

**Enamel bond durability of universal adhesives
in self-etch mode under different degradation conditions**

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This thesis was structured on the basis of the published article listed below with the additional data of adhesive treated SEM images.

Suzuki S, Takamizawa T, Imai A, Tsujimoto A, Sai K, Takimoto M, Barkmeier WW, Latta MA, Miyazaki M (2018) Bond durability of universal adhesive to bovine enamel using self-etch mode. Clin Oral Investig 22, 1113-1122.

Summary

Improvements in resin-based materials and adhesive technologies over the years have contributed to the ongoing development in the field of adhesive dentistry. However, biological and biomechanical degradations invariably result in such restorations deteriorating over time. It is therefore clinically important to investigate the degradation mechanism of these restorations in intraoral environmental situations. Long-term clinical trials of resin composite restorations might provide information about the bonding performance of the adhesive materials used. However, clinical trials are thought to be time consuming, expensive and difficult to achieve without significant biases. Long-term water storage (WS) and thermal cycling (TC) tests combined with bond strength tests have been common methods for testing the *in vitro* degradation of restored teeth, because the results before and after the degradation processes can be standardized and easily compared to previous reports. Although no specific correspondence has been found between intraoral period of time and WS period or the number of TC used, it is possible that the results from these tests might provide information on the long-term bonding durability of resin composite restorations.

The most recent advancement in the adhesive technology is the introduction of the universal adhesives, which is distinguished by both their suitability for different types of adherent substrates, and for uses in etch-&-rinse, selective-etch and self-etch modes with mineralized tooth tissue. Several studies have investigated the influence of biomechanical factors on the bonding effectiveness of different type of self-etch adhesives through shear fatigue testing. However, from a biodegradation perspective, there is limited information on the durability of the enamel bond strengths of universal adhesives. The purpose of this study was to determine the

enamel bond durability of universal adhesives in the self-etch mode using different simulated degradation methods.

Three commercially available universal adhesives and a gold standard two-step self-etch adhesive were used. Bond specimen preparation was performed according to the ISO 29022 specification. Bovine incisors were used as the substitutes for human teeth. The bonding enamel sites were wet ground using a sequence of SiC papers ending with #320-grit. For each prepared surface, the adhesives were applied in accordance with their manufacturer's instructions. Following the adhesive procedures, the specimens were secured by the Ultradent Bonding Jig and a plastic mold (2.4 mm in diameter, 2.0 mm in height) was set in place. A resin composite was placed into the mold-enclosed assembly on the enamel surfaces, and then light irradiated for 30 s. Ten specimens of each test group were prepared, and shear bond strength (SBS) was measured to determine the bonding durability after TC or WS. The bonded specimens were divided into three groups: 1) specimens subjected to TC, where the bonded specimens were stored in 37°C distilled water for 24 h before being subjected to 3,000, 10,000, 20,000 or 30,000 TC; 2) specimens stored in 37°C distilled water for 3-month, 6-month, 1-year or 2-year; and 3) specimens stored in 37°C distilled water for 24 h, serving as a baseline. The bonded specimens were loaded to failure at 1.0 mm/min using a universal testing machine. The SBS values were calculated from the peak load at failure divided by the bonded surface area.

Representative treated enamel surfaces with universal adhesives, restorative–enamel interfaces, and the de-bonded failure sites were observed by scanning electron microscopy (SEM). For the observation of treated enamel surfaces, they were treated in accordance with the experimental protocol, and then rinsed with acetone and water. Prior to the SEM observations of the restorative–enamel interfaces, the bonded specimens were embedded in epoxy resin and then

longitudinally sectioned. The sectioned surfaces were then polished to a high gloss with abrasive discs followed by diamond paste. The fracture sites from each storage condition were prepared directly for SEM observations. All of the SEM specimens were dehydrated with *tert*-butyl alcohol and then transferred to a critical-point dryer. The restorative–enamel interfaces were then subjected to argon-ion beam etching. All of the SEM specimens were coated with a thin film of Au. Observations were performed under operating voltage of 10 kV.

By defining the baseline enamel SBS values for each tested adhesive as being 100%, the SBS values ranged from 97.5 to 126.6% after TC. Although some universal adhesives did not show any significant differences, all of the adhesives showed higher SBS values in the early TC (for the 3,000 and 10,000 TC) than the baseline group, and the SBS values tended to decrease as the number of TC increased.

By defining the baseline enamel SBS value as being 100% for each of the tested adhesives, the SBS values ranged from 104.3% to 130.8% after WS. The two-step self-etch adhesive showed significantly higher SBS value ($p < 0.05$) than the other universal adhesives over all of the WS periods, and there was no significant difference in enamel SBS values among any WS periods. In contrast, the universal adhesives tested showed significantly higher SBS values at 3-month WS compared to those values of the baseline. In addition, the universal adhesives were found to have stable SBS values, during any WS periods.

In SEM images of treated enamel surfaces of different adhesives, although all the universal adhesives had similar morphological appearances with remaining scratch marks by grinding with SiC papers, demineralization status were different. When arranged in the order as demineralization status, $SE \cong GP > SU > AB$. In SEM images of resin–enamel interfaces, the thicknesses of the adhesive layer were materials dependent, and the two-step self-etch adhesive

formed thicker adhesive layer than the universal adhesives (approximately 40 μm). After the SBS test, at lower magnifications, all of the groups showed adhesive failures at the resin–enamel interface; however, at higher magnifications, cracks, cleavages, and cohesive failures could be observed more clearly in the enamel. These features were particularly apparent after long term degradation of TC and WS, regardless of the type of adhesive used.

This laboratory study showed that the degradation method, type of adhesive and degradation period significantly influence enamel SBS. The two-step self-etch adhesive showed significantly higher SBS than the universal adhesives tested, regardless of the type of degradation method. All universal adhesives showed no significant enamel SBS reductions in TC and WS, when compared to baseline and the other degradation conditions. Compared to the bond strengths obtained with the two-step self-etch adhesive, significantly lower bond strengths were obtained with universal adhesives. However, the enamel bond durability of universal adhesives was relatively stable under both degradation conditions tested. These results suggested that there ought to be no concerns about the durability of enamel bonds formed by universal adhesives in the self-etch mode. When considering degradation testing, it might be necessary for the degradation periods to be extended in some situations.

