Bond durability of a two-step adhesive with a universal-adhesive-derived primer in different etching modes under different degradation conditions

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Research Article

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Abstract

Objectives

This study investigated the enamel and dentin bond durability of a two-step adhesive system, using a universal adhesive-derived primer (G2-Bond Universal [GU]), and compared it with the two conventional, two-step, self-etch adhesive systems (Clearfil SE Bond 2 [CS] and OptiBond XTR [OX]) under thermal cycling (TC) and long-term water storage (WS).

Materials and Methods

The shear bond strengths to bovine enamel and dentin were determined using the etch-and-rinse and self-etch modes. Twelve specimens per test group were prepared and further divided into the following subgroups: 1) subjected to TC, 2) long-term WS, and 3) WS for 24 h (baseline). The cured adhesive layers' water contact angle and surface free energy were measured.

Results

Among the specimens in the baseline subgroups, the GU's enamel and dentin bond performances were equivalent to those of CS, regardless of the etching mode. Although GU's enamel bond durability was similar to that of CS, its dentin bond durability was superior to those of the other adhesive systems in both etching modes. GU's cured adhesive layer was more hydrophobic than those of the other adhesive systems.

Conclusion

The enamel and dentin bond durability of GU was superior to those of CS and OX in different etching modes and under different degradation conditions.

Clinical Relevance

The latest two-step adhesive system, which utilizes universal adhesives' benefits in its primer, might offer durable clinical bonding performances and can be widely used in a clinical setting.

Introduction

Universal adhesives are single-step, self-etch (SE) adhesive systems due to their composition and bonding process [1, 2]. They are widely used due to their multi-functional properties, versatility in clinical performance, and the reduced number of bonding steps involved [1, 2]. However, similar to the early single-step SE adhesive systems, universal adhesives' enamel bonding effectiveness in SE mode was lower than that in the etch-and-rinse (ER) mode due to their lower etching capability [3–5]. On the other hand, the dentin bond performance and universal adhesives' durability were similar in both the ER and SE modes [6–8]. Therefore, universal adhesives allow for different etching methods based on the clinical conditions and the configurations of the tooth cavity [3–8]. Also, recent studies have reported the achievement of acceptable bond effectiveness with some universal adhesives, despite reductions in the application time before light irradiation (quick bonding) [9–11].

Previous studies on universal adhesives' bonding performance showed an equal or greater bond performance in the enamel and dentin than those in the early versions of the single-step SE adhesive systems. However, they did not appear to reach the traditional two-step SE adhesive systems [6–8, 12]. The cured adhesive layer's thickness in most universal adhesives is generally less than 10 μm [13]. Also, they are hydrophilic due to water and solvents' presence in the adhesive layer. These properties may decrease resistance to mechanical stress and hydrolysis compared to the traditional two-step SE adhesive systems [14, 15]. Several approaches have improved universal adhesives' bonding effectiveness while maintaining their versatility and multi-functional properties [16–20]. Applying a hydrophobic bonding agent after universal adhesive application and a double-layer application technique have been reported to be effective in improving the bonding performance of universal adhesives [16–18]. These techniques could improve the cured adhesive layer's quality due to an increase in its thickness and improvements in its mechanical properties [19, 20]. Therefore, one of the developmental directions for the next generation of dental adhesive systems is using the benefits of universal adhesives and surpassing their shortcomings [21–24].

A new type of two-step SE adhesive system comprising a HEMA-free primer and a hydrophobic bonding agent has been developed using universal adhesive technology [24–26]. Unlike the traditional primer in three- or two-step adhesive systems, this new type of adhesive primer's components are similar to those of universal adhesives, containing 4-methacryloyloxyethyl trimellitate (4-MET) and 10-methacryloyloxydecyl dihydrogen phosphate (MDP) as functional monomers, in addition to a dimethacrylate monomer, filler, and photoinitiator. On the other hand, the bonding agent has simple components, such as dimethacrylate monomers, bis-GMA, fillers, and photoinitiators. Tamura et al. [24] investigated the bond effectiveness of this two-step adhesive system in the SE mode from its initial bond performance and bond durability under fatigue stress. They compared the outcome with the two-step SE adhesive system Clearfil SE Bond 2. They reported that the two-step adhesive system's bonding effectiveness was equivalent to that of the gold standard adhesive system. However, there is a lack of information about this new type of adhesive system's bond durability in different etching modes or under different degradation conditions, such as thermal stress and hydrolysis.

The purpose of this study was to compare the bond durability of the new two-step adhesive system using a universal adhesive-derived primer with those of conventional two-step SE adhesive systems in different etching modes and under different degradation conditions. The cured adhesive layer's water contact angle (WCA) and surface free energy (SFE) were determined to understand the cured adhesive layer's properties. The null hypotheses to be tested were as follows: (i) the bond durability of the universal adhesive-derived two-step adhesive system would not be different in different etching modes, under different degradation conditions; (ii) the bond durability of the universal adhesive-derived two-step adhesive system would not be different from those of the conventional two-step SE adhesive systems, and (iii) the SFE of the cured adhesive layer would not be different between the tested materials.

Materials And Methods

Study design

The enamel and dentin bond durability of the two-step adhesive systems in different etching modes were determined under different degradation conditions. The etching mode was either SE, or ER mode. Enamel and dentin bond durability were separately determined by measuring shear bond strength (SBS) after three different numbers of thermal cycles (TC): 0, 10,000, 20,000 or 30,000; and different periods of long-term water storage (WS): 24 h, 6-months, 1-year, or 2-year. The TC 0 (no cycling) group and WS 24 h group was set as a baseline group and were merged as a single group. Thus, there were a total of 42 treatment groups (3 adhesives × 2 etching modes × 7 degradation conditions (baseline group, 3 thermal cycling groups, and 3 water storage groups) in enamel and dentin. A total of 1092 bovine teeth were used in this study: 1008 teeth were used for enamel and dentin bond strength tests, 72 for the measurement of the surface free energy (SFE) of the cured bonding agent, and 12 for the scanning electron microscopy (SEM) observations. The

necessary sample sizes for the enamel and dentin SBS test were calculated using Sigma Plot version 13 (Systat Software, Chicago, IL, USA). Based on our previous study [27], we assumed that the minimum important difference in the mean SBS values was 10 MPa, the expected standard deviation of residuals was 4.0, and the number of groups was 42 for shear bond strength. Power = 0.9 and α = 0.05 were set for SBS test. The calculations indicated that 12 specimens were required to effectively measure the shear bond strength in each group.

For SFE measurement, a statistical power analysis (G Power calculator) indicated that 10.7 specimens were required to measure the SFE effectively. The parameters used were as follows: α = 0.05 and power = 0.9. Thus, the experiments were conducted using 12 specimens for SFE tests.

Study materials

Table 1 shows the materials used in this study. The two-step adhesive system using a universal adhesive-derived primer, G2-Bond Universal (GU; GC, Tokyo, Japan), was used. Clearfil SE Bond 2 (CS; Kuraray Noritake Dental, Tokyo, Japan) and OptiBond XTR (OX; Kerr, Orange, CA, USA) were the two traditional two-step SE adhesive systems used for comparison. Clearfil AP-X (Kuraray Noritake Dental) was used for bonding to the tooth substrate after bonding procedures. A light-emitting diode curing unit (Valo; Ultradent Products, South Jordan, UT, USA) was used with a 10 mm internal tip diameter, and a light irradiance of over 1,000 mW/cm^2 (standard mode) was checked during the experiment.

