE might be sufficient for clinical application in self-etch mode. However, the adhesion of universal adhesives to IE is more susceptible to thermal stress and hydrolytic degradation than that to GE, regardless of etching mode.

In this study, enamel bond durability treated using different degradation techniques was evaluated. The TC test can accelerate degradation near the adhesive layer through temperature changes (18). Thermal stress caused by differences between the thermal expansion rates of the substrates and hydrolytic degradation caused by the water bath can induce degradation of the restoration (8,16,19). In particular, the differences in thermal expansion between the enamel and adhesive might lead to percolations at bonded interfaces due to the mechanical stress from temperature changes (16,19). On the other hand, the SFS test simulates oral conditions from the perspective of biomechanical stress attributed to occlusal function (20). Repeated subcritical loading induces fatigue stress at the interface between the tooth structure and resin composite restorations over time. Fatigue can be defined as the deterioration or failure of mechanical properties after repeated loading of stress at a levels well below the ultimate fracture strength of the material and/or interface (21). Therefore, the SFS test provides useful information not only about the ability of a material or interface to resist the propagation of cracks but also about the endurance characteristics of a bonding system (20,22,23).

When comparing the results of CUQ and SBU, the trends of enamel bond effectiveness were similar. Although the chemical compositions of CUQ and SBU are quite different, both adhesives contain methacryloyloxydecyl dihydrogen phosphate (MDP) as a functional monomer and they are categorized as mild type adhesives in accordance with a previous study (24). Therefore, it is probable that the efficacy of enamel bonding due to chemical bond and mechanical interlocking is equivalent between CUQ and SBU. The SFS of CUQ and SBU in etch-and-rinse mode showed different trends when compared with CSB. It is probable that the differences in load stress might influence the crack propagations and failure pattern at the interface. Therefore, the SFS/SBS ratio was helpful for understanding the fracture mechanisms and the endurance of the interface in different adhesives or etching modes.

Based on the results of the present study, the null hypotheses that (i) the enamel bond durability of self-etch adhesive would not be influenced by whether the enamel adherent surface is IE nor GE, regardless of etching mode, and (ii) bond durability of IE in the etch-and-rinse mode would not be influenced by the type of self-etch adhesive, were both ultimately rejected. Therefore, to maintain resin composite restorations in a better condition in the intraoral environment, using self-etch adhesives (including universal adhesives) in etch-and-rinse mode may be preferable due to the increased enamel bond performance and marginal integrity in both IE and GE.

Conclusions

1. The universal adhesives in self-etch mode with IE tended lower initial enamel bond strength and durability than those with GE.
2. All of the examined adhesives in the etch-and-rinse mode displayed significantly higher initial enamel bond strength and durability than in self-etch mode regardless of IE and GE.
3. The universal adhesives in IE showed a tendency to be lower bond strength than those in GE after TC and SFS testing.
4. Selective phosphoric acid pre-etching or griding the enamel surface might be helpful to establish reliable initial and long-term bonds to enamel when using universal adhesives in self-etch mode.

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Tables and Figures

Table 1: Materials used in this study

Code	Adhesive	Main components	pH	Manufacturer
Universal adhesives				
CUQ	Clearfil Universal Bond Quick ER (CM0014)	bis-GMA, MDP, HEMA, hydrophilic amide monomer, filler, ethanol, water, NaF, photo initiators, chemical polymerization, accelerator, silane coupling agent, others	2.3	Kuraray Noritake Dental, Tokyo, Japan
SBU	Scotchbond Universal (609889)	MDP, HEMA, dimethacrylate resins, Vitrebond copolymer, filler, ethanol, water, initiators, silane	2.7	3M Oral Care, St. Paul, MN, USA
Two-step self-etch adhesive				
CSB	Clearfil SE Bond 2 (Primer: 5852494) (Adhesive: 5847004)	Primer: MDP, HEMA, water, initiators Adhesive: MDP, HEMA, bis-GMA, initiators, microfiller	2.0 (Primer)	Kuraray Noritake Dental
Pre-etching agent				
	Ultra-Etch (G017)	35% phosphoric acid		Ultradent Products, South Jordan, UT, USA
Resin composite				
	Clearfil AP-X (N416713)	bis-GMA, TEGDMA, silane barium glass filler, silane silica filler, silanated colloidal silica, CQ, pigments, others		Kuraray Noritake Dental

bis-GMA: 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy) phenyl) propane, MDP: 10-methacryloyloxydecyl dihydrogen phosphate, HEMA: 2-hydroxyethyl methacrylate, NaF: sodium fluoride, TEGDMA: triethyleneglycol dimethacrylate, CQ: *dl*-camphorquinone

