Immediate enamel bond strength of universal adhesives to intact enamel surface in different etching modes

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Contents

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Summary

Most laboratory enamel-bond studies have used ground flat surfaces, regardless of the type of bond-strength test. It is easy to standardize the methodology because an appropriate adherent area for the bonded assembly and uniform stress distribution can be achieved on a ground flat surface. However, in clinical situations, the outer surface of intact enamel is known to have indistinct and abnormal prism structures, or no prism structures at all, and is therefore often called the prismless layer. Therefore, it is important to investigate bond effectiveness to unground enamel as well as to ground enamel surface.

Universal adhesives can be used with either etch-&-rinse or self-etch approaches. Practitioners are able to select the optimal etching mode in accordance with cavity configuration and the proportion of enamel or dentin. On the other hand, little information is available on the enamel bond performance of universal adhesives to unground enamel, and on the performance in this respect in comparison with conventional self-etch systems. The purpose of the present study was to determine the enamel bond effectiveness of universal adhesives to unground and ground enamel in different etching modes, and to compare these bond performances with conventional two-step or single-step self-etch adhesives.

Five adhesives were used: three universal adhesives, Clearfil Universal Quick, Scotchbond Universal, and Prime & Bond Universal; a conventional two-step self-etch adhesive, Clearfil SE Bond; and a conventional single-step self-etch adhesive, Xeno JP. Two hundred extracted and de-identified human lower incisors were used in this study (Ethics Committee for Human Studies at Nihon University School of Dentistry, EP20D007). Each tooth was mounted in self-curing acrylic resin to expose a labial area of approximately 5 mm^2 at the center of the tooth surface. For unground enamel specimens, the enamel bonding surfaces were brushed with fluoride-free prophylaxis paste for 30 s and then rinsed with water spray.

For ground enamel specimens, the labial surfaces of embedded teeth were ground with #320-grit carbide polishing papers with a water coolant.

The shear bond strength (SBS) to enamel was measured using the notched-edge SBS test, as described in ISO 29022. Ten specimens were used for each test group to determine the enamel SBS in self-etch mode or in etch-&-rinse mode. Experimental specimens were divided into four groups: (i) unground enamel in self-etch mode; (ii) ground enamel in self-etch mode; (iii) unground enamel in etch-&-rinse mode; and (iv) ground enamel in etch-&-rinse mode. All bonding procedures were carried out in accordance with the manufacturers' instructions. Following application of adhesives, bonded resin-composite cylinders were formed on the surfaces by the bonding mold insert in bonding clamp against the enamel surfaces. Resin composite was packed into the mold and then light cured for 30 s. The bonded specimens were stored for 24 h in distilled water at 37°C before testing. Specimens were loaded to failure at 1.0 mm per min with a universal testing machine. The SBS values were calculated from the peak load at failure divided by the bonded surface area. After testing, the bonding sites were observed to determine the bond failure mode. Representative treated enamel surfaces, restorative/enamel interfaces, and failure sites of the debonded specimens were observed by scanning electron microscopy (SEM).

The mean SBS values of the unground enamel specimens treated in self-etch mode ranged from 14.0 to 21.8 MPa, while the corresponding values for the ground enamel specimens ranged from 23.1 to 34.5 MPa. The mean enamel SBS values of the unground enamel specimens in etch-&-rinse mode ranged from 40.8 to 43.1 MPa, while the corresponding values for the specimens in the ground enamel group ranged from 39.5 to 43.3 MPa. When comparing the different modes for each adhesive, all the adhesives showed significantly higher SBS values in etch-&-rinse mode than in self-etch mode, regardless of the adherent surface condition. The influence of enamel surface condition on bond strength was different in different etching modes. For the self-etch mode, all tested materials showed lower SBS values in unground than in ground enamel specimens. For the etch-&-rinse mode, no significant differences in SBS values were observed between unground and ground enamel specimens treated with any of the adhesives tested. For all adhesives in self-etch mode, adhesive failure was observed for all the de-bonded specimens, regardless of the adherent surface or adhesive. However, mixed failure and cohesive failure in enamel were observed in both unground and ground enamel specimens in etch-&-rinse mode.

In the SEM observations of the treated enamel surfaces, unground enamel specimens in self-etch mode showed similar morphological features, regardless of the type of adhesive. There were no clear signs of demineralization for any of the tested adhesives. The morphological features of unground enamel specimens in etch-&-rinse mode were varied and adhesive dependent. For the ground enamel specimens in self-etch mode, although scratch marks remained and no typical etching pattern was observed, the smear layer was dissolved in some areas, regardless of the type of adhesive. On the other hand, the morphological appearances of ground enamel specimens in etch-&-rinse mode were adhesive dependent.

In the SEM observations of the restorative-enamel interfaces, the morphological appearance of the specimens treated with universal adhesive was enamel surface condition and etching mode dependent. The unground enamel specimens in self-etch mode appeared relatively flat and uniform. On the other hand, unground enamel specimens treated in etch-&-rinse mode showed an irregular demineralized enamel surface, and infiltrated resin tags were observed. For the restorative-enamel interface in ground enamel specimens treated in self-etch mode, a smear layer was observed and resin monomers appeared to infiltrate through the smear layer. In the vicinity of the interface of ground enamel specimens treated in etch-&-rinse mode, the enamel surface was smoother than that of unground enamel specimens treated in etch-&-rinse mode, although an irregular enamel surface could be observed.

In representative SEM images of failure sites, the pattern of failure was dependent on the etching mode and enamel surface conditions. For the unground enamel condition in self-etch mode, the failure patterns were relatively flat and beach marks were not visible regardless of the type of adhesive. However, for the ground enamel condition, cracks and cleavages in the adhesives were observed in both universal adhesives and the two-step self-etch adhesive. For the ground enamel condition in etch-&-rinse mode, the tested adhesives exhibited more cracks and cleavages in the adhesives and the attached enamel fragments were clearly observable. The unground enamel condition in etch-&-rinse mode showed a similar morphological appearance to that of the ground enamel condition in etch-&-rinse mode in all the adhesives.

From the results of this laboratory study, the SBS of enamel bonds of the tested self-etch adhesives showed significantly higher values with pre-etching than without, regardless of the adhesive system or enamel surface condition. For all the self-etch adhesives with pre-etching, no significant difference was observed in SBS between unground and ground enamel specimens. On the other hand, although the two-step self-etch adhesive, Clearfil SE Bond had a significantly higher SBS value than the other adhesives on ground enamel without pre-etching, there was no significant difference in the SBS values between the two-step self-etch adhesive Clearfil SE Bond and the universal adhesives used without pre-etching on unground enamel specimens.