Introduction

Improvements in resin-based materials and adhesive technologies over the years have contributed to the ongoing development in the field of adhesive dentistry (1,2). Direct restorations have benefited from the use of resin composites, and the aesthetically pleasing results, simple treatment procedures and preservation of tooth structures have led them to be widely used technique (3). However, biological and biomechanical degradations invariably result in such restorations deteriorating over time (4–8). It is therefore clinically important to understand the degradation over time of these restorations in intraoral environmental situations.

Although long-term clinical trials of resin composite restorations might provide information about the bonding performance of the adhesive materials used, such trials are thought to be time consuming, expensive, and difficult to achieve without significant biases (9,10). Although it is difficult for all aspects of an intraoral conditions to be simulated simultaneously, *in vitro* simulated testing is thought to be an effective way of acquiring results (9,11). In addition, simulated oral condition tests can be designed so that the mechanisms governing specific aspects of the degradation processes happening in clinical situations can be precisely investigated (9); several simulation tests have been developed in order for certain degradation processes to be clarified, such as those of biofilm attack, hydrolytic degradation, enzymatic degradation by matrix metalloproteinases, thermal shock and fatigue stress (4–8,12).

Long-term water storage (WS) and thermal cycling (TC) tests combined with bond strength tests have been common methods for testing the *in vitro* degradation of restored teeth, because the results before and after the degradation processes can be standardized and easily compared to previous reports (9). The degradation mechanism that occurs near an adhesive during WS tests is thought to be mainly related to the hydrolytic degradation of the resinous

materials (11–14). TC tests, however, can accelerate degradation near the adhesive layers due to thermal stress; this is because of the discrepancies between the thermal expansion rates of the substrates, and the hydrolytic degradation caused by the water (9,12,15). Although no specific correspondence has been found between an intraoral period and a WS period or TC cycles used, it is possible that the results from these tests would help prediction of the long-term bonding durability of resin composite restorations *in vivo*.

The most recent advancement in the adhesive technology is the introduction of the universal adhesives. They are designed to be suitable for different types of adherent substrates, and for uses in etch-&-rinse, selective-etch and self-etch modes with mineralized tooth tissue (16–21). The versatilities of these adhesives allow clinicians to tailor clinical approaches to the prevailing cavity conditions; variables such as size, depth, location and proportion of the enamel and/or dentin, can be considered and optimized for. Several studies have investigated the influence of biomechanical factors on the bonding effectiveness of different type of self-etch adhesives through shear fatigue testing (7,8). Some of the reports claimed that the universal adhesives in the etch-&-rinse mode exhibited higher enamel bonding performances without affecting dentin bonding (18,19). However, from a biodegradation perspective, there is limited information on the enamel bonding durability of universal adhesives.

The purpose of this study was to determine the enamel bond durability of universal adhesives in the self-etch mode using different simulated degradation conditions. The null hypothesis to be tested was that different degradation processes would not be influenced by the type of adhesives.

Materials and Methods

1. Materials used

Three universal adhesives used were; Scotchbond Universal (SU; 3M Oral Care, St. Paul, MN, USA), G-Premio Bond (GP; GC, Tokyo, Japan), and All Bond Universal (AB; Bisco, Schaumburg, IL, USA). A conventional two-step self-etch adhesive, Clearfil SE Bond (SE; Kuraray Noritake Dental, Tokyo, Japan), was used for comparison. A resin composite, Clearfil AP-X (Kuraray Noritake Dental), was used for the bonding to enamel. The lot numbers and compositions of the materials used are listed in Table 1.

2. Specimen preparation

Specimen preparation was performed according to the ISO 29022 (22). Mandibular bovine incisors extracted from cattle were used as the substitutes for human teeth, and they were stored in frozen before being used. The root of each tooth was removed at the cemento–enamel junction (CEJ) using a low-speed saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). Pulpal tissue was removed, and then the labial surfaces were ground wet with 240-grit silicon carbide (SiC) paper (Fuji Star Type DDC, Sankyo Rikagaku, Saitama, Japan) in order to create flat enamel surfaces. Each tooth was then mounted in a self-curing acrylic resin (Tray Resin II, Shofu, Kyoto, Japan) so that the flattened enamel area could be exposed. The bonding enamel sites were wet ground using a sequence of SiC papers ending with 320-grit (Fuji Star Type DDC) and then the surfaces were dried with oil-free compressed air.

3. Storage condition and shear bond strength tests

The instruction of the bonding procedures according to each manufacture are described in Table 2. Ten specimens were used in each test group to determine their shear bond strength (SBS) to enamel without the use of phosphoric acid pre-etching (i.e. the self-etch mode). For

each prepared surface, the adhesives were applied in accordance with their manufacturer's instructions. Following the adhesive procedures, the specimens were secured by the Ultradent Bonding Jig (Ultradent Products, South Jordan, UT, USA) and a plastic mold (2.4 mm in diameter, 2.0 mm in height) was set in place. A condenser was used to insert a resin composite into the mold-enclosed assembly on the enamel surfaces, and then light irradiated for 30 s by a visible-light curing unit (Optilux 501, sds Kerr, Danbury, CT, USA).

The bonded assemblies were then subjected either to thermal cycling (TC group) or storage in distilled water at 37°C for long periods of time (WS group). For the TC groups, the bonded specimens were stored in distilled water at 37°C for 24 h before being treated with 3,000, 10,000, 20,000 or 30,000 TC between 5 and 55°C with dwell times of 30 s. The bonded specimens of the WS groups were stored in distilled water at 37°C for 3-month, 6-month, 1-year or 2-year before the SBS tests. The storage water, which did not contain antibiotics, was changed every week during the course of the experiment. Baseline specimens were stored in distilled water at 37°C for 24 h before the SBS tests (baseline group).

The SBS was measured using an Ultradent Bonding Assembly (Ultradent Products), as described by ISO 29022 (22). The bonded specimens were loaded to failure at 1.0 mm/min using a 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 bonded tooth surfaces and resin composite cylinders were observed through an optical microscope (SZH-131, Olympus, Tokyo, Japan) at a magnification of 10× to determine the failure mode. Based on the percentage of the substrate area observed on the debonded cylinders and bonded tooth sites, the types of bond failure were recorded as being either

1) adhesive failure, 2) cohesive failure in the composite, 3) cohesive failure in the enamel or 4) mixed failure—partially adhesive and partially cohesive.