Table 2: Application protocol for pre-etching and universal adhesives

Method	Pre-etching protocol
Etch-and-rinse	Enamel surface was etched with phosphoric acid for 15 s. Etched surface was rinsed with water for 15 s and air-dried (three-way dental syringe).
Self-etch	Phosphoric acid pre-etching was not performed.
Adhesive	**Adhesive application protocol**
CUQ	Adhesive was applied to air-dried enamel surface for 10 s, and then medium air pressure was applied over the liquid adhesive for 5 s or until the adhesive was no longer moved and the solvent had completely evaporated. Light irradiation was done for 10 s.
SBU	Adhesive was applied to air-dried enamel surface with rubbing motion for 20 s and then medium air pressure was applied to surface for 5 s. Light irradiation was done for 10 s.
CSB	Primer was applied to air-dried enamel surfaces for 20 s followed by medium air pressure for 5 s. Adhesive was then applied to primed surfaces and was air thinned gently. Adhesive was light irradiated for 10 s.

Table 3: The results of multivariable linear regression analysis of the SBS

		Estimated effect of the experimental factors on SBS (in MPa)	
Predictor	Values	β	95% CI for β
Enamel griding	Ground enamel (GE)	Reference	-
	Uncut enamel (IE)	-2.6	-4.2, -0.9
Adhesive used	SBU	Reference	-
	CSB	2.3	0.4, 4.3
	CUQ	-2.6	-4.5, -0.7
Etching mode	Etch-and-rinse	Reference	-
	Self-etch	-16.4	-18.1, -14.7
Aging	Baseline	Reference	-
	Aged (thermal cycling)	-4.6	-6.4, -2.8
Aging*Adhesive	Thermal cycling*CSB	3.0	0.9, 5.1
	Thermal cycling*CUQ	1.5	-0.5, 3.5
Aging *Enamel substrate	Thermal cycling*IE	-1.7	-3.4, -0.0
Aging *Etching mode	Thermal cycling*self-etch	3.2	1.5, 4.9
Aging *Etching mode	CSB*Self-etch	4.7	2.6, 6.8
	CUQ*Self-etch	0.2	-1.8, 2.2
Enamel substrate *Etching mode	IE*Self-etch	-9.1	-10.8, -7.4
Constant		44.8	43.4, 46.2

*Interaction between adhesive and enamel substrate was statistically insignificant.

Table 4: Influence of surface condition on enamel SFS (MPa)								
Code	Etch-and-rinse mode				Self-etch mode			
	GE	SFS/SBS ratio	IE	SFS/SBS ratio	GE	SFS/SBS ratio	IE	SFS/SBS ratio
CUQ	18.1 (1.3)^{aA}	0.43	14.5 (1.4)^{bB}	0.37	10.0 (1.5)^{bC}	0.41	7.8 (1.6)^{aC}	0.47
SBU	18.8 (2.9)^{aA}	0.42	14.4 (1.3)^{bB}	0.34	11.2 (1.9)^{bBC}	0.41	8.0 (1.4)^{aC}	0.45
CSB	20.0 (2.7)^{aA}	0.43	17.6 (1.4)^{aAB}	0.39	16.5 (1.9)^{aB}	0.42	8.4 (2.2)^{aC}	0.42

N = 15, mean (SD) in MPa. Same small case letter in vertical columns indicates no difference at 5% significance level.
Same capital letter in horizontal rows indicates no difference at 5% significance level.
Values in parenthesis indicate standard deviation.

