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ORIGINAL ARTICLE

Comparison of dentin bond durability of a universal adhesive and two etch-and-rinse adhesive systems

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Abstract

Objective This study aimed to compare dentin bond durability under different degradation conditions between two etch-and-rinse (ER) systems and a universal adhesive in ER mode.

Method This study used a universal adhesive [Scotchbond Universal (SU)], a three-step ER adhesive [Scotchbond Multi-Purpose Plus (SM)], and a two-step ER adhesive [Single Bond Plus (SB)]. A phosphoric acid-etching agent was applied to bovine dentin prior to the application of either a primer or the adhesive. After acid etching, bonding procedures were conducted. The specimens were divided into three group classes: (1) subjected to 10,000, 30,000, or 50,000 thermal cycles (TC); (2) stored in distilled water at 37 °C for 6 months or 1 year (WS); and (3) stored in distilled water for 24 h (baseline). Shear bond strength (SBS) tests were conducted.

Results SB showed a higher baseline SBS than the other adhesives. Defining the baseline SBS value for each adhesive system as 100%, TC groups ranged from 56.1 to 70.3% for SM, from 98.4 to 103.7% for SB, and from 120.3 to 126.7% for SU. WS groups ranged from 66.2 to 71.4% for SM, from 98.1 to 103.3% for SB, and from 102.5 to 118.1% for SU.

Conclusions Although SB showed relatively stable dentin bond performance under all degradation conditions, SM showed decreased dentin SBS with prolonged degradation. SU did not show any significant decrease in SBS from the baseline under any degradation condition.

Clinical relevance The universal adhesive showed comparable adhesive performance with the two-step ER adhesive.

Keywords Dentin bond durability · Universal adhesive · Etch-and-rise systems · Different degradation conditions

Introduction

Dental adhesive systems can be divided into two categories based on etching strategies: etch-and-rinse (ER) and self-etch (SE) [1]. An ER system is defined as including phosphoric acid etching of both the enamel and dentin prior to the application of adhesive [2]. On the other hand, the bonding procedures of SE systems omit this strong acid pre-etching of the dentin substrate. The bonding process of SE systems involves a chemical interaction between hydroxyapatite (HAp) and functional resin monomers, followed by the micromechanical interlocking of the etched dentin [1, 3]. Both systems have been developed over time to simplify their bonding procedures: three- and two-step ER systems and two and single-step SE systems. Omission of the priming procedure in both systems leads to changes not only in the adhesive compositions but also in the application procedures. The formation of a hybrid layer (HL) and resin tags in the dentinal tubules is critical for micromechanical interlocking, which is the main step in the dentin bond process involved in ER systems [4]. The HL is defined as the etched layer above the intact dentin where the adherent smear layer has been removed and the resin monomers have penetrated the demineralized region to form a collagen/resin structure [4]. On the other hand, the incomplete formation of a collagen/resin structure, because of the presence of collagen fibrils that are unprotected by resin monomers, can compromise dentin bond durability [5, 6].

Universal adhesives are fundamentally categorized as SE systems and are similar to single-step SE adhesive systems in

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terms of their adhesive compositions and bonding procedures. However, universal adhesives can be used with either an ER or SE approach for both enamel and dentin, unlike single-step SE systems [7]. Although the use of an ER approach prior to the application of SE adhesives is not a standard dentin-bonding procedure, previous studies of universal adhesives showed that the use of an ER approach for dentin bonding yields a bond strength equal to or greater than the use of an SE approach [8–10]. It is possible that the use of universal adhesives in ER mode may differ not only in the dentin-bonding mechanism but also in dentin bond durability compared with conventional three- or two-step ER systems. However, there have been no direct investigations of whether universal adhesives show better durability in ER mode than conventional ER adhesives.

The purpose of the present study was to compare dentin bond durability in two conventional ER systems and a universal adhesive in ER mode under different degradation conditions. Two different simulated degradation conditions, long-term water storage and thermal cycling, were applied before conducting a shear bond strength (SBS) test. The null hypotheses to be tested were that the universal adhesive in ER mode would not differ from the conventional three- and two-step ER systems in terms of dentin bond durability.

Materials and methods

Study materials

The materials used in this study are shown in Table 1. The universal adhesive used was Scotchbond Universal (SU; 3M Oral Care, St. Paul, MN, USA). The three-step ER adhesive, Scotchbond Multi-Purpose Plus (SM; 3M Oral Care), and the two-step ER adhesive, Single Bond Plus (SB; 3M Oral Care), were used as comparison adhesives. The 35% phosphoric acid pre-etching was performed using Ultra-Etch (Ultradent Products, South Jordan, UT, USA). The microhybrid resin composite, Clearfil AP-X (Kuraray Noritake Dental, Tokyo, Japan), was used for bonding to dentin. A visible-light curing unit with output wavelengths 400 to 505 nm (Optilux 501; sds Kerr, Danbury, CT, USA) was used, and the light irradiance (above 600 mW/cm^2) of the curing unit was checked using a dental radiometer (Model 100, sds Kerr) when making bonded specimens in every experimental group.