4. Scanning electron microscopy (SEM) observations

Representative treated enamel surfaces with universal adhesives, restorative–enamel interfaces, and the de-bonded failure sites were observed by field-emission SEM (ERA-8800FE, Elionix, Tokyo, Japan). For the observation of treated enamel surfaces, they were treated in accordance with the standard experimental protocol, and then rinsed with acetone and water. Prior to the ultrastructure observations of the restorative–enamel interfaces, the bonded specimens (stored in 37°C distilled water for 24 h) were embedded in an epoxy resin (Epon 812, Nisshin EM, Tokyo, Japan) and then longitudinally sectioned by the low-speed saw (Isomet 1000, Buehler). The sectioned surfaces were then polished to a high gloss with abrasive discs (Fuji Star Type DDC) followed by diamond paste having 0.25 μm particles (DP-Paste, Struers, Ballerup, Denmark). The fracture sites from each storage condition were prepared directly for SEM. All of the SEM 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 to a critical-point dryer (Model ID-3, Elionix) for 30 min. The restorative–enamel interfaces 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/cm²) directed perpendicular to the polished surfaces. Finally, all of the SEM specimens were coated with a thin film of Au (Quick Coater, Type SC-701, Sanyu Electron, Tokyo, Japan). Observations were performed under operating voltage of 10 kV.

5. Statistical analysis

Two-way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test (α level of 0.05) were used to analyse the SBS data. Two factors were considered; the degradation period (the number of TC or the WS period) and the adhesive system used.

According to a report by Gale *et al.* (23), approximately 10,000 TC were thought to be equivalent to 1-year in intraoral conditions. Therefore, in order to determine whether the influence of the type of degradation methods used interacted with the influence of the degradation period and adhesive system, three-way ANOVAs were also performed on all of the SBS data; the factors of it included the degradation method, the degradation period ((a): 3,000 TC/3-month WS, (b): 10,000 TC/1-year WS, and (c): 20,000 TC/2-year WS), and the adhesive system. The statistical analysis was performed using statistical software (Sigma Plot ver. 11.0; SPSS, Chicago, IL, USA).

Results

Influence of TC

The results of the enamel SBS under TC conditions are shown in Table 3. Two-way ANOVA revealed that the number of TC and the adhesive system significantly influenced the enamel SBS values ($p < 0.001$), and the interaction between the two factors was significant ($p < 0.05$).

For the baseline group, although the two-step self-etch adhesive SE showed a significantly higher SBS value ($p < 0.05$) than the other universal adhesives, no significant differences were found among the universal adhesives. Regarding the results in the TC groups, although some universal adhesives did not show any significant differences, all of the adhesives showed higher

SBS values in the early TC cycles (for the 3,000 and 10,000 TC) than the baseline group, and the SBS values tended to decrease as the number of TC increased. By defining the baseline enamel SBS values for each tested adhesive as being 100%, the SBS values ranged from 97.5 to 126.6% (Table 3).

Influence of WS

The results of the enamel SBS under WS conditions are shown in Table 4. Two-way ANOVA revealed that the WS period and adhesive system used significantly influenced the enamel SBS values ($p < 0.001$), but the interaction between the two factors was not significant ($p = 0.076$).

In the WS groups, SE showed significantly higher SBS value ($p < 0.05$) than the other adhesives over all of the WS periods, and there were no significant differences in enamel SBS values among any WS periods. In contrast, the universal adhesives tested showed significantly higher SBS values at 3-month WS compared to those values of the baseline. In addition, the universal adhesives were found to have stable SBS values, during any WS periods. By defining the baseline enamel SBS value as being 100% for each of the tested adhesives, the SBS values ranged from 104.3% to 130.8% (Table 4).

Three-way ANOVA

Three-way ANOVA for the enamel SBS values was performed to determine whether the degradation methods used interacted with the influence of the degradation periods and adhesive systems (Table 5). All three factors significantly influenced the SBS values ($p < 0.05$), but none of the interactions were significant ($p > 0.05$) apart from the interaction between the degradation methods and the adhesive systems ($p < 0.001$). The relationship between the TC and WS degradation methods on enamel SBS is shown in Fig. 1. When the baseline enamel SBS value

was defined as being 100% for each tested adhesive, 10,000 TC were assumed to be equivalent to WS period of 1-year. All the adhesives tested showed enamel SBS percentages above the baseline percentages during the degradation periods, for both TC and WS conditions; however, the trend in the changing over time depended on both the adhesive and degradation methods. In general, the enamel SBS values under TC were much more likely to vary than under the WS condition (Fig. 1).

Failure mode analysis

The frequencies of the different failure modes after the SBS tests are shown in Figs. 2 and 3. For all of the groups, the predominant failure mode was adhesive failure, regardless of the type of degradation conditions. In particular, all of the de-bonded specimens exhibited adhesive failure for the groups of 1- and 2-year WS.

SEM observations

SEM images of representative treated enamel surfaces of different adhesives are shown in Fig. 4. Although all the universal adhesive conditions tended to show similar morphological appearances with remaining scratch marks by grinding with SiC papers, demineralization status were different. SEM images of SE showed that the smear layer was completely removed, but clear etching pattern was not observed. When arranged in the order as demineralization status, $SE \cong GP > SU > AB$.

Representative SEM images of resin–enamel interfaces are shown in Fig. 5. The thicknesses of the adhesive layer were materials dependent, and SE formed thicker adhesive layer than the universal adhesives (approximately 40 μm in Fig. 5D). Conversely, SU and GP formed thin adhesive layers of approximately 10 μm (Figs. 5A and 5B), whereas AB formed layer of 4–5 μm (Fig. 5C). Although adhesive layer of AB was observed to be homogeneous (Fig.

5C), the adhesive layers of SU, GP, and SE were all found to contain nano-sized fillers (Figs. 5A, 5B and 5D).

Representative SEM images of the resin side of the de-bonded specimens after the SBS test are shown in Fig. 6. After the SBS test, at lower magnifications, all of the groups showed adhesive failures at the resin–enamel interface; however, at higher magnifications, cracks, cleavages, and cohesive failures were observed more clearly in the enamel. These features were particularly apparent after long term degradation of TC and WS, regardless of the type of adhesive observed.