Introduction

Clinical use of direct resin-composite restorations has been expanding as a result of improvements in resin composites and developments of adhesive technologies. Enhanced mechanical properties and wear resistance of resin composites and user-friendly matrix systems contribute to the broader application of resin composite in larger cavities, high-stress-bearing areas, and class II cavities (1, 2). In addition, highly esthetic resin composites can be used not only for all classes of cavities but also for discolored teeth, diastema, and extensively fractured teeth (3).

At present, dental adhesive systems can generally be classified into two categories: etch-&-rinse systems and self-etch adhesive systems (4, 5). Each adhesive system is also divided into two groups according to the bonding procedure, that is three-step or two-step in etch-&-rinse systems, and two-step or single-step in self-etch systems (4, 5). For etch-&-rinse systems, enamel bond durability is considered to be optimal as a result of the creation of micromechanical retention between the demineralized enamel structure and the hydrophobic adhesive layer (6). The chemical bonds play a key role in preventing secondary caries, sealing restoration margins, and promoting restoration durability (7, 8). However, many laboratory investigations have shown that self-etch adhesive systems revealed lower enamel bond strengths than etch-&-rinse adhesive systems as a result of their lower etching ability (9–11). In addition, the self-assembled nano layering created on enamel is significantly weaker than that created on dentin (12, 13). Therefore, selective etching with phosphoric acid before application of a self-etch adhesive has been recommended to achieve a strong and durable bond to enamel in clinical situations (14–17).

Most laboratory enamel-bond studies have used ground flat surfaces, regardless of the type of bond-strength test (18, 19). It is easy to standardize the methodology because an appropriate adherent area for the bonded assembly and uniform stress distribution can be achieved on a ground flat surface (18, 19). However, in clinical situations, the outer surface of intact enamel is known to have indistinct and abnormal prism structures, or no prism structures at all, and is therefore often called the prismless layer (20). Resin composite restorations are often extended beyond the margins or bevels of the cavity preparation, for example, in cases of pit and fissure sealing, diastema closure, and restoration of fractured teeth, without any enamel preparation (21). Therefore, it is important to investigate the bond effectiveness to unground enamel as well as to ground enamel surface.

Universal adhesives can be used with either etch-&-rinse or self-etch approaches (16). This flexibility may be used to enhance the durability of the enamel bond with an etch-&-rinse approach or to reduce technique sensitivity with a self-etch approach to dentin. Practitioners are able to select the optimal etching mode in accordance with cavity configuration and the proportion of enamel or dentin. A few years have passed since early universal adhesives were first introduced, and *in-vivo* and *in-vitro* data have gradually been gathered. However, newly developed universal adhesives with different characteristics are constantly being released onto the market. Therefore, further research is needed on aspects of universal adhesives that have yet to be studied. In particular, little information is available on the enamel bond performance of universal adhesives to unground enamel, and on the relative merits of these systems and conventional self-etch systems.

The purpose of the present study was to determine the enamel bond effectiveness of universal adhesives to unground and ground enamel in different etching modes, and to compare these bond performances with conventional two-step or single-step self-etch adhesives. The null hypotheses to be tested were: (i) enamel bond performance in different adherent enamel surface treatments would not be influenced by type of adhesive system (i.e., two-step, single-step, or universal); and (ii) the adherent enamel surface treatments would not affect the enamel bond performance of universal adhesives, regardless of the etching mode.

Materials and methods

Study materials

The materials used in this study are shown in Table 1. Five adhesives were used: three universal adhesives, Clearfil Universal Quick (CUQ, Kuraray Noritake Dental, Tokyo, Japan), Scotchbond Universal (SBU, 3M Oral Care, St Paul, MN, USA), and Prime & Bond Universal (PBU, Dentsply Sirona, Konstanz, Germany); a conventional two-step self-etch adhesive, Clearfil SE Bond (CSE, Kuraray Noritake Dental); and a conventional single-step self-etch adhesive, Xeno JP (XJP, Dentsply Sirona, Tokyo, Japan). The latter two (CSE and XJP) were used as comparison adhesives. The phosphoric acid pre-etching agent used was Ultra-Etch (Ultradent Products, South Jordan, UT, USA). 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, sds Kerr, Danbury, CT, USA) was used, and the power density (average 600 mW cm^2) of the curing unit was checked using a dental radiometer (Model 100, sds Kerr).

Specimen preparation

In order to obtain a flat enamel surface, 200 extracted and de-identified human lower incisors were selected for use in this study. This study protocol was reviewed and approved by the Ethics Committee for Human Studies at Nihon University School of Dentistry, Tokyo, Japan (EP20D007). Before the experiment, extracted teeth with any signs of cracking of enamel, caries, or restoration, and teeth with obviously irregular enamel surfaces, erosion, or abrasion, were discarded. The enamel bonding sites were prepared by removing approximately two-thirds of the apical root structure using a low speed precision saw with diamond impregnated disk (IsoMet 1000 Precision Sectioning Saw, Buehler, Lake Bluff, IL, USA). Pulp tissues were removed and the pulp chamber of each tooth was filled with cotton. Each tooth was then mounted in self-curing acrylic resin (Tray Resin II, Shofu, Kyoto, Japan) to expose a labial area of approximately 5 mm^2 at the center of the tooth surface. For unground enamel specimens, the enamel bonding surfaces were brushed with fluoride-free prophylaxis paste (Merssage Fine, Shofu, Kyoto, Japan) for 30 s and then rinsed with water spray. For ground enamel specimens, the labial surfaces of embedded teeth were ground with #320-grit silicon carbide (SiC) polishing papers (Struers, Cleveland, OH, USA) with a water coolant.

Shear bond strength tests

The shear bond strength (SBS) to enamel was measured using the notched-edge SBS test, as described in ISO 29022 (22). The experimental protocols for the bonding procedures are shown in Table 2. Ten specimens were used for each test group to determine the enamel SBS in self-etch mode (i.e., without phosphoric acid etching) or in etch-&-rinse mode (i.e., with phosphoric acid application for 15 s prior to adhesive application). Therefore, experimental specimens were divided into four groups: (i) unground enamel in self-etch mode; (ii) ground enamel in self-etch mode; (iii) unground enamel in etch-&-rinse mode; and (iv) ground enamel in etch-&-rinse mode. All bonding procedures were carried out in accordance with the manufacturers' instructions (Table 2). Ultradent bonding kit (Ultradent Products) was used for determining SBS. Following application of adhesives, bonded resin-composite cylinders were formed on the surfaces by the bonding mold insert (2.4 mm in internal diameter, approximately 2.5 mm in height) in the bonding clamp against the enamel surface. Resin composite was packed into the mold insert and then light cured for 30 s. The bonded specimens were removed from the mold and stored for 24 h in distilled water at 37°C before testing. Specimens were loaded to failure at 1.0 mm per min with the universal testing machine (Type 5500R, Instron, Canton, MA, USA). The SBS values were calculated from the peak load at failure divided by the bonded surface area. After testing, the bonding sites of tooth surfaces and resin-composite cylinders were observed under an optical microscope (SZH-131, Olympus, Tokyo, Japan), at a magnification of ×10, to determine the bond failure mode. Based on the percentage of substrate area (adhesive – resin composite – enamel) observed on the de-bonded cylinders and tooth bonding sites, the types of bond failure were recorded as adhesive failure, cohesive failure in composite, cohesive failure in enamel, or mixed failure (partially adhesive and partially cohesive).