Table 5: Failure mode analysis of de-bonded enamel specimens after TC								
	SBS (Baseline)				After TC 30,000			
	Etch-and-rinse mode		Self-etch mode		Etch-and-rinse mode		Self-etch mode	
	GE	IE	GE	IE	GE	IE	GE	IE
CUQ	[80/0/13.3/6.7]	[80/0/0/20]	[100/0/0/0]	[100/0/0/0]	[73.4/0/13.3/13.3]	[80/0/13.3/6.7]	[100/0/0/0]	[100/0/0/0]
SBU	[73.4/0/20/6.7]	[73.4/0/20/6.7]	[100/0/0/0]	[100/0/0/0]	[80/0/20/0]	[73.3/0/20/6.7]	[100/0/0/0]	[100/0/0/0]
CSB	[73.4/0/13.3/13.3]	[73.3/0/26.7/0]	[80/0/20/0]	[100/0/0/0]	[66.7/0/20/13.3]	[80/0/13.3/6.7]	[93.4/0/0/6.7]	[100/0/0/0]

Failure mode [adhesive failure/cohesive failure in resin composite/cohesive failure in enamel/mixed failure] percentage of each failure mode

Table 6: Failure mode analysis of de-bonded enamel specimens after SFS test								
	SBS (Baseline)				SFS			
	Etch-and-rinse mode		Self-etch mode		Etch-and-rinse mode		Self-etch mode	
	GE	IE	GE	IE	GE	IE	GE	IE
CUQ	[80/0/13.3/6.7]	[80/0/0/20]	[100/0/0/0]	[100/0/0/0]	[76.9/0/23.1/0]	[90/0/0/10]	[100/0/0/0]	[100/0/0/0]
SBU	[73.4/0/20/6.7]	[73.4/0/20/6.7]	[100/0/0/0]	[100/0/0/0]	[72.7/0/18.2/9.1]	[73.3/0/20/6.7]	[100/0/0/0]	[100/0/0/0]
CSB	[73.4/0/13.3/13.3]	[73.3/0/26.7/0]	[80/0/20/0]	[100/0/0/0]	[76.9/0/23.1/0]	[83.3/0/16.7/0]	[84.6/0/7.7/7.7]	[100/0/0/0]

Failure mode [adhesive failure/cohesive failure in resin composite/cohesive failure in enamel/mixed failure] percentage of each failure mode