Discussion

In this study, the changes in enamel SBS under the different degradation conditions were investigated using three universal adhesives and a two-step self-etch adhesive. The statistical analysis assumed that 10,000 TC was equivalent to 1-year of WS, and it was revealed that the degradation method, type of adhesive used, and degradation period significantly influenced the SBS values (Table 5). Therefore, the null hypothesis that the influence of the different degradation methods would not depend on the type of adhesive used was rejected. The two-step self-etch adhesive was found to be shown significantly higher SBS values than all the universal adhesives tested, regardless of the degradation methods and degradation periods as reported in previous studies (7,8).

To make the trends in bond strength clearer, the 24 h SBS value was defined as 100% for each adhesive tested, and the percentages at different degradation periods were determined. When observing the changes in SBS rates, SE showed similar trends under different degradation conditions, in that the rates increased early in the degradation processes before decreasing

afterwards. However, rates of the universal adhesives were found to show different trends over time. This disparity might be due to the complex compositions and different bonding procedures of these adhesives (24,25). Moreover, the compositions of the universal adhesives were highly diverse; for example, they varied in whether containing 2-hydroxyethyl methacrylate (HEMA) and inorganic nano-fillers. Other things pointed out were pH values, the types of solvent, amount of water contents, and resin monomers used. All these differences influenced the characteristics of the adhesive layers, leading to different trends in enamel SBSs.

With regards to the influence of the degradation methods on the enamel SBS values, the values were not found to be significantly lower than the baseline values for the adhesives in either the TC or WS conditions during all the test time periods. All the adhesives showed less changing in SBS values for WS than for TC. Unlike the mechanisms of dentin bond degradation, enamel bond degradation in WS is thought to mainly rely on the hydrolysis of resinous components, which absorb water over time (26–28). However, in the TC test, the main factor behind the deterioration of adhesive interfaces was thermal stress caused by discrepancies in the thermal expansion rates in the vicinity of the adhesive layer (9,15). Defects or cracks created by thermal stress have been found to induce percolation and the breakdown of poorly polymerized oligomers in an adhesive layer (15). The present study indicated that thermal stress might induce much greater damage in the vicinity of an adhesive layer than hydrolytic degradation. Therefore, although there was no clear difference between the HEMA-containing and HEMA-free universal adhesives in the WS condition, the enamel SBS values of the HEMA-containing adhesive was found to decrease more than the HEMA-free adhesive in the TC conditions.

The most interesting results obtained in this study was that the inter-relationship between the two common degradation methods investigated. By assuming that 10,000 TC are equivalent

to 1-year of WS (Fig. 1), it might be possible to consider how these degradation methods would relate to the longevity of bonds. Previous studies have indicated that the geometry, size and storage conditions of bonded specimens influence the degradation of the bonds (9,29). In addition, the time at which water is changed and the additives in the water might influence the degradation process in the WS condition (9,30). Early in the degradation process of WS (3,000 TC/3-month WS in the present study), the SBS values were found to be adhesive dependent; however, most of the adhesives exhibited similar trends, in that the SBS values increased above those of the baseline SBS values, this particular trend was remarkable in the universal adhesives, as described in previous studies (8,12). It can be speculated that the mechanical properties of the adhesive layer of the universal adhesives might improve after 24 h due to the post-curing effects within these adhesives, and the functional monomers might increase their chemical bonding to the tooth substrate (8,31).

For enamel bonds, although the changes over time were adhesive and degradation methods dependent, each adhesive was found to have had enamel SBS values that were higher than those of the baseline SBS values until 20,000 TC/2-year of WS. In addition, the morphological appearances of the failure sites by the SEM observations after both degradation methods showed no clear differences. Therefore, for the enamel bonds of the universal adhesives, it might be desirable for the degradation period of both TC and WS conditions to be extended beyond the point as described by ISO TR 11405 (32).

Conclusion

This laboratory study showed that the degradation method, type of adhesive and degradation period significantly influence enamel SBS. In all the degradation conditions, a two-

step self-etch adhesive showed significantly higher enamel SBS values than all the universal adhesives tested. The changes in time of the SBS values of the universal adhesives were both adhesive and degradation method dependent. However, the enamel bond disabilities of the universal adhesives were relatively stable under both the thermal cycling and water storage degradation conditions. These results suggested that there ought to be no concerns about the durability of enamel bonds formed by universal adhesives in the self-etch mode. When considering degradation testing, it might be necessary for the degradation periods to be extended in some situations.

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Table 1 Materials used in this study.

Code	Adhesive (Lot No.)	Main components	pH* mean (SD)	Manufacturer
SU	Scotchbond Universal (41256)	MDP, HEMA, imethacrylate resins, Vitrebond copolymer, filler, ethanol, water, initiators, silane	2.7 (0.03)	3M Oral Care St. Paul, MN, USA
GP	G-Premio Bond (1501221)	MDP, 4-MET, MEPS, BHT, acetone, dimethacrylate resins, initiators, water	1.5 (0.02)	GC. Tokyo, Japan
AB	All-Bond Universal (1300008503)	MDP, bis-GMA, HEMA, ethanol, water, initiators	3.2 (0.03)	Bisco. Schaumburg, IL, USA
SE	Clearfil SE Bond (011613)	Primer: MDP, HEMA, water, initiators Bond: MDP, HEMA, bis-GMA, initiators, microfiller	2.5 (0.02) (primer)	Kuraray Noritake Dental Tokyo, Japan
Resin composite				
	Clearfil AP-X (CC0043)	bis-GMA, TEGDMA, silanated barium glass filler, silanated silica filler, silanated colloidal silica catalysts, accelerators, CQ, pigments, others		Kuraray Noritake Dental
		Filler Load: 84.5 wt%		

MDP: 10-methacryloyloxydecyl dihydrogen phosphate, HEMA: 2-hydroxyethyl methacrylate,
4-MET: 4-methacryloxyethyl trimellitate, MEPS: methacryloyloxyalkyl thiophosphate, BHT: butylated
hydroxytoluene, bis-GMA: 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy) phenyl] propane, TEGDMA:
triethyleneglycol dimethacrylate, CQ: *dl*-camphorquinone

* pH measurement was conducted five times for each adhesive using a compact pH meter (LAQUAtwin-pH-33,
Horiba, Tokyo, Japan)

Table 2 Adhesive application protocol.