Scanning electron microscopy (SEM) observations

Representative treated enamel surfaces, restorative–enamel interfaces, and debonded fracture sites after SBS test were observed by field-emission SEM (ERA-8800FE, Elionix, Tokyo, Japan). 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 and then longitudinally sectioned with the low-speed precision saw. The sectioned surfaces were polished to a high gloss with abrasive discs (Fuji Star Type DDC, Sankyo Rikagaku, Saitama, Japan) followed by diamond pastes down to 0.25 μm particle size (DP-Paste, Struers, Ballerup, Denmark). For observation of 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, samples ground with wet #320-grit SiC paper, with and without phosphoric acid pre-etching, were also observed as a baseline, but these samples were not rinsed with acetone.

All SEM specimens of the treated enamel surfaces and restorative–enamel interfaces 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 (Model ID-3, Elionix) for 30 min. Restorative–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. Debonded fracture sites were prepared directly for SEM. Finally, all of the scanning electron microscopy specimens were coated in an automatic ion spatter (Quick Coater, Type SC-701, Sanyu Electron, Tokyo, Japan) with a thin film of gold. Observation was carried out using SEM (FE-8800, Elionix) at an operating voltage of 10 kV.

Statistical analysis

A statistical power analysis indicated that at least nine samples were necessary for effective measurement of bond strength. Therefore, this experiment was initially performed with sample sizes of 10. After gathering the data, post-hoc power tests were performed, and these tests indicated that the sample size was adequate. Three-way ANOVA followed by Tukey's honest significant difference (HSD) test ($\alpha = 0.05$) was used for analysis of all the bond-strength data. Factors included etching mode, adherent surface characteristic (unground or ground), and adhesive system. The statistical analysis was performed with a statistical analysis software Sigma Plot (ver. 11.0, SPSS, Chicago, IL, USA).

Results

Shear bond strength tests

The results for the SBS tests are shown in Table 3. The three-way ANOVA revealed that all factors, namely etching mode, adherent surface condition (unground or ground), and adhesive system, significantly influenced the SBS values ($p < 0.001$), while the three-way interaction was not significant ($p = 0.35$). However, all six pairwise interactions were significant ($p < 0.05$).

The mean SBS values of the unground enamel specimens treated in self-etch mode ranged from 14.0 to 21.8 MPa, while the corresponding values for the ground enamel specimens ranged from 23.1 to 34.5 MPa. In the unground enamel specimens, there were no significant differences in SBS among most of the adhesives, although specimens treated with the XJP adhesive showed a significantly lower SBS value than the specimens treated with the other adhesive systems. On the other hand, in the ground enamel specimens, specimens treated with the CSE showed a significantly higher SBS value than specimens treated with the other adhesives, while specimens treated with the XJP showed a lower SBS value than specimens treated with the other adhesives, as was also observed for the unground enamel specimens. The mean enamel SBS values of the unground enamel specimens in etch-&-rinse mode ranged from 40.8 to 43.1 MPa, while the corresponding values for the specimens in the ground enamel group ranged from 39.5 to 43.3 MPa. For the both the unground and the ground enamel groups, there were no significant differences in the SBS values among the adhesive systems tested.

When comparing the different bonding modes for each adhesive, all the adhesives showed significantly higher SBS values in etch-&-rinse mode than in self-etch mode, regardless of the adherent surface condition (ground or unground). The influence of enamel surface condition on bond strength was different in different etching modes. For the self-etch mode, all tested materials showed lower SBS values in unground than in ground enamel specimens. For the etch-&-rinse mode, no significant differences in SBS values were observed between unground and ground enamel specimens treated with any of the adhesives tested.

Failure mode analysis of SBS debonded specimens

The frequency of different failure modes is shown in Fig. 1. For all adhesives in self-etch mode, adhesive failure was observed for all the de-bonded specimens, regardless of the adherent surface or adhesive. However, mixed failure and cohesive failure in enamel were observed in both unground and ground enamel specimens in etch-&-rinse mode.

SEM observations

Representative SEM images of untreated and treated enamel surfaces of unground and ground enamel specimens treated with different etching modes are shown in Figs. 2–5. To classify the morphological features observed after phosphoric acid pre-etching, the Silverstone report (23) were referred. A type I etching pattern was defined as demineralized enamel rods with remaining interprismatic substance; a type II etching pattern was defined as demineralized interprismatic substance with remaining enamel rods; and a type III etching pattern was defined as mixed type I and II. The untreated and unground enamel specimens showed a smooth and flat surface (Fig. 2A). On the other hand, unground enamel specimens etched with phosphoric acid were difficult to classify in accordance with the Silverstone definitions because the surface prismless enamel layer was not completely removed, while a type I etching pattern was observed in the same area (Fig. 2B). The untreated specimens ground with SiC papers showed scratch marks from the carbide polishing paper, and the smear layer and some fragments on the smear layer were observed (Fig. 2C). In the untreated ground enamel specimens with phosphoric acid etching, the smear layer was completely removed, and a type II etching pattern was observed (Fig. 2D).

The unground enamel specimens in self-etch mode showed similar morphological features, regardless of the type of adhesive. There were no clear signs of demineralization for any of the tested adhesives (Figs. 3A, 4A, and 5A). The morphological features of unground enamel specimens in etch-&-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. 3B, 4B, and 5B). For the ground enamel specimens in self-etch mode, there were similarities in morphological appearance to the untreated specimens in the ground enamel group, regardless of the type of adhesive. The scratch marks remained, and no typical etching pattern was observed. However, the smear layer was dissolved in some areas, regardless of the type of adhesive. On the other hand, the morphological appearances of ground enamel specimens in etch-&-rinse mode were adhesive dependent. Although specimens treated with the SBU did not show a clear etching pattern, the smear layer was completely dissolved and the treated surface was irregular. Specimens treated with the CSE showed clear type II etching patterns, and those treated with the XJP showed type III etching patterns.