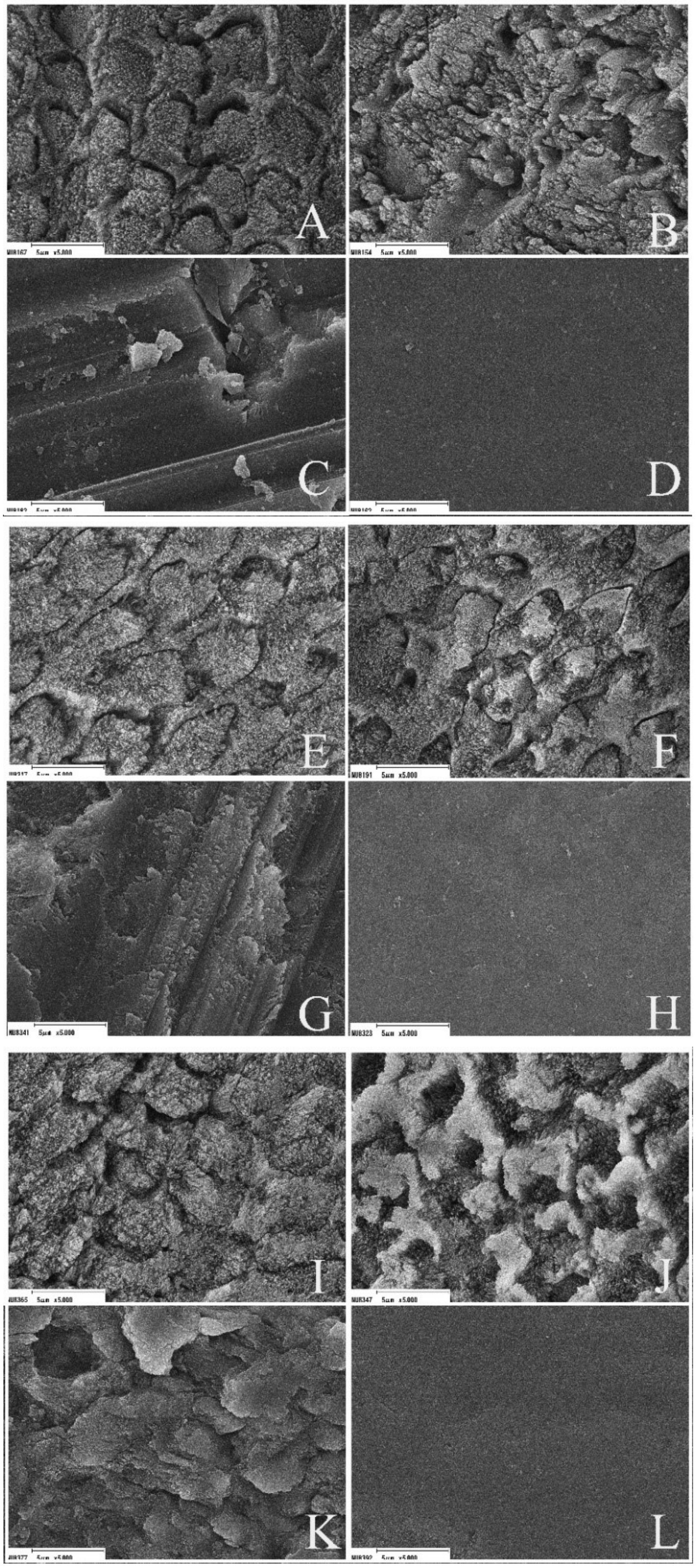

Fig. 1 Representative SEM images of baseline and adhesive treated enamel surfaces in different enamel grinding (×5,000). (A) GE with phosphoric acid etching. (B) IE with phosphoric acid etching. (C) Enamel surface ground by silicon carbide (SiC) paper. (D) Intact enamel without any treatment. (E) GE with CUQ in etch-and-rinse mode. (F) IE with CUQ in etch-and-rinse mode. (G) IE with CUQ in self-etch mode. (H) IE with CUQ in self-etch mode. (I) GE with CSB in etch-and-rinse mode. (J) IE with CSB in etch-and-rinse mode. (K) IE with CSB in self-etch mode. (L) IE with CSB in self-etch mode.

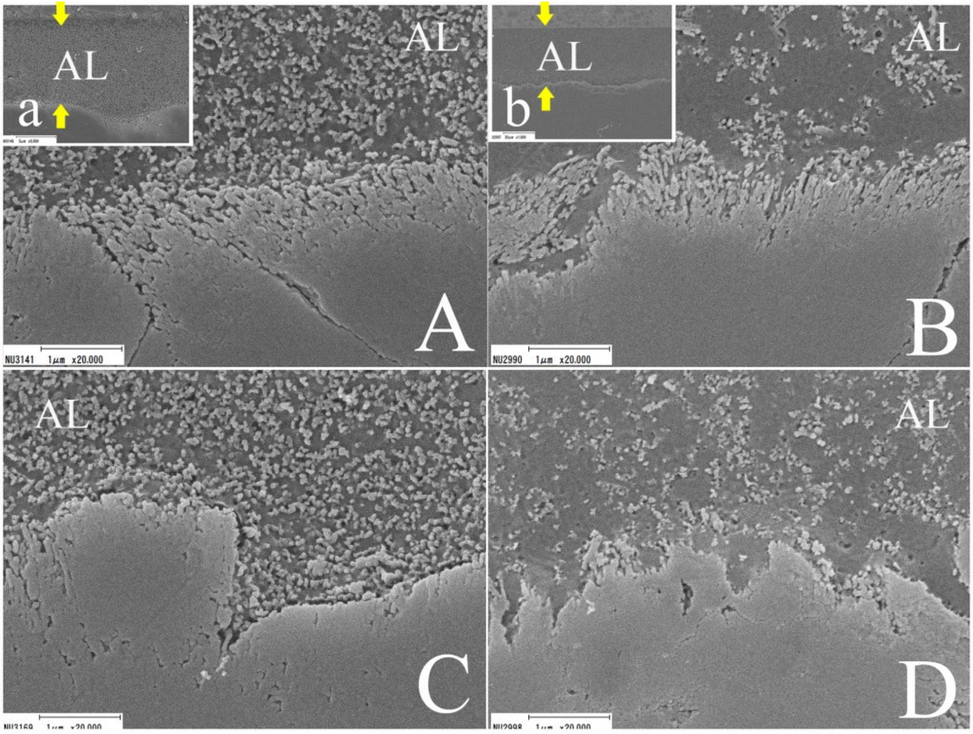

Fig. 2 Representative SEM images of resin/enamel interface in etch-and-rinse mode.
(A) GE with CUQ (×5,000 and ×20,000). (B) GE with CSB (×1,000 and ×20,000).
(C) IE with CUQ (×20,000). (D) IE with CSB (×20,000).
The visible material is indicated by abbreviations: AL, adhesive layer (yellow arrows indicate thickness of AL)