SU	Adhesive applied to air-dried enamel surface with rubbing action for 20 s and then medium air pressure applied to surface for 5 s. Light irradiated for 10 s.
GP	Adhesive applied to air-dried enamel surface for 10 s. Strong stream of air applied over the liquid adhesive for 5 s or until adhesive no longer moved and the solvent had completely evaporated. Light irradiated for 10 s.
AB	Adhesive applied to enamel surface with rubbing action for 10-15 s per coat. No light cure between coats. Gentle stream of air applied over the liquid for at least 10 s. Light irradiated for 10 s.
SE	Primer applied to air-dried enamel surface for 20 s. Medium air pressure applied to surface for 5 s. Adhesive applied to primed surface and then air thinned for 5 s. Light irradiated for 10 s.

Table 3 Influence of thermal cycling on enamel SBS (MPa (SD)).

	24 h	3,000 TC	10,000 TC	20,000 TC	30,000 TC
SU	27.1 (3.8) ^{bB} [100%]	32.4 (4.0) ^{bA} [119.6%]	34.3 (2.6) ^{bA} [126.6%]	30.2 (2.7) ^{bAB} [111.4%]	26.9 (1.2) ^{cB} [99.3%]
GP	30.2 (4.1) ^{bB} [100%]	35.0 (3.8) ^{bA} [115.9%]	33.3 (4.0) ^{bAB} [110.3%]	33.0 (1.5) ^{bAB} [109.3%]	33.9 (1.4) ^{bAB} [112.3%]
AB	28.3 (4.7) ^{bA} [100%]	28.4 (4.4) ^{cA} [100.4%]	30.8 (2.4) ^{bA} [108.8%]	30.1 (3.1) ^{bA} [106.4%]	27.6 (4.1) ^{cA} [97.5%]
SE	40.0 (3.1) ^{aB} [100%]	47.7 (3.0) ^{aA} [119.3%]	46.5 (1.6) ^{aA} [116.3%]	45.3 (1.9) ^{aA} [113.3%]	42.6 (1.6) ^{aB} [106.5%]

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.

[%] defined the 24-h SBS value as 100% for each tested adhesive and recorded the percentage at different degradation periods.

Table 4 Influence of long-term water storage on enamel SBS (MPa (SD)).

	24 h	3-month WS	6-month WS	1-year WS	2-year WS
SU	27.1 (3.8) ^{bb} [100%]	32.1 (2.7) ^{ca} [123.6%]	33.5 (3.9) ^{ca} [123.6%]	32.6 (2.4) ^{ca} [120.3%]	32.7 (2.1) ^{ba} [120.7%]
GP	30.2 (4.1) ^{bc} [100%]	39.5 (3.3) ^{ba} [130.8%]	37.5 (1.5) ^{baB} [124.2%]	36.7 (1.5) ^{baB} [121.5%]	35.0 (1.4) ^{bb} [115.9%]
AB	28.3 (4.7) ^{bb} [100%]	32.8 (1.1) ^{ca} [115.9%]	33.5 (1.4) ^{ca} [118.4%]	33.2 (1.3) ^{ca} [117.3%]	33.4 (1.5) ^{ba} [118.0%]
SE	40.0 (3.1) ^{aa} [100%]	43.3 (2.4) ^{aa} [108.3%]	43.6 (2.2) ^{aa} [109.0%]	42.7 (2.3) ^{aa} [106.8%]	41.7 (4.9) ^{aa} [104.3%]

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.

[%] defined the 24-h SBS value as 100% for each tested adhesive and recorded the percentage at different degradation periods.

Table 5 Three-way analysis of variance for the SBS tests.

Factors	Sum of squares	df	Mean square	<i>F</i> -ratio	<i>p</i>
Degradation method	55.4	1	55.4	6.7	0.010
Degradation period	94.5	2	47.2	5.7	0.004
Adhesives	6320.7	3	2106.9	254.8	<0.001
Methods × Period	4.9	2	2.47	0.3	0.742
Methods × Adhesives	611.6	3	203.8	24.6	<0.001
Period × Adhesives	103.4	6	17.2	2.1	0.056
Methods × Period × Adhesives	96.8	6	16.1	2.0	0.074

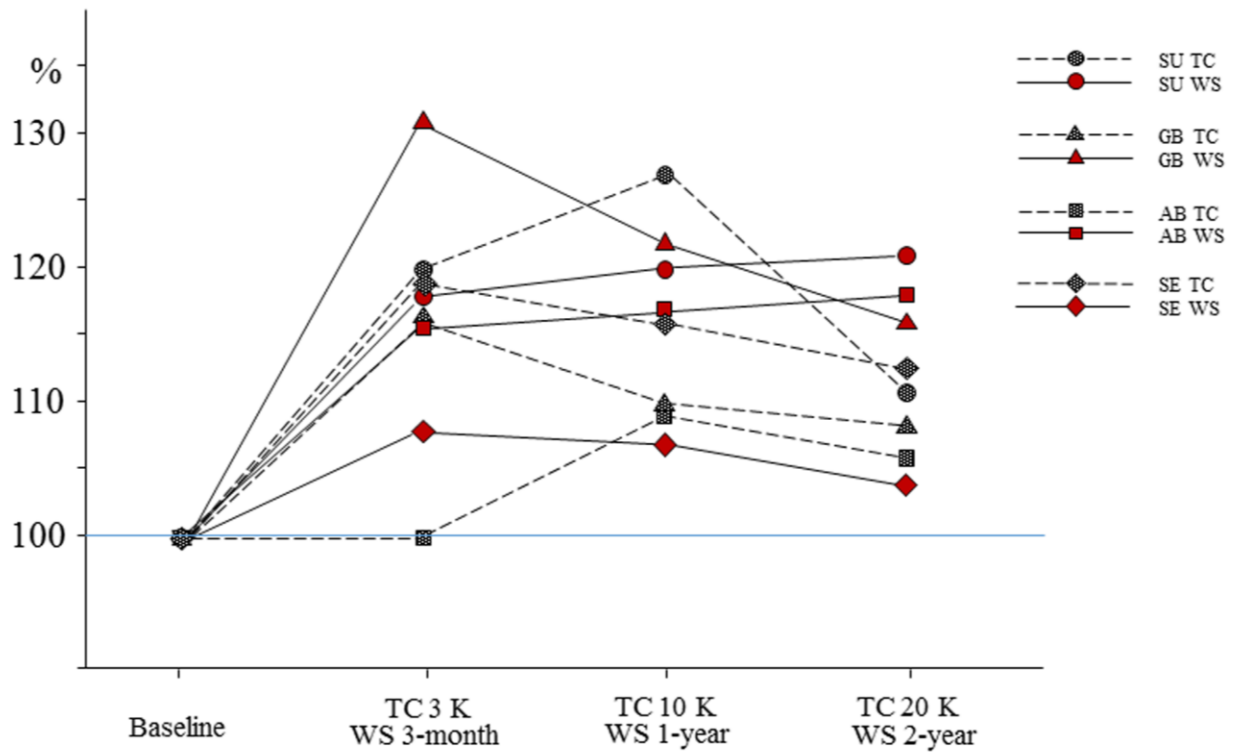


Fig. 1: Influence of the degradation method on enamel SBS values (where 10,000 TC is assumed to be equivalent to 1-year of WS).