Representative SEM images of the restorative–enamel interfaces of the unground and ground enamel specimens treated in different etching modes are shown in Fig. 6. The morphological appearance in the vicinity of the adhesive–enamel interfaces of specimens treated with PBU was enamel surface condition and etching mode dependent. The thickness of adhesive layers of PBU was approximately 1–3 μm. The unground enamel specimens in self-etch mode appeared relatively flat and uniform (Fig. 6A). On the other hand, unground enamel specimens treated in etch-&-rinse mode showed an irregular demineralized enamel surface, and infiltrated resin tags were observed (Fig. 6C). For the restorative–enamel interface in ground enamel specimens treated in self-etch mode, a smear layer was observed and resin monomers appeared to infiltrate through the smear layer (Fig. 6B). In the vicinity of the enamel–adhesive layer of ground enamel specimens treated in etch-&-rinse mode (Fig. 6D), the enamel surface was smoother than that of unground enamel specimens treated in etch-&-rinse mode, although an irregular enamel surface could be observed.

Representative SEM images of the resin side of the debonded specimens after SBS testing are shown in Figs. 7 and 8. The failure pattern was dependent upon the etching mode and enamel surface condition. However, a similar morphological appearance was observed for the universal adhesives and the two-step self-etch adhesive. In the self-etch mode, different morphological features were observed in different surface conditions. For the unground enamel condition, the failure patterns were relatively flat and beach marks were not visible in either CUQ and CSE (Figs. 7A and 8A). However, for the ground enamel condition, cracks and cleavages in the adhesives were observed in both CUQ and CSE (Figs. 7B and 8B). The unground enamel condition in the etch-&-rinse mode showed a similar morphological appearance to that of the ground enamel condition in the self-etch mode in both CUQ and CSE (Figs. 7C and 8C). For the ground enamel condition in the etch-&-rinse mode, CUQ and CSE exhibited attached enamel fragments that were clearly observable (Figs. 7D and 8D).

Discussion

The focus of the present study was to elucidate the immediate enamel bond performance of newer universal adhesives under different etching modes on unground intact enamel, and to compare this with ground enamel through bond strength tests. The etch-&-rinse mode resulted in significantly higher SBS values than the self-etch mode, regardless of the type of adhesive or the adherent enamel surface condition. On the other hand, no significant differences in SBS values were observed between unground and ground enamel specimens when treated in etch-&-rinse mode, irrespective of the adhesives used. When self-etch adhesives were introduced to the profession, routine phosphoric acid pre-etching of enamel was not recommended by manufacturers. However, phosphoric acid pre-etching before application of adhesives was very beneficial for obtaining higher enamel bond strength with the adhesives tested in this study.

In the SEM observations, the morphological appearances of enamel surfaces treated in etch-&-rinse mode were dependent on the adhesive used and the adherent enamel surface condition. In contrast to ground enamel, unground specimens showed varying and complex features. That is, in untreated unground enamel etched with phosphoric acid, the prismless enamel layer was not completely removed, so the surfaces treated with adhesive after phosphoric acid etching showed variable types of etching patterns. In permanent teeth, the thickness of the prismless layer depends on the type and region of the tooth or shape of the layer. Different studies have found the thickness of the surface prismless enamel to be 30, 15–20, or 10–30 μm (24). In the present study, the center of the labial surface of the lower anterior tooth was used as the adherent enamel surface, and the main reason why the teeth had been extracted was severe periodontitis in elderly people. Therefore, the thickness of the prismless layers in the present study might be thinner than observed in previous reports as a result of variation in the type of tooth, region, and age.

A previous study showed that the phosphoric acid etching depth in ground enamel was 20–25 μm when using the same etching agent as in this study (25). Kuroiwa (24) reported that a prismless enamel structure tended to have a stronger resistance to acid than a prismatic enamel surface. Burrow *et al*. (26) suggested removing the prismless layer of enamel at pits and fissures mechanically to improve the bonding of fissure sealant, because this region seems resistant to etching. This phenomenon can be explained not only by structure but also by uptake of fluoride ions or other trace elements. Therefore, it can be inferred that the standard phosphoric acid etching method might not remove the prismless layer of enamel completely, and areas of prismless layer might still remain on the etched surface.

When comparing the SBS values in self-etch mode between unground and ground enamel specimens, all adhesives tested showed significantly lower SBS values with unground enamel than with ground enamel. The first null hypothesis is that each type of adhesive system (self-etch adhesive or universal adhesive) would show similar differences between its bonding to ground enamel and its bonding to unground enamel. All the adhesives showed a significantly lower SBS to unground enamel, and thus did show the same pattern. Therefore, this hypothesis was not rejected. The pH values of the self-etch adhesives tested in this study range from 2.3 to 2.7, and are categorized as mild or ultra-mild systems (27). In the SEM images of enamel surfaces treated in self-etch mode, it was difficult to find morphological changes in the unground enamel specimens. However, although the smear layer remained on ground enamel specimens after application of adhesives, the prismatic layer was observed in some areas as a result of demineralization. Therefore, it seems that the lower etching ability of ultra-mild adhesives could not demineralize the prismless layer effectively. Thus, micro-mechanical retention in the self-etch mode with unground enamel may be weak.

When looking at the SBS values for different adhesive systems, no significant differences were observed among the tested adhesives in etch-&-rinse mode, regardless of the type of enamel surface to which bonding was made. However, in the self-etch mode, the SBS values were adhesive system-dependent in both ground and unground enamel specimens. Therefore, the second null hypothesis, that the adherent enamel surface characteristics would not affect the enamel bond performance of universal adhesives, regardless of the etching mode, was partly rejected.

No significant difference was observed in the unground enamel specimens between CSE and the universal adhesives. Mine *et al*. (28) observed the prismless layer of the third molar using transmission electron microscopy, and reported that resin impregnation of single-step self-etch adhesive was limited to a depth ranging from almost zero to 400 nm, and for the most part the infiltration did not extend beyond the prismless layer. However, a much deeper resin infiltration (from 500 nm up to 1.5 μm) was found for enamel ground using SiC paper. Therefore, the prismless layer may work not only as an acid-resistant layer inhibiting demineralization, but also as a physical obstacle to the infiltration of the functional monomers, even when the two-step self-etch adhesive CSE is applied.

The results of this study suggested that care should be taken when bonding to unground enamel with the use of self-etch adhesive systems without phosphoric acid pre-etching or universal adhesives in self-etch mode. If resin composite is placed beyond the cavity margins, marginal integrity might deteriorate as a result of weaker adhesion, and this might lead to marginal discoloration, marginal gap formation, and secondary caries. On the other hand, phosphoric acid pre-etching before application of self-etch adhesives appears to be effective in obtaining superior initial enamel bond performance, regardless of enamel surface treatment. The clinical relevance of this laboratory study was that phosphoric acid pre-etching before application of universal adhesives to unground enamel surface should be done to establish adequate initial enamel bond effectiveness. Therefore, further research is needed to investigate the enamel bond durability of self-etch adhesive systems and universal adhesives in etch-&-rinse mode to unground enamel.