Specimen preparation

Bovine teeth were used as a substitute for human teeth. Approximately two-thirds of each tooth's apical root was removed using a diamond-impregnated disk in a precision sectioning saw (IsoMet 1000, Buehler, Lake Bluff, IL, USA). The labial surfaces were subjected to mechanical grinding/polishing (Ecomet 4, Buehler) with a wet 180-grit silicon carbide (SiC) paper (Fuji Star Type DDC, Sankyo Rikagaku, Saitama, Japan) to create a flat enamel and dentin surface. The prepared tooth was then mounted in self-curing acrylic resin (Tray Resin II, Shofu, Kyoto, Japan) to expose the flattened enamel and dentin surfaces. The adherent bonding surfaces were polished using 240-grit SiC paper followed by 320-grit SiC paper (Fuji Star Type DDC) under running water.

Adhesive application protocol and storage

Table 2 shows the adhesives' bonding procedures. Twelve specimens were prepared for each test group to measure the shear bond strength (SBS) to the enamel and dentin in the SE mode (without phosphoric acid pre-etching) and the ER mode (phosphoric acid pre-etching for 15 s). For the SE mode, the adhesives were applied to the adherent surface following the manufacturers' instructions. In contrast, for the ER mode, 35% phosphoric acid (Ultra-Etch, Ultradent Products, South Jordan, UT, USA) was applied to the enamel and dentin surfaces for 15 s, followed by rinsing with a water spray using a three-way dental syringe for 15 s. Bonded assemblies were fabricated by clamping plastic molds (height, 2.0 mm; internal diameter, 2.38 mm; Ultradent Products) to a fixture against the adherent surfaces. The resin composite was condensed into the mold and irradiated for 30 s. The bonded specimens were subjected to thermal cycling (TC groups) or underwent water storage (WS group) in distilled water (37°C). In the TC groups, the bonded assemblies were stored in distilled water at 37°C for 24 h and then subjected to 5,000, 10,000, 20,000, or 30,000 TCs (between 5°C and 55°C), with a dwell time of 30 s. The bonded assemblies in the WS groups were stored in distilled water (37°C) for 6-months, 1-year, or 2-year before the SBS tests (long-term storage). The storage water was changed every week during the experimental period. Baseline specimens were stored in distilled water at 37°C for 24 h before the SBS tests (baseline subgroups).

Shear bonding strength test

To reduce ununiform distribution stress on the bonded specimen when using shear bond strength test, the notched-edge SBS test measured the SBS of the bonded specimens after undergoing the different degradation conditions according to ISO 29022 [28]. The bonded specimens were fixed with the Ultradent shearing fixture and loaded until bonding failure occurred at 1.0 mm per min using a universal testing machine (Type 5500R, Instron, Canton, MA, USA). The SBS values (MPa) were obtained by dividing the peak load at failure by the bonded adherent area. The de-bonded sites on the tooth surfaces and resin composite rods were observed under an optical microscope (SZH-131; Olympus, Tokyo, Japan) at a magnification of ×10 to determine the bond failure mode. The failure modes were categorized as an adhesive failure, cohesive failure in resin, or cohesive failure in enamel or dentin when more than 80% of the adherent area comprised the adhesive, resin composite, or tooth substrate, respectively. Other failure patterns, such as partially adhesive and partially cohesive, were categorized as a mixed failure.

WCA and SFE measurements

Twelve enamel and dentin specimens in each adhesive were prepared as described for the SBS tests. The cured adhesive layer's hydrophilicity is related to the bond hydrolysis; hence, the cured adhesive layer's WCA and SFE were evaluated to determine the degree of hydrophilicity. An oxygen inhibited layer is normally present on the top surface of a cured adhesive layer. Therefore, SFE measurements of the cured adhesive layers were performed without the oxygen inhibited layer to measure the interior layer's hydrophilicity. According to the manufacturers' instructions, each adhesive was applied to the enamel and dentin in the SE mode, followed by light irradiation for 10 s. The cured adhesive's upper surface was removed with ethanol-impregnated cotton pellets to remove the oxygen inhibited layer. Subsequently, each group's SFE values were obtained by measuring three test liquids' surface contact angles with known SFE parameters, 1-bromonaphthalene, di-iodomethane, and distilled water. The equilibrium contact angle (θ) of each test liquid on the specimens was then measured at room temperature (23°C ± 1°C) via the sessile-drop method using a contact angle meter (Drop Master DM500; Kyowa Interface Science, Saitama, Japan) with a built-in charge-coupled device camera. Sessile liquid drops (1.0 μL) were dispensed with a micropipette, and the surface characteristics were calculated based on the fundamental concepts of wetting, as described previously [4,11]. The components of the SFE (γs) arising from the dispersion force (γ_s^d), the polar (permanent and induced) force (γ_S^p), and the hydrogen-bonding force (γ_S^h) were obtained using an add-on software (FAMAS, Kyowa Interface Science).

Scanning electron microscopy observations

Representative restorative/tooth interfaces were observed via scanning electron microscopy (SEM; ERA-8800FE, Elionix, Tokyo, Japan). Bonded specimens stored in 37ºC distilled water for 24 h were embedded in epoxy resin (Epon 812, Nisshin EM, Tokyo, Japan) and then longitudinally sectioned with a precision sectioning saw (IsoMet 1000 Precision Sectioning Saw). The resin/tooth interfaces' sectioned surfaces were polished to a high gloss using abrasive disks (Fuji Star Type DDC), followed by diamond pastes down to 0.25 μm in particle size (DP-Paste, Struers, Ballerup, Denmark); this was followed by ultrasonic cleaning for 30 min. The interface and treated surface 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 transferred to a freeze dryer (Model ID-3, Elionix) for 30 min. The resin/tooth interface specimens were subjected to argon-ion beam etching (EIS-200ER, Elionix) for 45 s with the ion beam (accelerating voltage 1.0 kV, ion current density 0.4 mA/cm^2) directed perpendicular to the polished surfaces. Finally, all the SEM specimens were coated with a thin film of gold in an automatic ion sputter (Quick Coater Type SC-701, Sanyu Electron, Tokyo, Japan). The observations were carried out using an SEM at an operating voltage of 10 kV.

Statistical analysis

The homogeneity of variance (Bartlett's test) and distribution (Shapiro-Wilk test) for each test data set was confirmed before analysis of variance (ANOVA) was performed. In the SBS data in the TC and WS groups, the enamel and dentin data were analyzed separately, and three-way ANOVA followed by Tukey's honest significant difference (HSD) test (α = 0.05) was used to analyze each data set. The factors evaluated were: the etching mode, storage period, and adhesive system. One-way ANOVA followed by Tukey's HSD test (α = 0.05) was used for comparisons within subsets.

For the CA and total SFE data, two-way ANOVA followed by Tukey's HSD test (α = 0.05) was used to analyze the full data set. The tooth substrate and adhesive system were evaluated. A one-way ANOVA followed by Tukey's posthoc test (α = 0.05) was used to analyze the results for the components of SFE. All statistical analyses were performed using a statistical analysis software system (Sigma Plot 13; SPSS, Chicago, IL, USA).