SU TC: Scotchbond Universal with thermal cycling; SU WS: Scotchbond Universal with water storage; GP TC: G-Premio Bond with thermal cycling; GP WS: G-Premio Bond with water storage; AB TC: All Bond Universal with thermal cycling; AB WS: All Bond Universal with WS; SE TC: Clearfil SE Bond with thermal cycling; SE WS: Clearfil SE Bond with water storage.

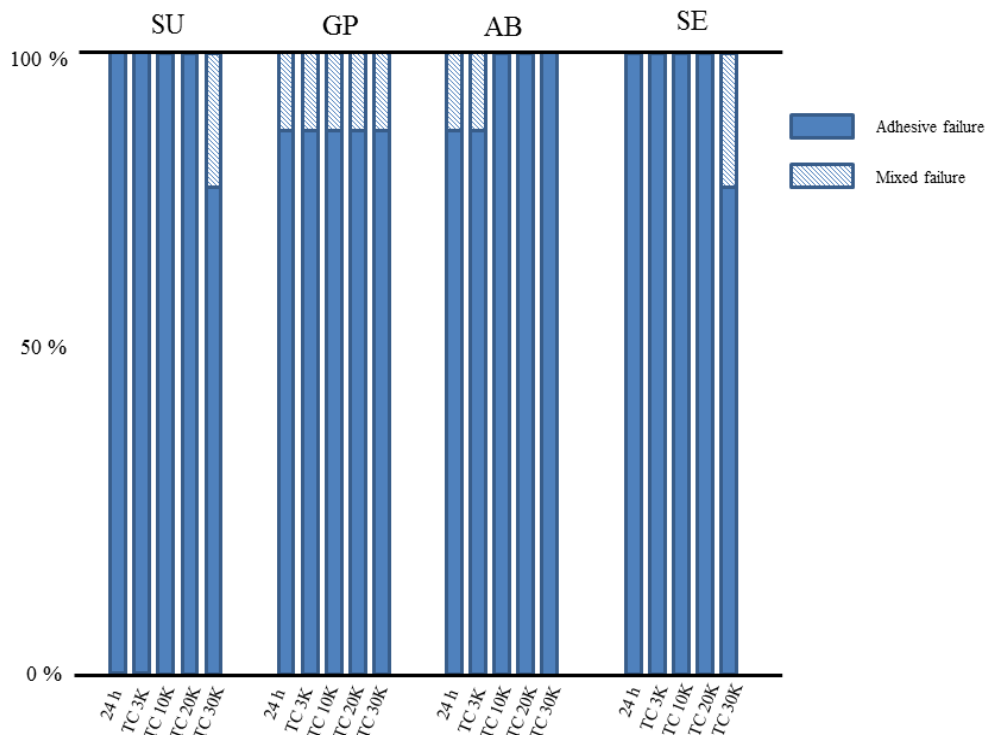


Fig. 2: Failure mode analysis of the de-bonded specimens under TC.

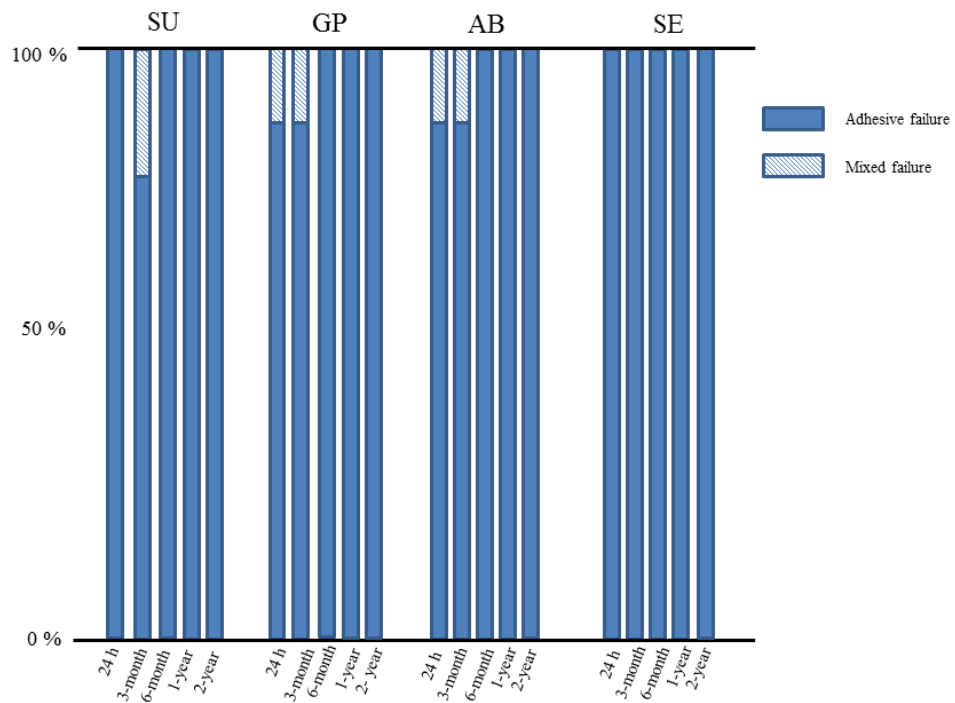


Fig. 3: Failure mode analysis of the de-bonded specimens under WS.