Conclusions

From the results of this laboratory study, the SBS to enamel of the tested self-etch adhesives showed significantly higher values with pre-etching than without, regardless of the adhesive system or enamel surface condition. For all the self-etch adhesives with pre-etching, no significant difference was observed in SBS between unground and ground enamel specimens. On the other hand, although the two-step self-etch adhesive CSE had a significantly higher SBS value than the other adhesives on ground enamel without pre-etching, there was no significant difference in the SBS values between the two-step self-etch adhesive CSE and the universal adhesives used without pre-etching on unground enamel specimens.

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Table 1: Materials used in this study

Code	Adhesive	Main components	pH	Manufacturer
Universal adhesives				
CUQ	Clearfil Universal Quick (CM0014)	bis-GMA, MDP, HEMA, hydrophilic amide monomer, filler, ethanol, water, NaF, photo initiators, chemical polymerization, accelerator, 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
PBU	Prime & Bond Universal (1706006938)	Bi- and multifunctional acrylate, MDP, PENTA, initiator, stabilizer, isopropanol, water	2.5	Dentsply Sirona, Konstanz, Germany
Two-step self-etch adhesive				
CSE	Clearfil SE Bond (Primer: 5852494) (Adhesive: 5847004)	Primer: MDP, HEMA, water, initiators Adhesive: MDP, HEMA, bis-GMA, initiators, microfiller	2.5 (primer)	Kuraray Noritake Dental
Single-step self-etch adhesive				
XJP	Xeno JP (00027792)	4-MET, Pyro-EMA, fluoride, alcohol, silica filler, photo initiator, water	2.5	Dentsply Sirona, Tokyo, Japan
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, PENTA: dipentaerythritol pentacrylate phosphate,
4-MET: 4-methacryloxyethyl trimellitate, Pyro-EMA: Pyro-ethyl methacrylate, TEGDMA: triethyleneglycol dimethacrylate, CQ: *dl*-camphorquinone.

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

Method	Pre-etching protocol
Self-etch mode	Phosphoric acid pre-etching was not performed.
Etch-&-rinse mode	Enamel surface was phosphoric acid etching for 15 s. Etching surface was rinsed with water for 15 s (three-way dental syringe) and air-dried.
Adhesive	**Adhesive application protocol**
Universal adhesives	
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 no longer moved and the solvent was completely evaporated. Light irradiated 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. Adhesive was light irradiated for 10 s.
PBU	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. Adhesive was light irradiated for 10 s.
Two-step self-etch adhesive	
CSE	Primer was applied to air-dried enamel surface for 20 s followed by medium air pressure for 5 s. Adhesive was then applied to primed surface and was air thinned gently. Adhesive was light irradiated for 10 s.
One-step self-etch adhesive	
XJP	Adhesive was applied to air-dried enamel surface for 10 s and then medium air pressure was applied to surface for 5 s. Adhesive was light irradiated for 10 s.

Table 3: Immediate enamel bond strength (MPa)

	Self-etch mode (no pre-etching)		Etch-&-rinse mode (pre-etching)	
	Unground enamel	Ground enamel	Unground enamel	Ground enamel
CUQ	19.1 (4.4)^{aC}	26.8 (3.8)^{bcB}	42.2 (3.7)^{aA}	42.4 (3.6)^{aA}
SBU	21.8 (3.8)^{aC}	28.5 (2.6)^{bB}	41.2 (5.4)^{aA}	40.1 (2.5)^{aA}
PBU	18.4 (2.0)^{aC}	25.6 (3.5)^{bcB}	40.8 (6.3)^{aA}	39.5 (3.0)^{aA}
CSE	21.2 (1.8)^{aC}	34.5 (2.5)^{aB}	43.1 (4.4)^{aA}	43.3 (2.9)^{aA}
XJP	14.0 (3.8)^{bC}	23.1 (2.0)^{cB}	41.3 (5.8)^{aA}	40.0 (3.2)^{aA}

N=10, mean (SD) in MPa
Same lower 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 indicates standard deviation.