Results

SBS in the TC groups

The enamel and dentin SBS values in SE and ER mode under the different degradation conditions are shown in Figs. 1 and 2. The SBS values under TC for the enamel are presented in Table 3. Three-way ANOVA showed that all the factors significantly influenced the SBS values ($p < 0.001$). The three-way interactions among the etching mode, TC period, and adhesive system type were significant ($p = 0.002$). Likewise, the two-way interactions were significant ($p < 0.001$), apart from that between the etching mode and TC period ($p = 0.350$).

The mean SBS values ranged from 41.8 to 51.6 MPa in CS, 45.0 to 53.8 MPa in GU, and 25.6 to 44.8 MPa in OX. GU showed significantly higher SBS values in the ER mode than in the SE mode, regardless of the TC period. Among the ER mode groups, OX showed a significantly lower SBS value in the TC 30,000 subgroup compared to those in the other TC subgroups. Nonetheless, the ER mode groups showed significantly higher SBS values than those in the SE mode. There were no significant differences in SBS among the different TC periods in the same etching mode in the GU specimens. Although CS showed a similar trend to GU, the SBS values in the TC 20,000 and 30,000 subgroups were significantly lower than those in the TC 5,000 and TC 10,000 subgroups in the ER mode. In the OX specimens, the SBS values decreased with the increase in the TC period, regardless of the etching mode. Although no significant differences in SBS values were observed between GU and CS during any of the TC periods in the SE mode, significantly lower values were observed in OX compared to the other adhesive systems. In the ER mode, GU presented with the highest SBS values. In contrast, OX presented with significantly lower SBS values than the other adhesive systems during all TC periods, except for the specimens in the baseline subgroups.

The SBS values under TC for dentin are presented in Table 4. Three-way ANOVA revealed that the TC period and adhesive system, but not the etching mode, significantly influenced the SBS values ($p < 0.001$). The three-way interactions among the etching mode, TC period, and the type of adhesive system were not statistically significant. Still, the two-way interactions were significant (except for that between the etching mode and TC period).

The mean SBS values ranged from 33.4 to 46.5 MPa in CS, 39.6 to 45.4 MPa in GU, and 32.9 to 43.7 MPa in OX. GU did not show any significant differences in SBS values, regardless of the etching mode or TC period, except for the TC 30,000 subgroup in the ER mode. However, CS and OX demonstrated decreased SBS values with the increase in the TC period, regardless of the etching mode. Furthermore, the TC 20,000 and TC 30,000 subgroups presented with significantly lower SBS values when compared to those of the baseline groups, regardless of the etching mode. A similar trend was observed in both SE and ER modes. However, there were no significant differences between the tested adhesive systems at baseline and, during the early TC phase, GU demonstrated significantly higher SBS values than the other adhesive systems in TC 30,000 subgroup (Table 4).

SBS inthe WS groups

Table 5 presents the SBS values of the enamel under WS. Three-way ANOVA revealed that all the factors significantly influenced the SBS values ($p < 0.001$). The three-way interactions among the etching mode, WS period, and type of adhesive system used were not statistically significant ($p = 0.067$). No statistically significant two-way interactions were observed between etching mode and WS period ($p = 0.375$), but the other two-way interactions were significant ($p < 0.05$).

The mean enamel SBS values ranged from 40.9 to 45.8 MPa in CS, 41.8 to 50.3 MPa in GU, and 22.0 to 44.8 MPa in OX. Comparisons of the different etching modes using the same adhesive revealed that the SBS values of OX significantly higher in the ER mode than in the SE mode. Similarly, the other adhesives tended to show higher SBS values in the ER mode than those in the SE mode. Although GB showed any significant differences among WS periods when the same etching mode was used, OX in the 2-year subgroup in SE mode and CS in the 2-year subgroup in ER mode showed significantly lower SBS values than the other WS groups with the same etching mode. When comparing the adhesive systems in SE mode, OX showed significantly lower SBS values than the other adhesives, regardless of the WS period. For the ER mode, although there was no significant differences among the adhesive systems in the 1-year subgroup, significant differences were observed among the adhesive systems in the other WS groups and GU presented with the highest SBS values.

The SBS values under WS for dentin are presented in Table 6. Three-way ANOVA revealed that the WS period and type of adhesive system, but not the etching mode ($p = 0.063$), significantly influenced the SBS values ($p < 0.001$). The three-way interactions among the etching mode, WS period, and type of adhesive system were not statistically significant ($p = 0.809$). Significant two-way interactions were observed ($p < 0.001$), except for that between the etching mode and WS period ($p = 0.450$).

The mean dentin SBS values ranged from 31.7 to 45.4 MPa in CS, 41.1 to 46.5 MPa in GU, and 25.5 to 43.7 MPa in OX. For CS, in the 2-year subgroup in the SE mode showed siginificantly lower SBS when compared to baseline and 6-month groups, and significant differences were observed in SBS in the 1-year and 2-year subgroups in the ER mode when compared to those in the other WS groups. GU did not demonstrate any significant differences in SBS values, regardless of the etching mode or WS period apart from between 6-month subgroup in ER mode and 2-year subgroups in the SE and ER mode. In the OX specimens, the SBS values significantly decreased with the WS period increase, regardless of the etching mode. When comparing the adhesive systems in the SE mode, no significant differences in SBS values were observed between CS and GU during any of the WS periods; however, OX showed significantly lower SBS values than the other adhesive systems, except for that in the baseline group. GU showed a significantly higher SBS value in the ER mode than the other adhesive systems in the 1-year and 2-year subgroups. OX presented with significantly lower SBS values than the other adhesive systems in 6-month, 1-year, and 2-year subgroups.

Failure mode analysis of de-bonded specimens after SBS

Adhesive failure was predominant for all the adhesive systems in the SE mode in the de-bonded enamel specimens in the TC groups, regardless of the TC period. On the other hand, mixed or cohesive enamel failures were more frequent in the ER mode than in the SE mode. There were no clear differences in failure modes among the different adhesive systems or TC periods. The frequencies of mixed and cohesive failure in dentin were more frequently observed in the SE mode than in the ER mode, but adhesive failure appeared to increase with the increase in the TC period, regardless of the etching mode or the type of adhesive system used.

In the de-bonded enamel and dentin specimens in the WS groups, the trend of failure was similar to that observed in the TC groups. However, the frequencies of mixed failure and cohesive failure in the enamel and dentin were less frequent

than those in the TC groups. In particular, adhesive failure was the predominant failure mode in OX, regardless of the tooth substrate or WS period.

WCA and SFE

Table 7 shows the WCAs of the cured adhesives without an oxygen inhibited layer on the enamel and dentin. Two-way ANOVA revealed that the adhesive type ($p < 0.001$), but not the tooth substrate, significantly influenced the WCA. The interactions between the factors were not significant ($p = 0.114$). GU showed significantly higher WCA values, whereas OX presented significantly lower WCA values than the other adhesive systems in both enamel and dentin. OX presented with significantly lower WCA values in the dentin than in the enamel, but there were no significant differences between enamel and dentin with the other adhesive systems.

Table 8 shows the total SFE (γ_S), and each component (γ_S^d, γ_S^p, and γ_S^h) of the cured adhesives on the enamel and dentin. Two-way ANOVA revealed that the type of adhesive system ($p < 0.001$) and tooth substrate ($p = 0.012$) significantly influenced the γ_S. Also, the interaction between the factors was significant ($p < 0.05$). GU showed significantly lower γ_S values than the other adhesive systems in both enamel and dentin. The dispersion force (γ_S^d) was similar at approximately 39 (mN m^{-1}), regardless of the adhesive system or tooth substrate. However, the polar (γ_S^p) and hydrogen-bonding forces (γ_S^h) were dependent on the adhesive system used. OX presented with significantly lower γ_S^p and higher γ_S^h values than the other adhesive systems in both enamel and dentin. Likewise, GU showed significantly lower γ_S^h values than the other adhesive systems in both enamel and dentin.