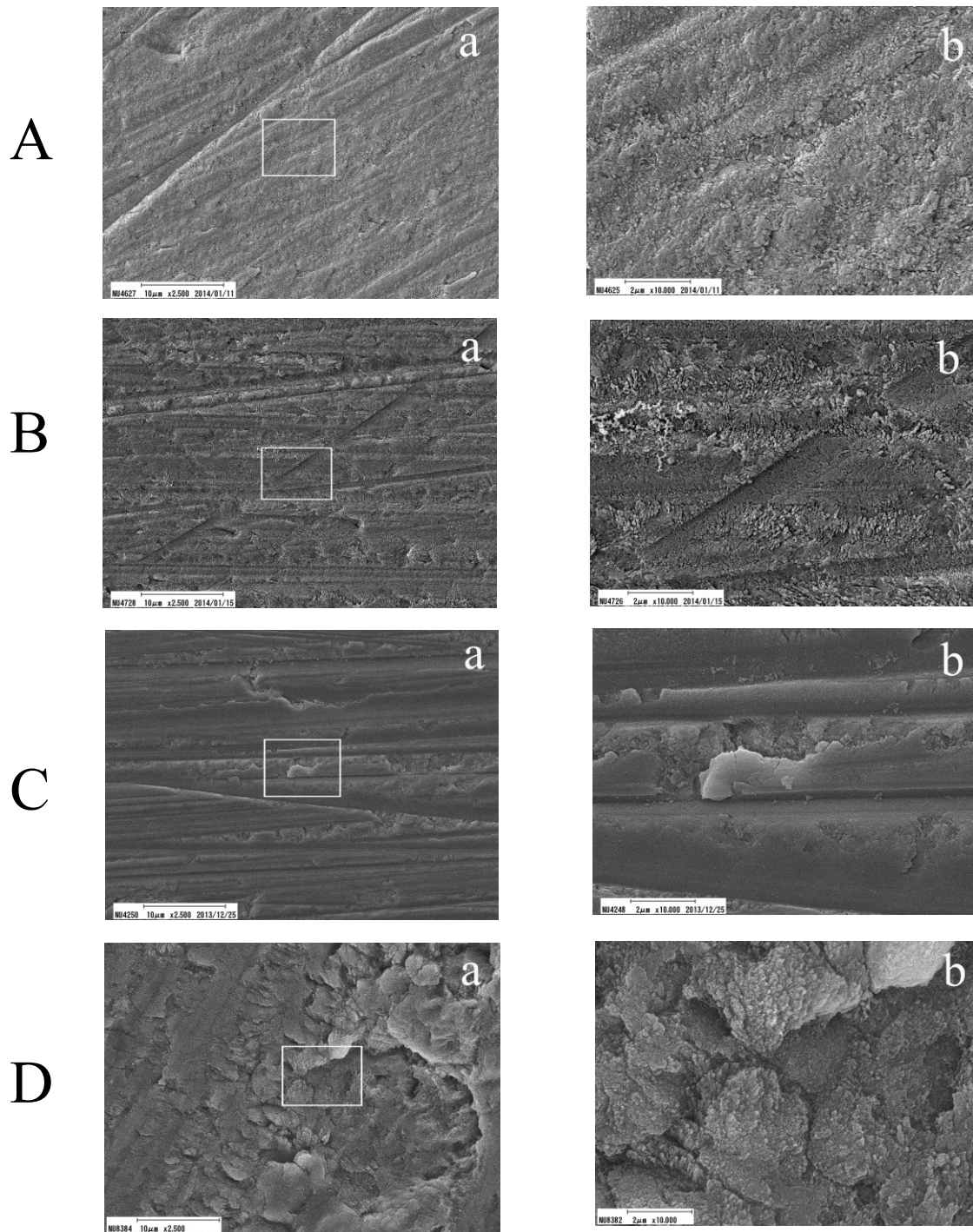


Fig. 4: Representative SEM micrograms of treated enamel surfaces.

- A: SU at magnifications of (a) 2,500 \times and (b) 10,000 \times .
- B: GP at magnifications of (a) 2,500 \times and (b) 10,000 \times .
- C: AB at magnifications of (a) 2,500 \times and (b) 10,000 \times .
- D: SE at magnifications of (a) 2,500 \times and (b) 10,000 \times .