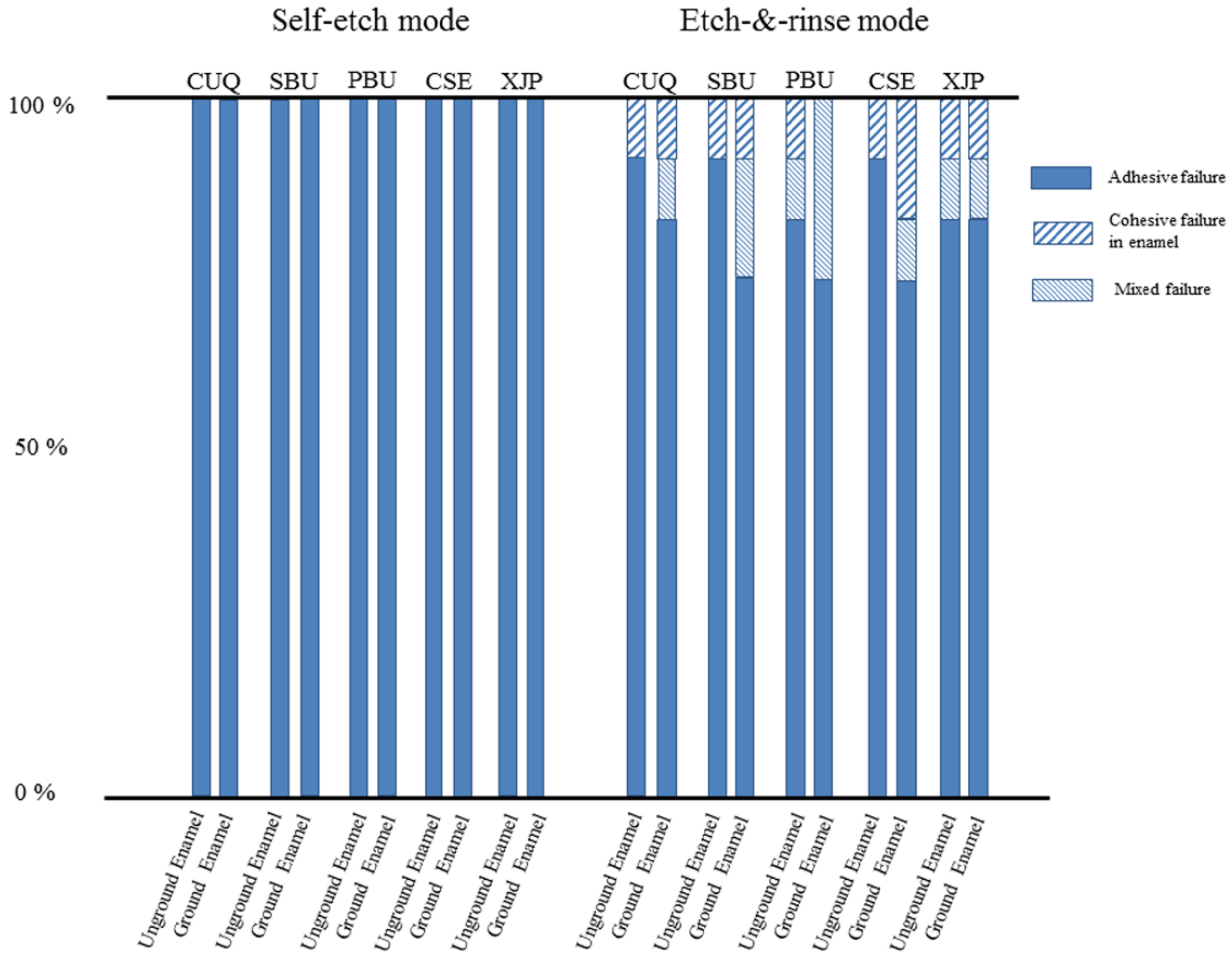

Fig. 1: Failure mode analysis of the debonded enamel specimens. CUQ, Clearfil Universal Quick; SBU, Scotchbond Universal; PBU, Prime & Bond Universal; CSE, Clearfil SE Bond; XJP, Xeno JP.

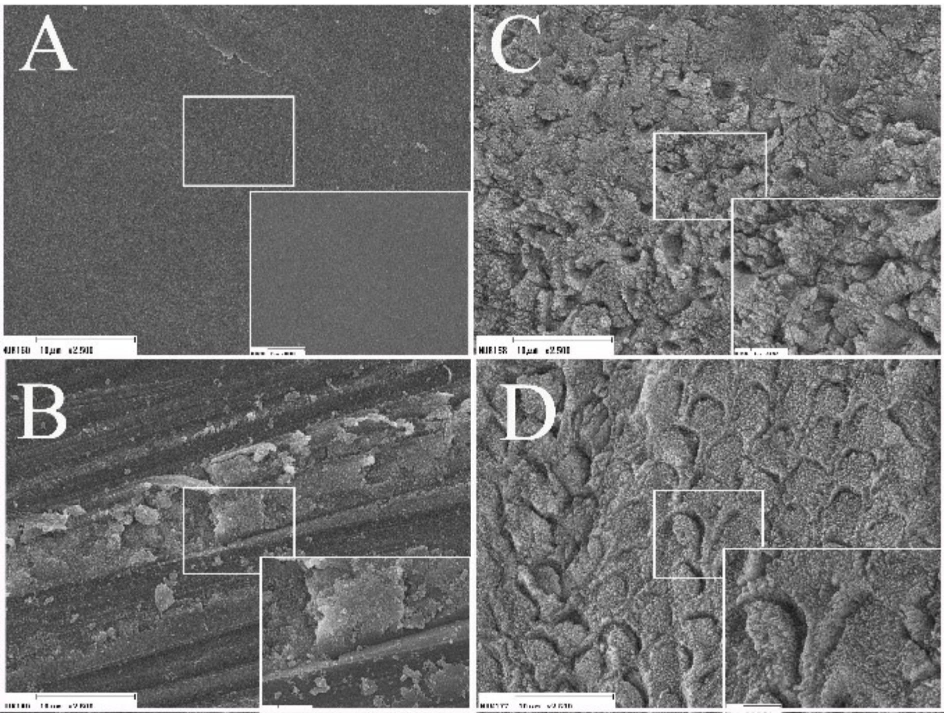

Fig. 2: Representative SEM images of untreated enamel surfaces. (A) Unground enamel surface (Behind image, ×2,500 and Front image, ×10,000). (B) Enamel surface ground by silicon carbide (SiC) paper (Behind image, ×2,500 and Front image, ×10,000). (C) Unground enamel surface after phosphoric acid pre-etching (Behind image, ×2,500 and Front image, ×10,000). (D) Ground enamel surface after phosphoric acid etching (Behind image, ×2,500 and Front image, ×10,000).

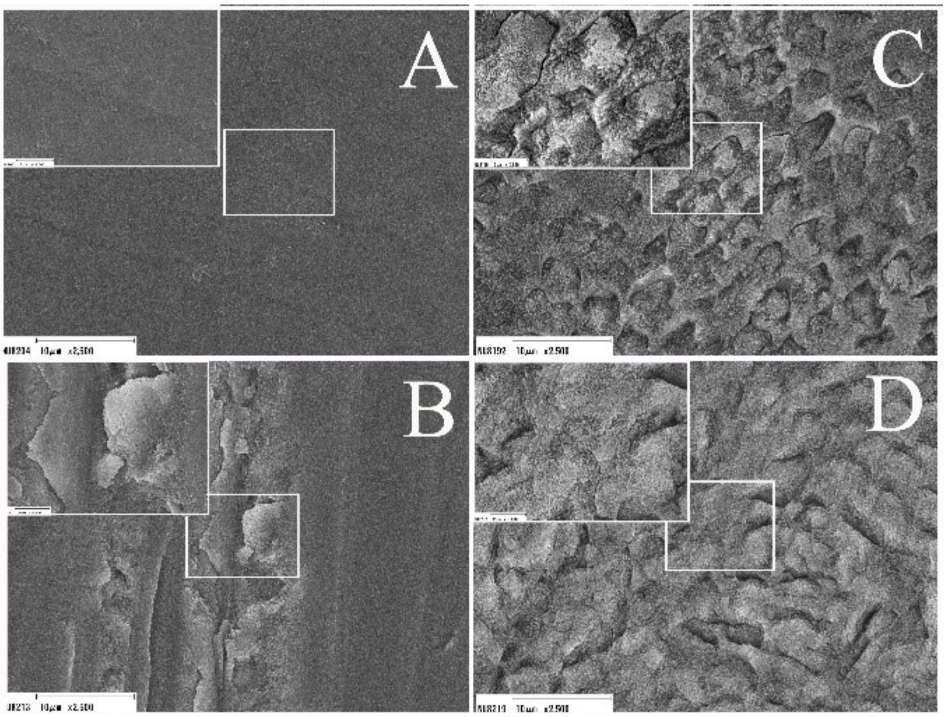

Fig. 3: Representative SEM images of treated enamel surfaces: Scotchbond Universal (SBU). (A) Unground enamel in self-etch mode (Behind image, ×2,500 and Front image, ×10,000). (B) Ground enamel in self-etch mode (Behind image, ×2,500 and Front image, ×10,000). (C) Unground enamel in etch-&-rinse mode (Behind image, ×2,500 and Front image, ×10,000). (D) Ground enamel in etch-&-rinse mode (Behind image, ×2,500 and Front image, ×10,000).