SEM observations

Figures 3 and 4 are representative SEM images of the resin/tooth interfaces. All the adhesive systems exhibited excellent adaptation between the mineralized tissue and the adhesive layer in the enamel and dentin. Although the morphological features at the interface between the adhesive and enamel substrate showed no differences among the adhesive systems, there were different ultrastructure features between the two etching modes used. The smear layer was completely dissolved, and adhesive interpenetration within the enamel was visible in the ER mode, but not eh SE mode, regardless of the adhesive system used.

For the resin/dentin interfaces, although all the adhesive systems exhibited a similar thickness (1–2 μm) of the hybrid layer (HL) in the ER mode, no clear HLs were observed for any of the adhesive systems in the SE mode. However, both CS and GU presented with a thin (0.5–1 μm) high-density layer above the intact dentin in both the SE (the layer between yellow circles) and ER modes (the layer between blue stars).

The thickness of the adhesive layer and its morphological appearance depended on the adhesive system, but the adhesive layer did not differ between the enamel and dentin using the same adhesive system. CS and GU (layer between the arrowheads, Figs. 3A, 3C, 4A–D) showed similar adhesive thickness (40–60 μm) in both the SE and ER modes; however, the adhesive layers in the OX specimens were approximately 20 μm thick (layer between the arrowheads, Fig. 3B and 3D). Nano-sized fillers were observed in CS and GU, whereas OX employed somewhat larger irregular fillers (approximately 0.1–0.5 μm) along with the nano-sized fillers.

Discussion

A review study regarding using bovine teeth as substitute for human teeth in bond strength tests showed that there have been many previous studies that found no significant differences in SBS test results between bovine teeth and human teeth [29]. The reasons for employing bovine teeth in this study are that they have a less variable composition than

human teeth, and it is easy to obtain large quantities of teeth in good condition. Furthermore, bovine teeth have large flat areas and have not had prior acid challenges that may affect the test results.

The bond strength test in this study was performed in accordance with ISO 29022 [28]. Because the making bonded specimens for this test is easy, and it is probable to avioid risk of damage to bonded specimens before and during testing. The μ-TBS (tensile bond strength) test is a well-established method and it is thought to be useful when human teeth are used [30]. However, there are concerns about inconsistent geometry, technique sensitivity, and labour intensity when fabricating specimens [31]. External forces applied to the bonded specimen during cutting or shaping may damage the interface of the specimen under degaradation condition or cause pre-failure before testing, thus distorting the results.

This study aimed to determine the bond durability of a recently introduced two-step adhesive system using the universal adhesive-derived primer and comparing it with those of two conventional two-step SE adhesive systems different degradation tests. The main deterioration factor regarding TC for the bonded assemblies could be the thermal stress at the resin/tooth interface [32]. Frequent temperature fluctuations may induce dimensional alterations in the material and generate internal strain at the interface due to differences in each material's thermal expansion. This discrepancy might also affect the adhesive layer's interior due to the presence of filler particles and the resin matrix, in addition to the water absorption by the adhesive layer in an aqueous environment [12]. Similarly, hydrolysis of the hydrophilic resin components could be the main degradation factor following long-term WS. Water uptake might induce a reduction in frictional forces between the polymer chains and promote resin matrix expansion. Pathways created by gaps between the fillers and the resin matrix might accelerate water diffusion and weaken the resin matrix and filler de-bonding [32]. Moreover, endogenous enzymatic degradation of the dentin is considerable following WS owing to the presence of host-derived proteases with collagenolytic activity [33–35]. Probably, TC might not provide appropriate conditions for the activation of matrix-bound matrix metalloproteinases and cysteine cathepsins due to frequent temperature fluctuations, which impair enzymatic activity [36]. Therefore, this study used different degradation conditions before the SBS test to investigate the effects of the different deterioration mechanisms.

The bond strength test results revealed different trends regarding the different adhesive systems, tooth substrates, and degradation conditions in this study. Although CS and OX showed significantly lower SBS values in some degradation subgroups compared to those of the baseline subgroups, GU did not show any significant decrease in SBS in the enamel and dentin under both TC and WS when compared to the specimens in the baseline subgroups. Furthermore, GU did not significantly decrease SBS in the enamel and dentin, regardless of etching mode. Therefore, the first null hypothesis that GU's bond durability would not be different in the different etching modes used was not rejected. Also, the second null hypothesis, that the bond durability of GU would not be different from that of conventional two-step SE adhesive systems was rejected.

All the adhesive systems showed higher enamel SBS values in the ER mode than in the SE mode, regardless of the conditions. The enamel bond durability in each adhesive system appeared to be more stable than that in the dentin. These results were in line with those reported previously [27, 37]. Mechanical interlocking enabled by phosphoric acid pre-etching of the homogenous enamel surface is still important to establish sufficient initial bond performance and bond durability [3, 4, 6, 12]. On the other hand, dentin is heterogeneous and comprises hydroxyapatite and collagen fibrils. The water content in dentin is much higher than that in enamel, which might influence the effectiveness of water and solvent evaporation in the primer; this, in turn, could affect the mechanical properties of the adhesive layer [38]. Additionally, the collagenolytic activity at the interface between the demineralized dentin and the adhesive layer reduces the dentin bond's durability over time [33–35].

GU's excellent bond durability might be related to the primer, the bonding agent's components, and the adhesive layer's properties. Although universal adhesives' bond effectiveness is not as high as those of two-step SE adhesive systems, many previous studies have indicated their superior versatility and bond reliability [1, 2, 12, 27, 37]. GU was designed to expand the range of clinical use by utilizing the benefits of universal adhesives as a primer and employing a hydrophobic bonding agent [24]. The WCA and SFE measurements' outcomes demonstrated significantly higher WCA and lower γS values in GU compared to the other adhesive layers. Furthermore, GU presented with significantly lower γ_S^h values, representing the water and hydroxyl components of the materials [4, 11], than the other adhesive layers. Therefore, the adhesive layer in GU is more hydrophobic than those in the other adhesive layers. The hydrophobicity of GU might be attributed to the simple components of the bonding agent. GU is a 2-hydroxyethyl methacrylate (HEMA)-free adhesive system, and the bonding agent does not have any functional monomers or solvents, in contrast to the other adhesive systems used in this study.

The stable dentin bond strength of GU in the ER mode may be attributed to the universal adhesive-derived primer's bond effectiveness. Unlike conventional two- and single-step SE adhesive systems, the dentin bond durability of universal adhesives is not affected by the etching mode [1, 2, 11, 13]. The GU primer does not contain HEMA, which might remain at the interface between the primed tooth surface and the adhesive layer. The hydrophilic HEMA helps the resin monomer infiltrate the demineralized dentin because of its compatibility with water-rich conditions. Therefore, it might be susceptible to hydrolytic degradation over time [39]. Therefore, the HEMA-free universal adhesive-derived primer in GU could inhibit dentin bond degradation at the interface between the demineralized dentin and the adhesive layer.