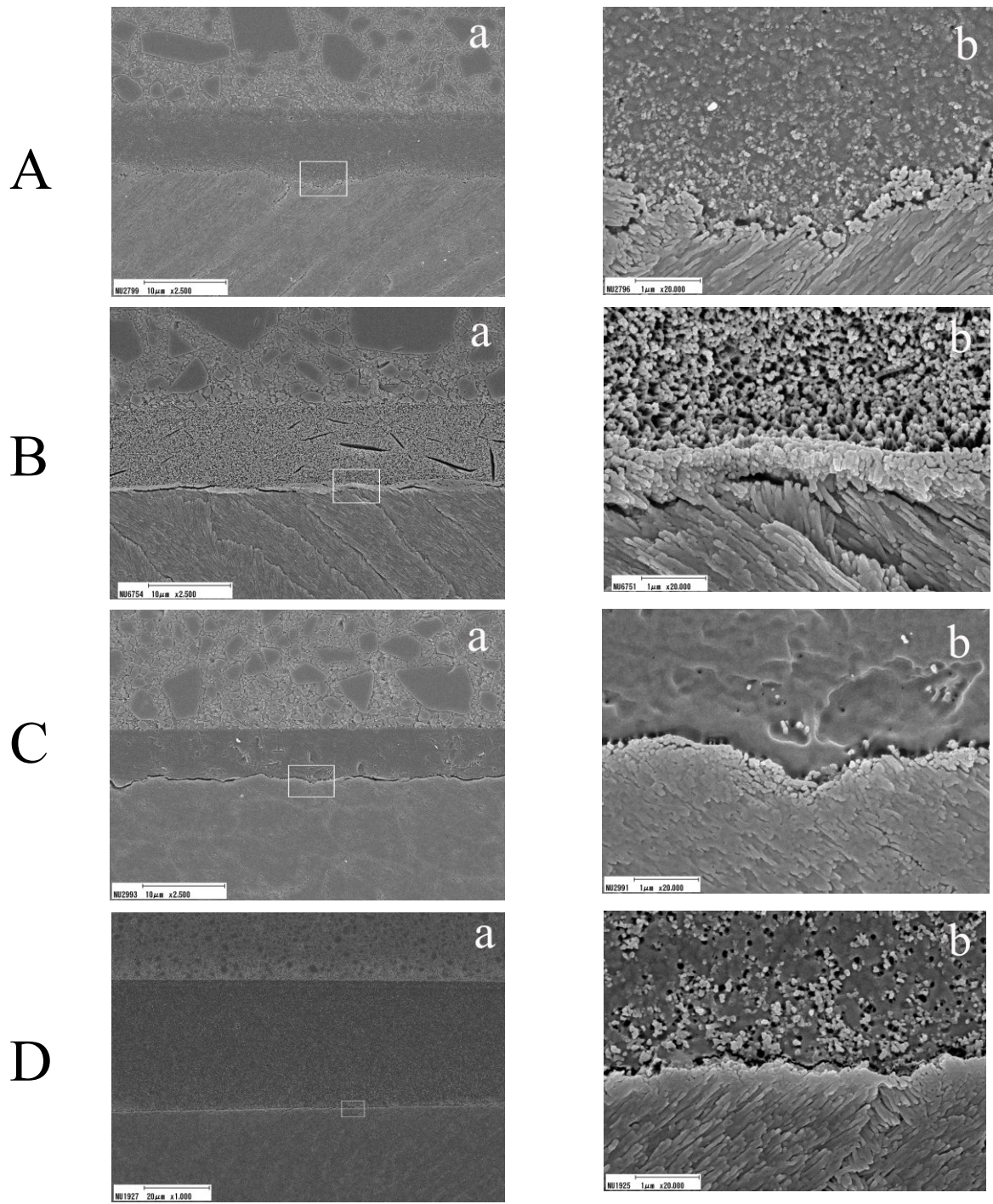


Fig. 5: Representative SEM micrograms of resin–enamel interfaces.

A: SU at magnifications of (a) 2,500× and (b) 20,000×.

B: GP at magnifications of (a) 2,500× and (b) 20,000×.

C: AB at magnifications of (a) 2,500× and (b) 20,000×.

D: SE at magnifications of (a) 1,000× and (b) 20,000×.

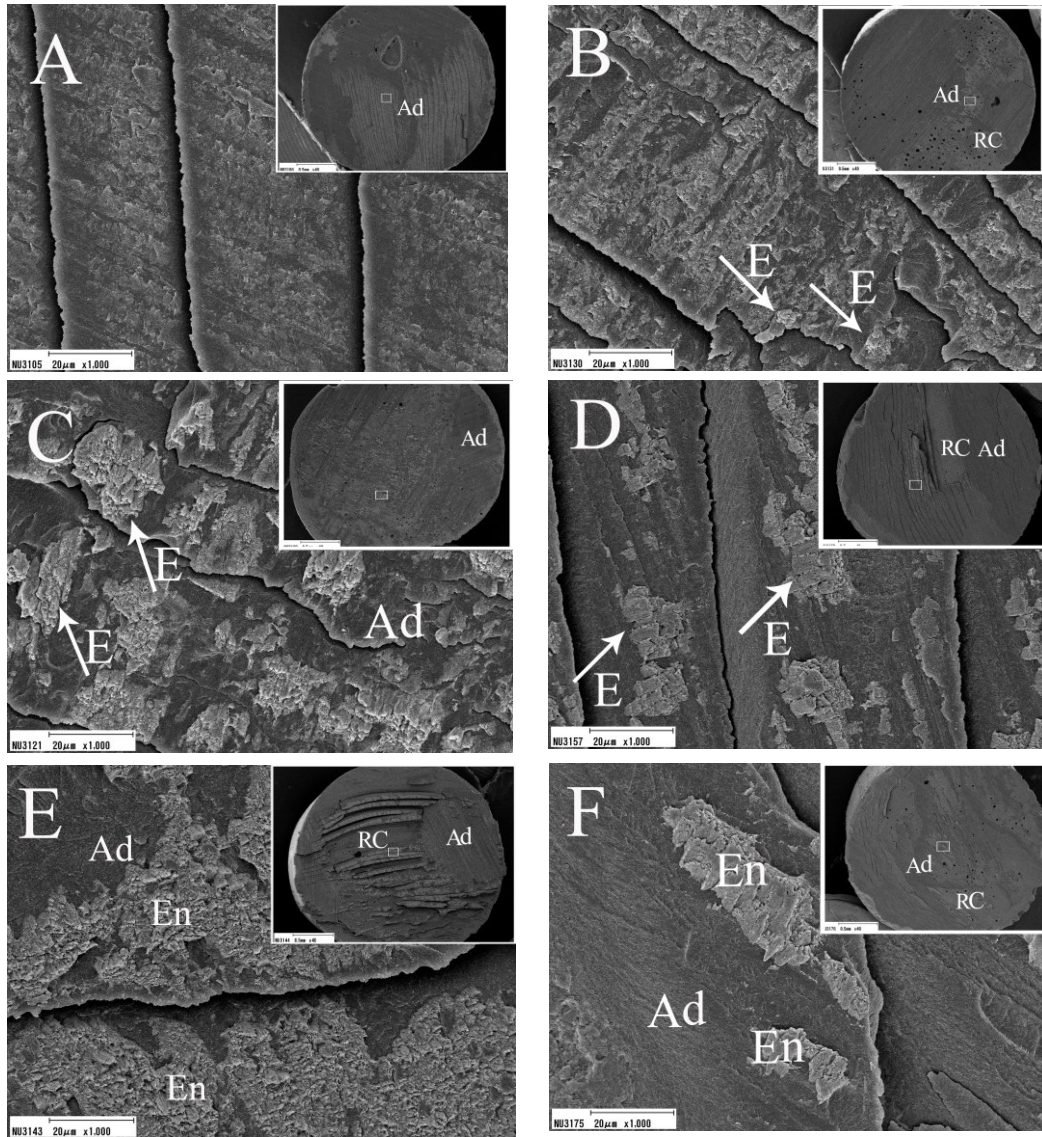


Fig. 6: Representative SEM micrographs of the de-bonded resin sides of bonds after the SBS testing of SU and SE.

- A: De-bonded failure site of SU for 24 h at magnifications of (a) 40× and (b) 1,000×.
- B: De-bonded failure site of SU for 30,000 TC at magnifications of (a) 40× and (b) 1,000×.
- C: De-bonded failure site of SU for 2-year WS at magnifications of (a) 40× and (b) 1,000×.
- D: De-bonded failure site of SE for 24 h at magnifications of (a) 40× and (b) 1,000×.
- E: De-bonded failure site of SE for 30,000 TC at magnifications of (a) 40× and (b) 1,000×.
- F: De-bonded failure site of SE for 2-year WS at magnifications of (a) 40× and (b) 1,000×.

The visible materials are indicated with the following abbreviations—Ad: adhesive, En: enamel, RC: resin composite. The arrows indicate the enamel.