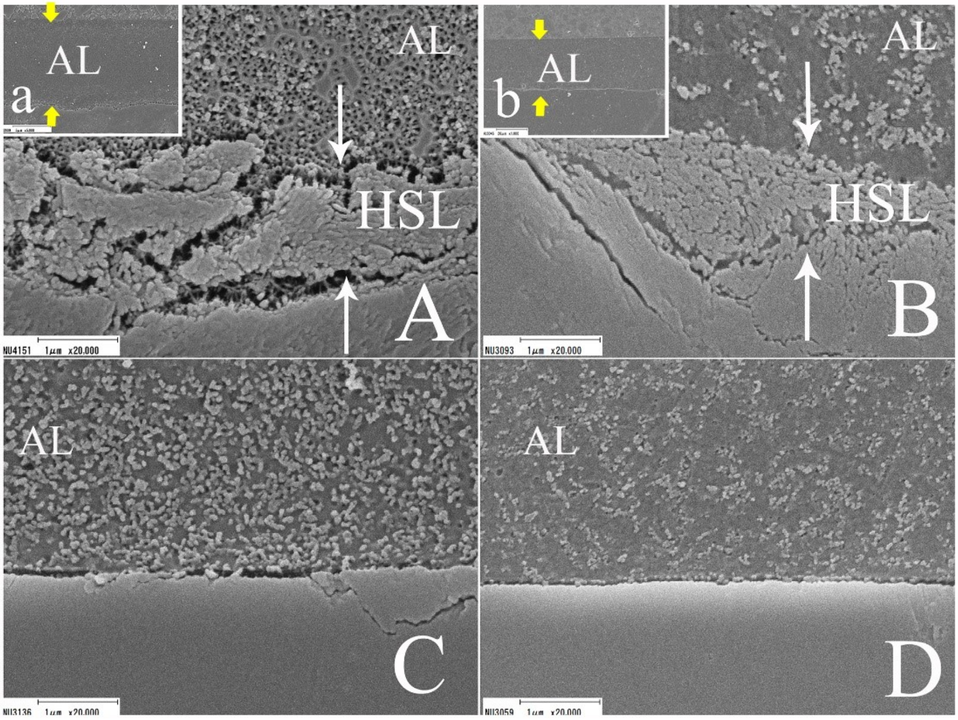

Fig. 3 Representative SEM images of resin/enamel interface in self-etch mode.
(A) GE with CUQ (×5,000 and ×20,000). (B) GE with CSB (×1,000 and ×20,000).
(C) IE with CUQ (×20,000). (D) IE with CSB (×20,000).
The visible material is indicated by abbreviations: AL, Adhesive layer (yellow arrows indicate thickness of AL); HSL, Hybrid smear layer (white arrows indicate HSL).