CS showed a similar trend to GU regarding the enamel bond durability. However, significant reductions in dentin SBS appeared in both etching modes under TC and the ER mode under WS compared to those in the baseline groups. OX showed significantly decreased enamel and dentin SBS values with prolonged degradation periods (except for the enamel SBS in ER mode under WS), regardless of the etching mode. OX and CS contain HEMA in the primer and bonding agent. Additionally, the bonding agent in CS contains 10-methacryloyloxydecyl dihydrogen phosphate (MDP) with hydrophobic and hydrophilic moieties [40]. Matsui et al., [40] reported that, although an MDP-containing bonding agent had a higher immediate μ-TBS value than an MDP-free bonding agent, it demonstrated a lower dentin bond durability than the MDP-free bonding agent after 10,000 thermal cycles. Therefore, the MDP- and HEMA-containing bonding agents in CS might induce hydrolytic degradation of the adhesive layer and the area around the interface between the water-rich dentin and adhesive layer over time. GU and CS exhibited excellent adaptation between the dentin substrate and the adhesive layer in the SEM observations. They presented with a thin high-density layer above intact the dentin in both SE and ER modes. This thin high-density layer might be evidence of a chemical interaction between the functional monomer and the intact dentin; thus, it is possible to observe sufficient infiltration of the resin monomers with phosphoric acid pre-etching before the application of the primer to the dentin [13]. However, unlike in the SE mode, CS showed a significantly lower dentin SBS after 1 year of WS periods compared to those at baseline and after 6 months of WS in the ER mode. Therefore, the hydrolysis of the dentin bonds in CS might occur in the HL vicinity under WS conditions.

The SFE value of the adhesive layer was dependent on the adhesive system. Therefore, the third null hypothesis, that the SFE of the adhesive layer would not be different among the tested materials was rejected. OX showed a significantly lower CA and a higher γ_S value than the other adhesive layers, and the extent of enamel and dentin SBS reduction in the degradation groups was greater than that in the other adhesives. OX contains a functional monomer, glycerol dimethacrylate dihydrogen phosphate (GPDM), which is a rather short molecule with two hydrophobic methacrylate groups and one hydrophilic phosphate group, but a long carbon spacer group does not separate these functional groups as in MDP [41]. A previous investigation on the adsorption of GPDM and MDP onto hydroxyapatite reported that, in contrast to MDP, GPDM was quickly removed after washing with water [41]. It also concluded that dentin treated with

GPDM appeared more hydrophilic than that treated with MDP. The relatively hydrophilic nature of the adhesive layer indicates that GPDM-Ca salts are more susceptible to hydrolytic degradation than the other adhesive systems. Moreover, OX's dentin bond durability was lower than those of the other adhesive systems, regardless of the etching mode or degradation condition. Besides, OX showed significantly higher γ_S and γ_S^h values on the dentin surface than on the enamel. This phenomenon might be related to the somewhat thinner adhesive layer and the method of application. The thickness of the adhesive layer in OX's case was almost half of those of the other adhesive layers. Active application for both priming and bonding might induce hydrophilicity in the adhesive layer from primer components and dentin surface moisture.

Conclusion

GU showed immediate enamel and dentin bond performance equivalent to those of CS and OX, regardless of the etching mode used. Although the enamel bond durability of GU was similar to that of CS, its dentin bond durability under different degradation conditions was superior to those of the other adhesive systems in both etching modes. Moreover, the cured adhesive layer of GU was more hydrophobic than those of the other adhesive systems. Thus, within this laboratory study's limitations, the two-step adhesive system utilizing a universal adhesive-derived primer showed superior enamel and dentin bond durability than the two conventional two-step SE adhesive systems in both SE and ER etching modes. Therefore, the latest two-step adhesive system utilizing universal adhesives' benefits in its primer might demonstrate a durable clinical bond performance and can be widely used in the clinical setting.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

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Ethical approval

This study does not contain any studies with human participants and subjects or animals performed by any of the authors.

Informed consent

For this type of study, formal consent is not required

Author contributions

Toshiki Takamizawa: Conceptualization, Methodology, Writing-Original draft preparation, Visualization. Data curation**.** **Eizo Hirokane and Ryota Aoki**: Visualization, Investigation. **Keiichi Sai and Ryo Ishii:** Investigation, Data curation. **Wayne W Barkmeier**: Methodology, Writing- Reviewing and Editing. **Mark A Latta:** Methodology, Writing-Reviewing and Editing. **Masashi Miyazaki**: Supervision, Writting- Reviewing and Editing.

All authors: Writing-reviewing and Editing.

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Tables

Table 1: *Materials used in this study*

Code	Adhesive Lot. No	Main components	pH (Primer)	Manufacturer
CS	Clearfil SE Bond 2 Primer: 5852494 Adhesive: 5847004	Primer: MDP, HEMA, water, initiators Adhesive: MDP, HEMA, bis-GMA, initiators, microfiller	2.0†	Kuraray Noritake Dental, Tokyo, Japan
GU	G2-Bond Universal Primer: 190711 Adhesive: 190711	Primer: 4-MET, MDP, MDTP, dimethacrylate monomer, acetone, water, photoinitiator, filler Adhesive: dimethacrylate monomer, bis-GMA, filler, photoinitiator	1.5*	GC, Tokyo, Japan
OX	OptiBond XTR Primer: 58470004 Adhesive: 5852494	Primer: GPDM, HEMA (10-30%), dimethacrylate monomers, acetone (10-30%), ethyl alcohol (5-10%), water, initiator Adhesive: ethyl alcohol (10-30%), HEMA (10-30%), dimethacrylate monomers, barium aluminoborosilicate glass, fumed silica, disodium hexafluorosilicate	1.6†	Kerr, Brea, CA, USA
Resin Composite		**Main Components**		**Manufacturer**
Clearfil AP-X N416713		bis-GMA, TEGDMA, silane barium glass filler, silane silica filler, silanated colloidal silica, CQ, pigments, others		Kuraray Noritake Dental

† [42]

* information from GC

MDP: 10-methacryloyloxydecyl dihydrogen phosphate, HEMA: 2-hydroxyethyl methacrylate,

bis-GMA: 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy) phenyl] propane, 4-MET: 4-Methacryloyloxyethyl trimellitate, MDTP:10-methacryloyloxydecyl dihydrogen thiophosphate,

GPDM: glycerol dimethacrylate dihydrogen phosphate, TEGDMA: triethyleneglycol dimethacrylate, CQ: *dl*-camphorquinone,

Table 2: *Application protocols for pre-etching and two-step adhesive systems*

Etching mode	***Pre-etching protocol***
SE (self-etch)	Phosphoric acid pre-etching was not performed.
ER (etch-and-rinse)	Enamel and dentin surfaces were phosphoric acid etched for 15 s. Etched surface was rinsed with water for 15 s and air-dried.
Adhesive	***Adhesive Application Protocol***
CS	Primer was applied to air-dried tooth surfaces for 20 s followed by medium air pressure for 5s. Adhesive was then applied to the primed surface and was gently air thinned. Adhesive was light irradiated for 10 s.
GU	Primer was applied to air-dried tooth 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. Bonding agent was then applied to the primed surface and was gently air thinned. Bonding agent was light irradiated for 10 s.
OX	Primer was applied to air-dried tooth surface with rubbing action for 20 s. Medium air pressure was applied to the surface for 5 s. Adhesive was applied to the primed surface with rubbing action for 15 s and then air thinned for 5 s. Adhesive was light irradiated for 10 s.