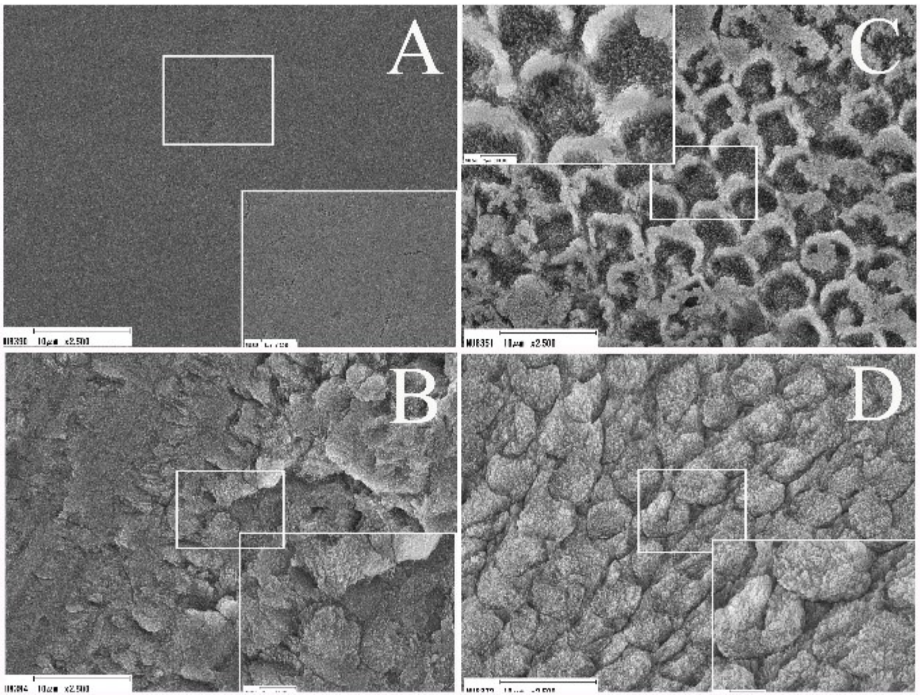

Fig. 4: Representative SEM images of treated enamel surfaces: Clearfil SE Bond (CSE). (A) Unground enamel in self-etch mode (Behind image, ×2,500 and Front image, ×10,000). (B) Ground enamel in self-etch mode (Behind image, ×2,500 and Front image, ×10,000). (C) Unground enamel in etch-&-rinse mode (Behind image, ×2,500 and Front image, ×10,000). (D) Ground enamel in etch-&-rinse mode (Behind image, ×2,500 and Front image, ×10,000).

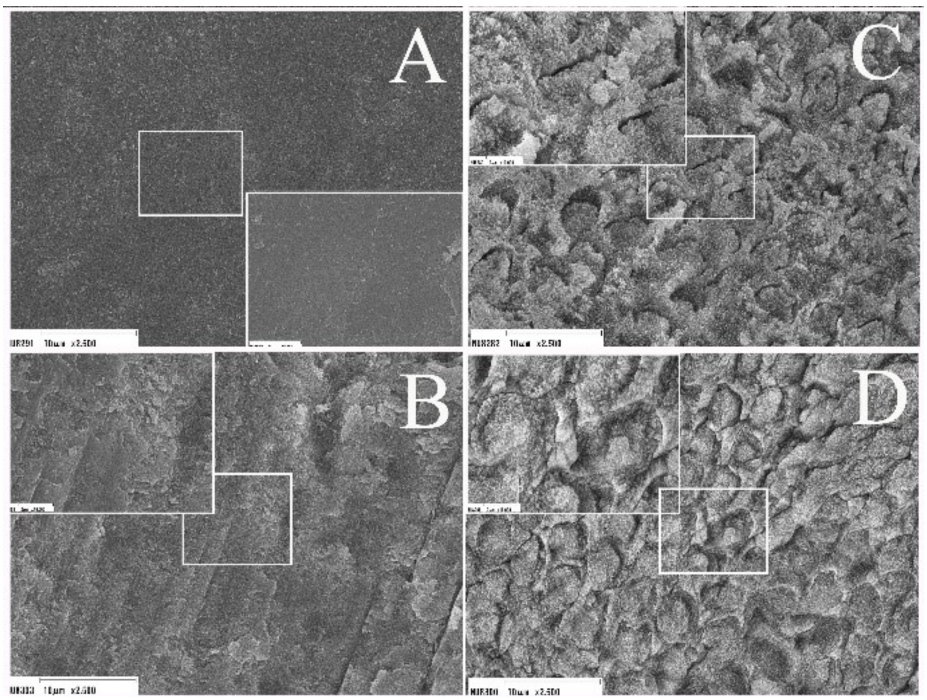

Fig. 5: Representative SEM images of treated enamel surfaces: Xeno JP (XJP). (A) Unground enamel in self-etch mode (Behind image, ×2,500 and Front image, ×10,000). (B) Ground enamel in self-etch mode (Behind image, ×2,500 and Front image, x10,000). (C) Unground enamel in etch-&-rinse mode (Behind image, ×2,500 and Front image, ×10,000). (D) Ground enamel in etch-&-rinse mode (Behind image, ×2,500 and Front image, ×10,000).