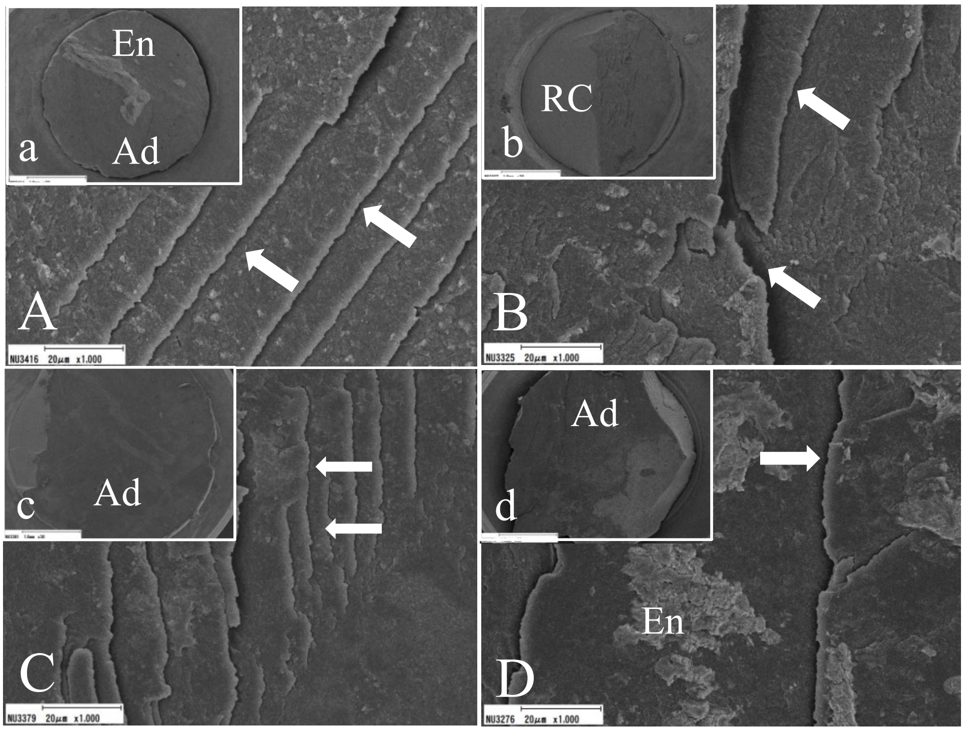

Fig. 4 Representative SEM images of de-bonded specimens in etch-and-rinse mode after SFS test.
(A) GE with CUQ (×30 and×1,000). (B) GE with CSB (×30 and×1,000).
(C) IE with CUQ (×30 and ×1,000). (D) IE with CSB (×30 and ×1,000).
The visible material is indicated by abbreviations: Ad, adhesive; En, enamel; RC, resin composite.
White arrows indicate cracks and cleavages in the adhesives.

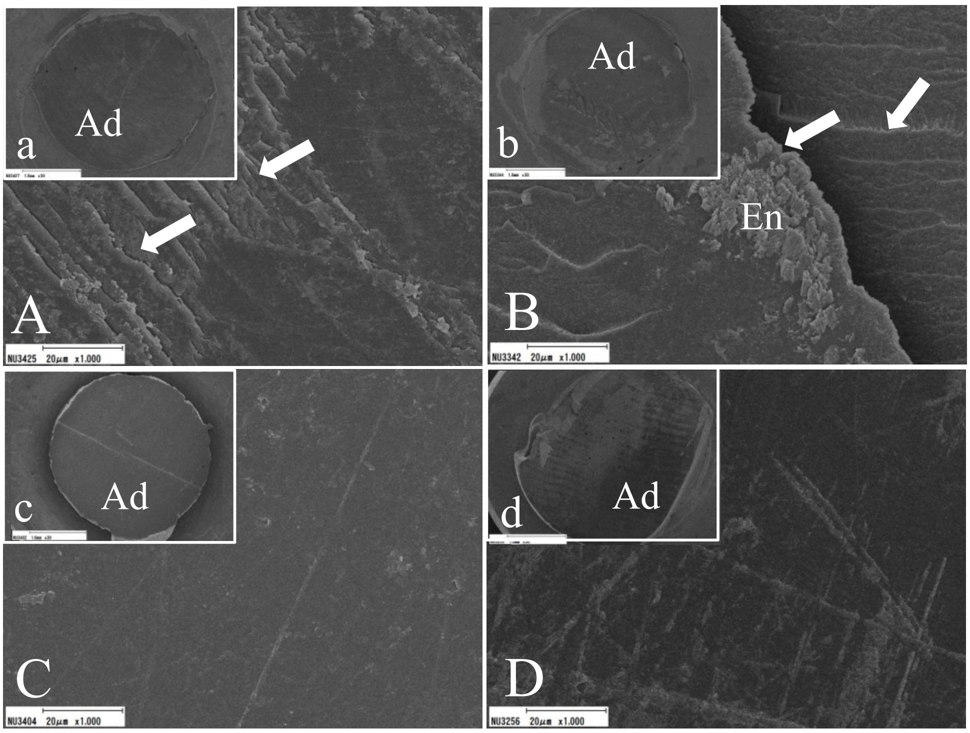

Fig. 5 Representative SEM images of de-bonded specimens in self-etch mode after SFS test.
(A) GE with CUQ (×30 and a: ×1,000). (B) GE with CSB (×30 and ×1000).
(C) IE with CUQ (×30 and ×1,000). (D) IE with CSB (×30, and ×1,000).
The visible material is indicated by abbreviations: Ad, adhesive; En, enamel.
White arrows indicate cracks and cleavages in the adhesives.