Table 3: *Influence of TC on enamel SBS*

Code	**SE mode**					**ER mode**				
	Baseline (24h)	TC 5000	TC 10000	TC 20000	TC 30000	Baseline (24h)	TC 5000	TC 10000	TC 20000	TC 30000
CS	42.4 (1.7)^{aCD}	42.8 (3.1)^{aCD}	43.8 (1.7)^{aCD}	44.1 (2.7)^{aCD}	41.8 (2.8)^{aD}	45.5 (2.3)^{bBC}	51.6 (1.6)^{aA}	51.1 (3.4)^{aA}	47.6 (2.6)^{aB}	45.0 (3.2)^{bBC}
GU	45.0 (4.5)^{aB}	45.4 (4.5)^{aB}	45.6 (2.4)^{aB}	46.7 (3.1)^{aB}	45.3 (5.7)^{aB}	50.3 (4.3)^{aA}	53.8 (3.4)^{aA}	51.8 (3.3)^{aA}	50.8 (3.6)^{aA}	51.4 (3.7)^{aA}
OX	32.9 (3.8)^{bD}	34.6 (4.6)^{bCD}	37.6 (3.3)^{bBC}	27.6 (3.1)^{bE}	25.6 (2.8)^{bE}	44.8 (4.1)^{bA}	43.4 (3.6)^{bA}	42.7 (3.3)^{bA}	41.4 (3.0)^{bAB}	34.8 (3.8)^{cCD}

N=12, 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 4: *Influence of TC on dentin SBS*

Code	SE mode					ER mode				
	Baseline (24h)	TC 5000	TC 10000	TC 20000	TC 30000	Baseline (24h)	TC 5000	TC 10000	TC 20000	TC 30000
CS	44.0 (3.8)^{aA}	46.1 (3.0)^{aA}	45.5 (4.2)^{aA}	39.6 (2.4)^{aBC}	36.7 (2.3)^{bCD}	40.2 (3.4)^{aB}	45.5 (2.5)^{aA}	45.9 (3.4)^{aA}	35.7 (2.5)^{bD}	33.4 (3.0)^{bD}
GU	42.6 (3.2)^{aAB}	41.6 (3.8)^{bAB}	42.8 (3.9)^{aAB}	41.0 (3.4)^{aAB}	40.4 (3.3)^{aAB}	42.3 (3.6)^{aAB}	45.4 (4.0)^{aA}	43.9 (4.3)^{aAB}	41.1 (3.6)^{aAB}	39.6 (2.9)^{aB}
OX	42.9 (4.2)^{aA}	43.3 (2.0)^{abA}	42.2 (2.9)^{aA}	34.3 (3.4)^{bC}	34.1 (3.0)^{bC}	43.7 (4.4)^{aA}	43.2 (2.0)^{aA}	38.4 (3.8)^{bB}	34.2 (1.8)^{bC}	32.9 (4.8)^{bC}

N=12, 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: *Influence of WS on enamel SBS*

Code	SE mode				ER mode			
	Baseline (24h)	6-month	1-year	2-yera	Baseline (24h)	6-month	1-year	2-year
CS	42.4 (2.1)^{aAB}	43.7 (2.3)^{aAB}	44.8 (2.0)^{aA}	40.9 (2.2)^{aB}	45.5 (2.3)^{bA}	45.8 (2.5)^{abA}	45.5 (3.5)^{aA}	41.2 (2.9)^{bB}
GU	45.0 (4.5)^{aAB}	45.2 (2.8)^{aAB}	45.4 (5.5)^{aAB}	41.8 (4.8)^{aB}	50.3 (4.3)^{aA}	48.6 (4.1)^{aA}	48.1 (5.4)^{aA}	46.9 (4.2)^{aA}
OX	32.9 (3.8)^{bB}	32.5 (1.4)^{bB}	32.5 (4.1)^{bB}	22.0 (2.8)^{bC}	44.8 (4.1)^{bA}	44.4 (3.4)^{bA}	44.7 (2.6)^{aA}	40.6 (4.3)^{bA}

N=12, 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 6: *Influence of WS on dentin SBS*

Code	SE mode				ER mode			
	Baseline (24h)	6-month	1-year	2-year	Baseline (24h)	6-month	1-year	2-year
CS	44.0 (3.8)^{aA}	45.4 (3.0)^{aA}	41.0 (4.2)^{aAB}	37.8 (3.2)^{aB}	40.2 (3.4)^{aAB}	42.6 (5.0)^{aA}	33.9 (5.1)^{bC}	31.7 (4.3)^{bC}
GU	42.6 (3.2)^{aAB}	45.0 (4.9)^{aAB}	43.7 (4.5)^{aAB}	41.1 (4.2)^{aB}	42.3 (3.6)^{aAB}	46.5 (4.5)^{aA}	43.9 (4.6)^{aAB}	41.2 (4.9)^{aB}
OX	42.9 (4.2)^{aA}	33.6 (5.3)^{bB}	26.4 (5.4)^{bC}	26.3 (1.4)^{bC}	43.7 (4.4)^{aA}	36.3 (1.7)^{bB}	28.1 (1.5)^{cC}	25.5 (4.3)^{cC}

N=12, 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 7: WCA of the cured adhesives

Code	Enamel	Dentin
CS	51.7 (2.9)^{bA}	52.7 (3.1)^{bA}
GU	72.8 (2.5)^{aA}	72.0 (2.2)^{aA}
OX	47.1 (2.3)^{cA}	44.8 (1.4)^{cB}

N=12, mean (SD) in °.

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 8: Total SFE and each component SFE of the cured adhesives

Code	γ_S		γ_S^d		γ_S^p		γ_S^h	
	Enamel	Dentin	Enamel	Dentin	Enamel	Dentin	Enamel	Dentin
CS	55.0 (2.3)^{aA}	54.7 (2.1)^{bA}	39.2 (0.6)^{aA}	38.7 (1.1)^{aA}	3.3 (0.7)^{aA}	2.7 (0.7)^{bA}	12.5 (1.7)^{bA}	13.3 (1.8)^{bA}
GU	46.8 (2.5)^{bA}	48.5 (2.5)^{cA}	38.7 (0.6)^{aA}	38.3 (0.8)^{aA}	2.5 (0.1)^{aB}	4.8 (1.4)^{aA}	5.6 (2.2)^{cA}	5.4 (1.9)^{cA}
OX	56.7 (2.5)^{aB}	60.0 (2.1)^{aA}	38.3 (1.2)^{aA}	38.5 (0.7)^{aA}	0.3 (0.1)^{bA}	0.1 (0.1)^{cA}	18.1 (1.3)^{aB}	21.4 (2.0)^{aA}

N=12, mean (SD) in mN m^{-1}.

Same small case letter in vertical columns indicates no difference at 5% significance level. Same capital letter in horizontal rows in each parameter indicates no difference at 5% significance level. Values in parenthesis indicates standard deviation.