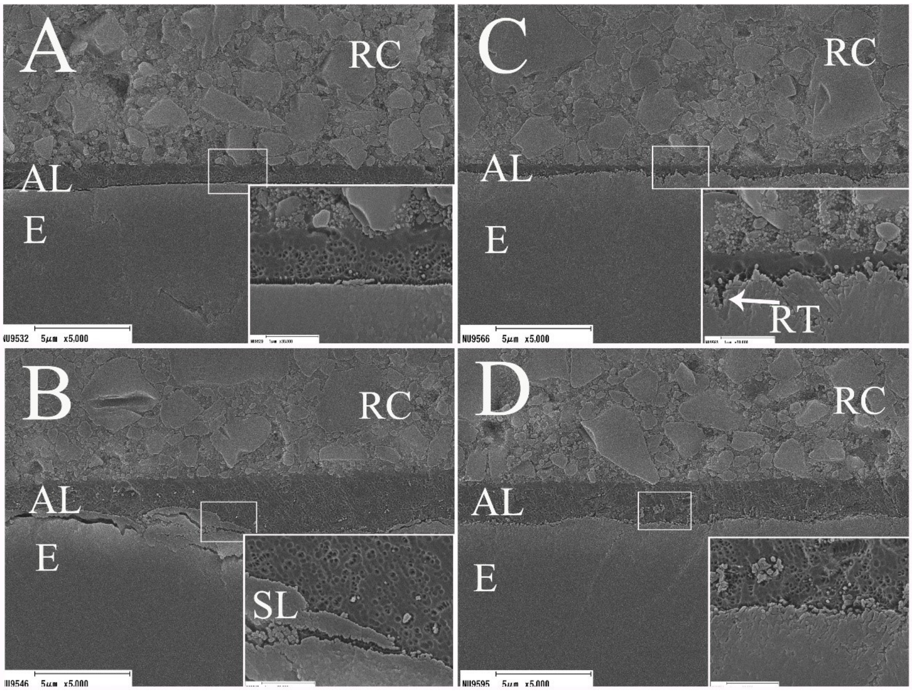

Fig. 6: Representative SEM images of the resin–enamel interfaces of Prime & Bond Universal (PBU). Arrow indicates RT. The visible material is indicated by the following abbreviations: AL, adhesive layer; E, enamel; RC, resin composite; RT, resin tag; SL, smear layer. (A) Unground enamel in self-etch mode (×5,000 and ×30,000). (B) Ground enamel in self-etch mode (×5,000 and ×30,000). (C) Unground enamel in etch-&-rinse mode (×5,000 and ×30,000). (D) Ground enamel in etch-&-rinse mode (×5,000 and ×30,000).

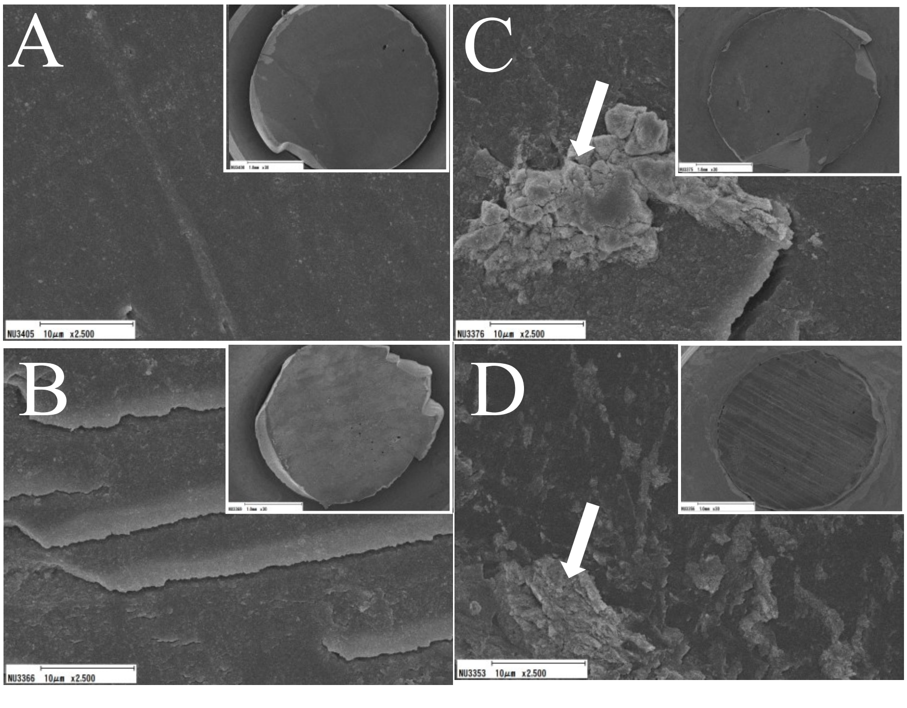

Fig. 7: Representative SEM images of debonded specimens of CUQ after shear bond strength test. White arrows indicate attached enamel fragments. (A) Unground enamel in self-etch mode (×30 and ×2,500). (B) Ground enamel in self-etch mode (×30 and ×2,500). (C) Unground enamel in etch-&-rinse mode (×30 and ×2,500). (D) Ground enamel in etch-&-rinse mode (×30 and ×2,500).

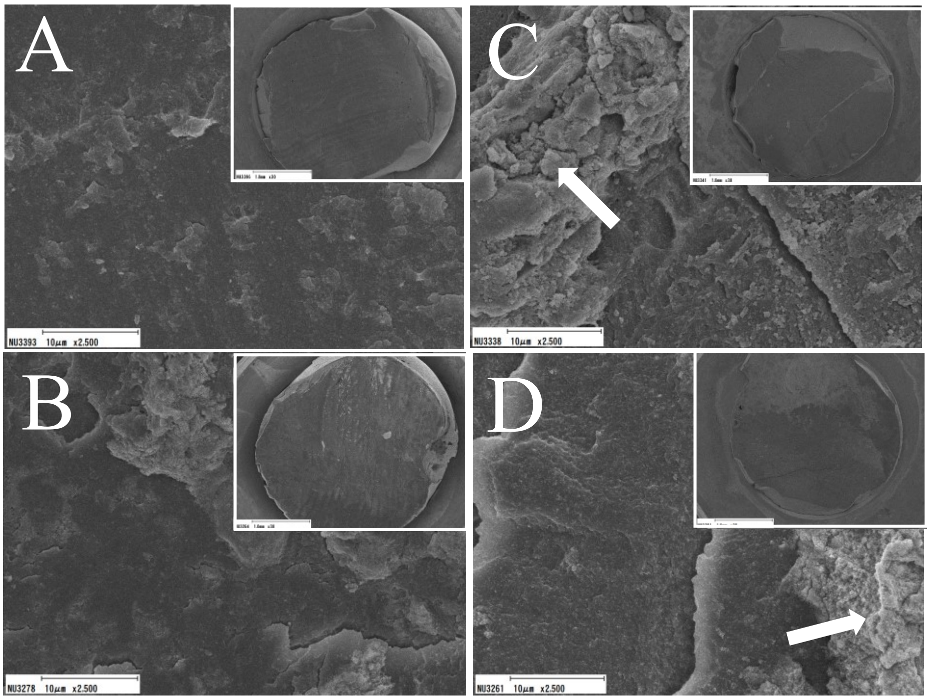

Fig. 8: Representative SEM images of debonded specimens of CSE after shear bond strength test. White arrows indicate attached enamel fragments. (A) Unground enamel in self-etch mode (×30 and ×2,500). (B) Ground enamel in self-etch mode (×30 and ×2,500). (C) Unground enamel in etch-&-rinse mode (×30 and ×2,500). (D) Ground enamel in etch-&-rinse mode (×30 and ×2,500).