Figures

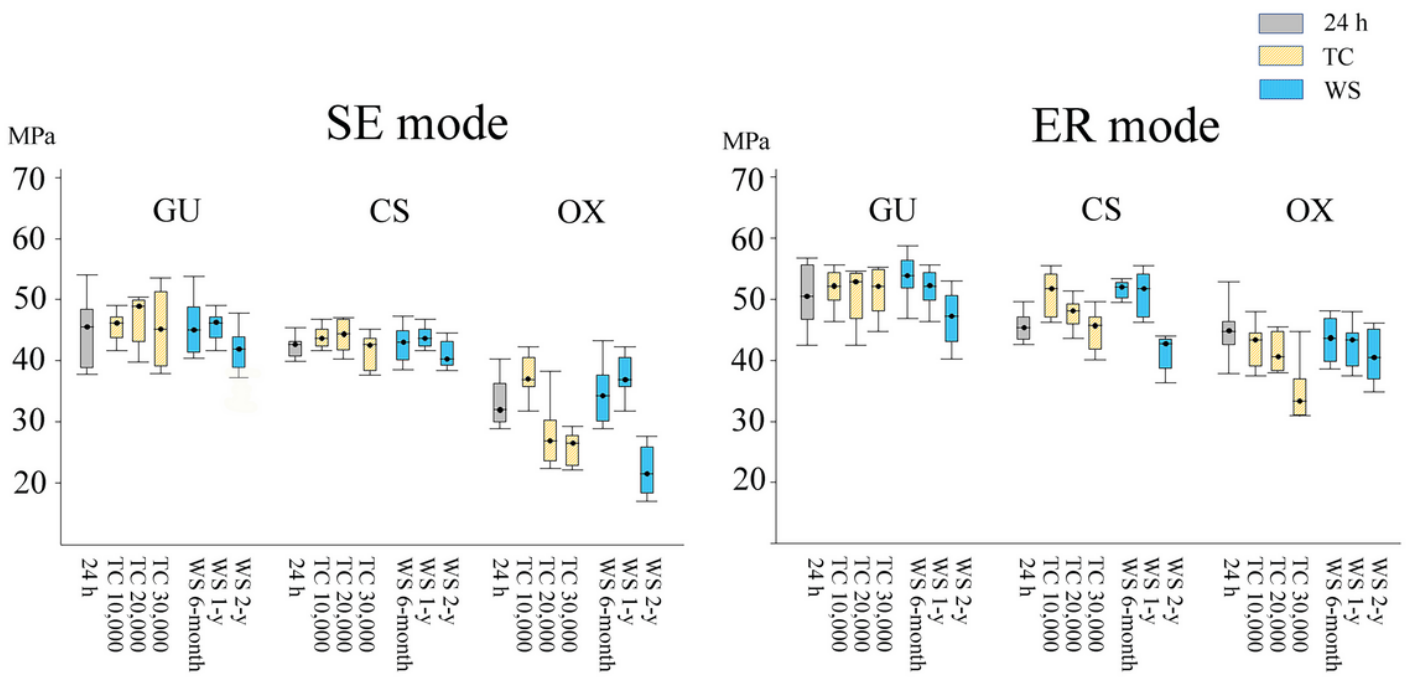

Figure 1

The enamel SBS values under different degradation conditions (TC or WS) in different etching mode (SE or ER mode).

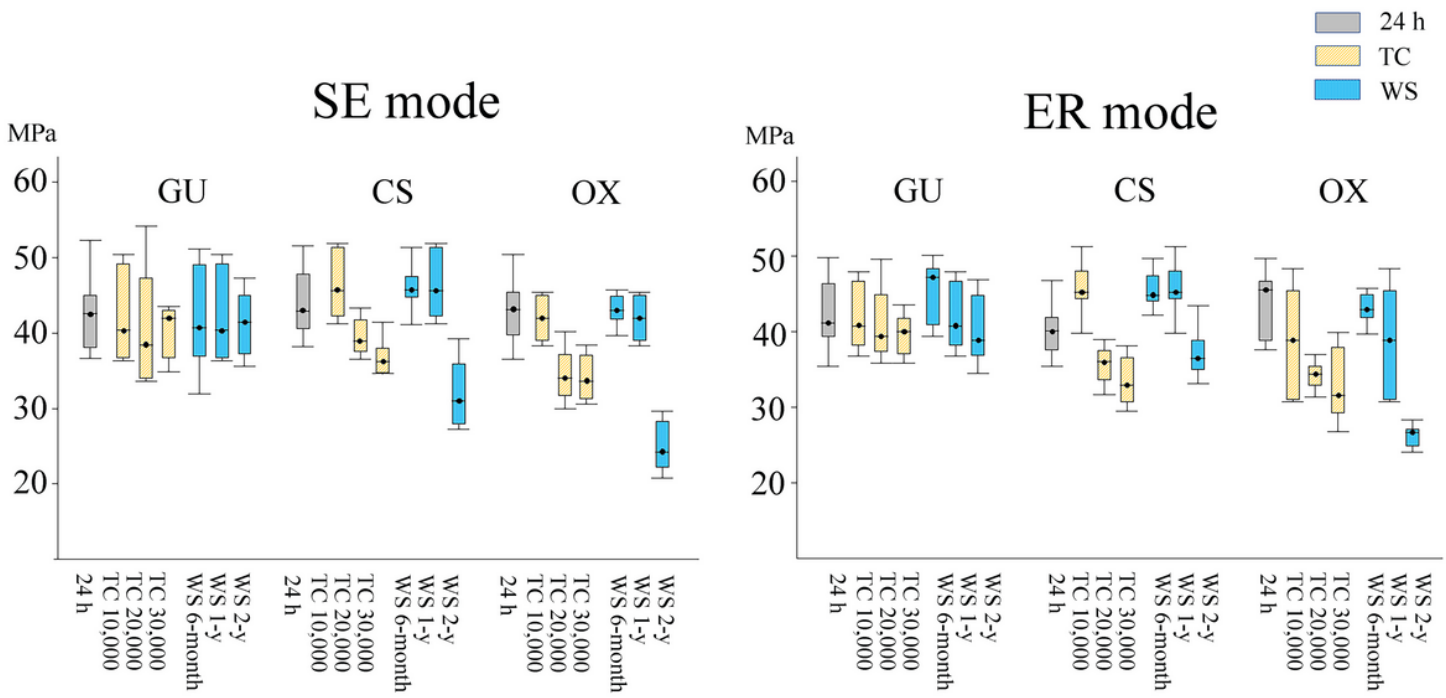

Figure 2

The dentin SBS values under different degradation conditions (TC or WS) in different etching mode (SE or ER mode).

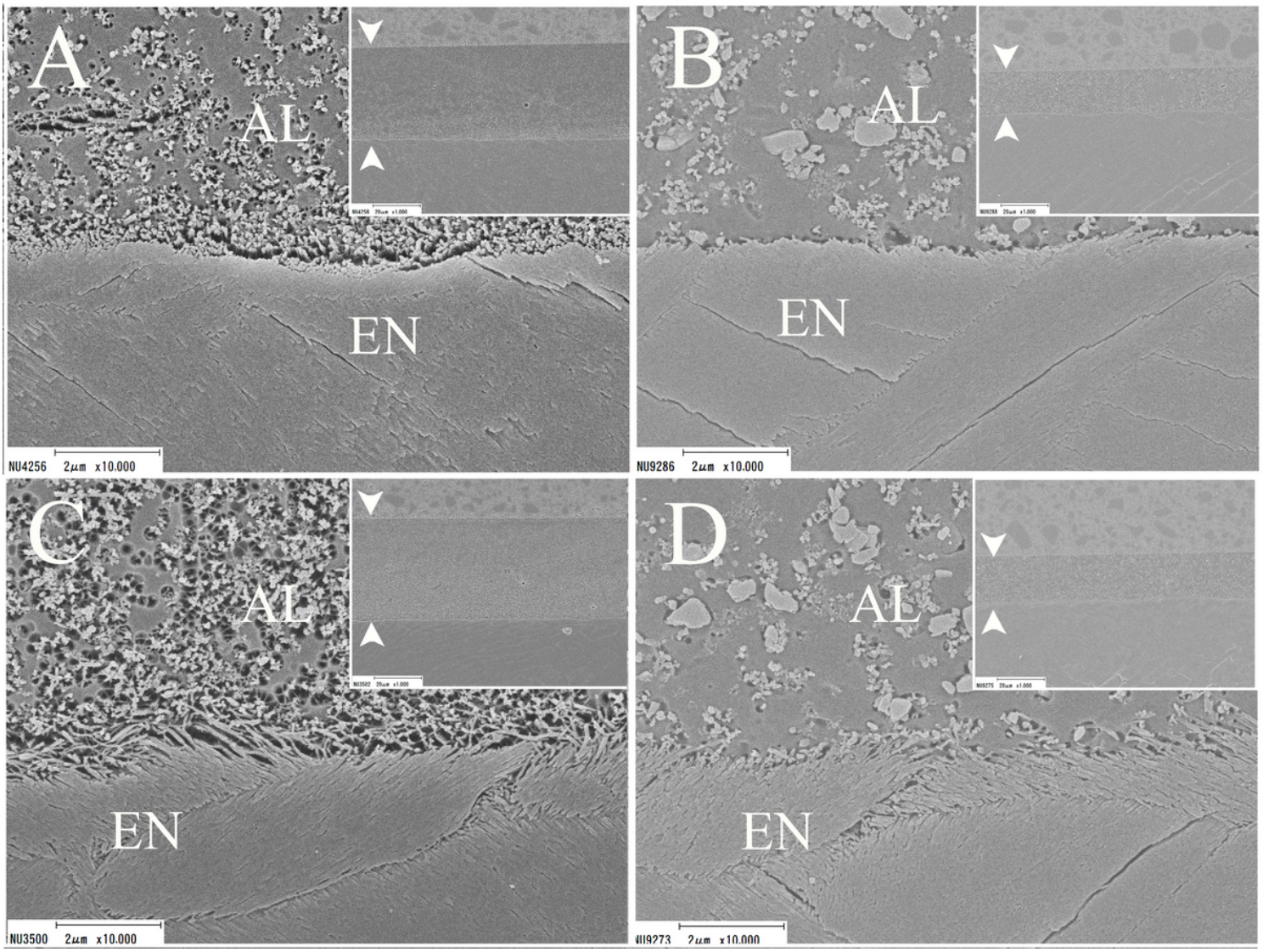

Figure 3

Representative SEM images of the resin/enamel interfaces. A. GU in SE mode (´1,000, ´10,000). B. OX in SE mode (´1,000, ´10,000). C. GU in ER mode (´1,000, ´10,000). D. OX in ER mode (´1,000, ´10,000). GU, G2-Bond Universal; OX, OptiBond XTR; EN, enamel; AL, adhesive layer. The yellow stars indicate the adhesive layer. The arrows indicate a thin high-density layer.

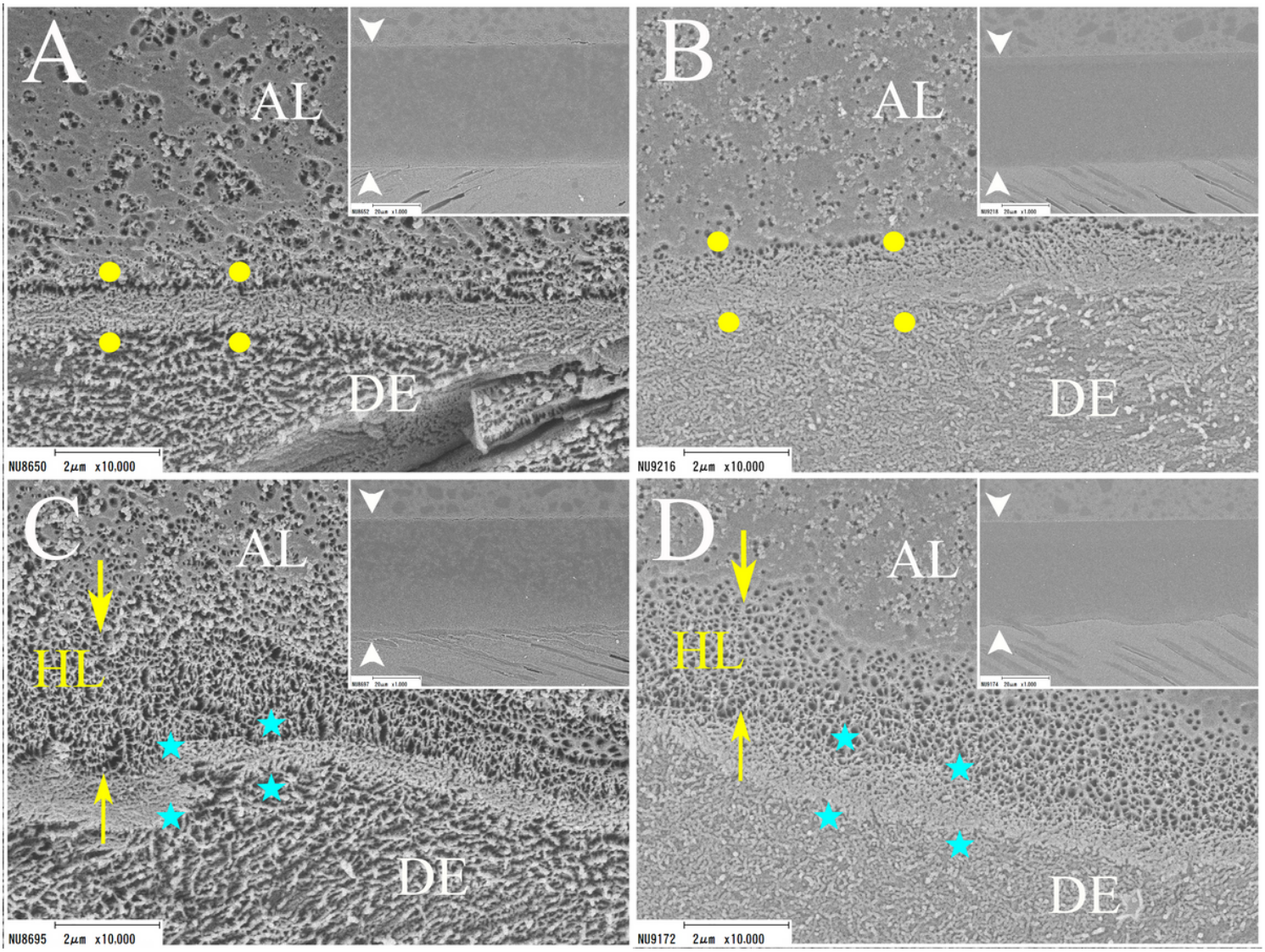

Figure 4

Representative SEM images of resin/dentin interfaces. A. GU in SE mode (´1,000, ´10,000). B. CS in SE mode (´1,000, ´10,000). C. GU in ER mode (´1,000, ´10,000). D. CS in ER mode (´1,000, ´10,000). GU, G2-Bond Universal; CS, Clearfil SE Bond 2; DE, dentin; AL, adhesive layer; HL, hybrid layer. The yellow cycles indicate a thin high-density layer in SE mode. The blue stars indicate a thin high-density layer in ER mode (reaction layer).