Specimen preparation

Extracted permanent bovine incisors were used as substitutes for human teeth. Approximately two-thirds of the apical root structure of each tooth was removed with a diamond disk in a low-speed saw (IsoMet 1000 Precision Sectioning Saw; Buehler, Lake Bluff, IL, USA). The labial surfaces were ground with wet #240-grit silicon carbide (SiC) paper (Fuji Star Type DDC, Sankyo Rikagaku, Saitama, Japan) to create a flat dentin surface. Each tooth was then mounted in self-curing acrylic resin (Tray Resin II; Shofu, Kyoto, Japan) to expose the flattened area. The dentin-bonding surfaces were polished using a water coolant and 240 grit followed by 320-grit SiC paper (Fuji Star Type DDC).

Storage conditions and SBS tests

The SBS values of the adhesives to dentin were determined in accordance with ISO 29022 [11]. The dentin-bonding protocols for each adhesive are shown in Table 2. Thirty-five percent phosphoric acid (Ultra-Etch, Ultradent Products) was applied for 15 s prior to the application of the primer or adhesive, and then removed with water rinsing for 15 s. After phosphoric acid pre-etching, bonding procedures were conducted in accordance with the manufacturer's instructions (Table 2). Regarding the drying techniques used after phosphoric acid etching, we followed the manufacturers' instructions and monitored surface moisture. For SB, excess water remaining on the etched dentin surface was removed by blotting with a small piece of cotton pellet, leaving the surface visibly moist. For SU, rinsed dentin surfaces were air-blown with medium air pressure for 5 s, and no remaining water was visible. For SM, the dentin surface condition after air blowing for 2 s was intermediate between SB and SU. An Ultradent bonding assembly (Ultradent Products) was used to make bonded specimens. Following the application of the adhesive to the dentin-bonding sites, bonded resin composite cylinders were formed on the adherent surfaces by clamping plastic molds (2.38 mm internal diameter and 2.0 mm height, Ultradent Products) in a fixture against the dentin surfaces. The resin composite was placed into the mold, and light irradiation was performed for 30 s. After removal of the mold, the bonded specimens were subjected to various numbers of thermal cycles (TCs; TC groups) or storage for various times in distilled water at 37 °C (WS groups). For the TC groups, bonded specimens were stored in distilled water at 37 °C for 24 h and then subjected to 10,000, 30,000, or 50,000 TCs between 5 and 55 °C, with a dwell time of 30 s. Bonded specimens of the WS groups were stored in distilled water at 37 °C for 6 months or 1 year prior to the SBS tests. The antibiotic-free storage water was changed every week during the experiments. Baseline specimens were stored in distilled water at 37 °C for 24 h before the SBS tests (baseline or control group).

After thermal cycling or storage, 15 bonded specimens per test group were loaded until failure at a rate of 1.0 mm/min using a universal testing machine (Type 5500R; Instron, Canton, MA, USA). The SBS values (in MPa) were calculated from the peak load at failure divided by the bonded surface area. After testing, the bonded tooth surfaces and resin composite were observed under an optical microscope (SZH-131;

Table 1 Materials used in this study

Code	Universal adhesive	Main components	pH	Manufacturer
SU	Scotchbond Universal Lot No. 666964	bis-GMA (15–25 wt%), HEMA (15–25 wt%), silane treated silica (nanofiller; 10–20 wt%), ethanol (10–15 wt%), water (10–15 wt%), MDP (5–15 wt%), Vitrebond copolymer (1–5 wt%), CQ, silane	2.7	3M Oral Care, St. Paul, MN, USA
Code	Etch-and-rinse adhesive			
SM	Scotchbond Multi-purpose plus (three-step) Lot No. N852287 (Primer) Lot No. N86909 (Adhesive)	Primer: water (40–50 wt%), HEMA (35–45 wt%), polyalkenoic acid (10–20 wt%), adhesive: bis-GMA (60–70 wt%), HEMA (30–40 wt%) , triphenylantimony, amines	Primer: 3.3	3M Oral Care
SB	Single Bond Plus (two-step) Lot No. N898889	Ethanol (25–35 wt%), bis-GMA (10–20 wt%), silane-treated silica (nanofiller; 10–20 wt%), HEMA (5–15 wt%), Vitrebond copolymer (5–10 wt%), GDMA (5–10 wt%), UDMA (< 5 wt%), water (< 5 wt%), diphenyliodonium hexafluorophosphate (< 1 wt%), EDMAB (< 1 wt%), CQ	4.7	3M Oral Care
	Pre-etching agent			
	Ultra-Etch Lot No. G017	35% phosphoric acid		Ultradent Products, *South Jordan, UT, USA

MDP 10-methacryloyloxydecyl dihydrogen phosphate, *bis-GMA* 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy)phenyl] propane, *HEMA* 2-hydroxyethyl methacrylate, *GDMA* glycerol dimethacrylate, *EDMAB* ethyl 4-dimethyl aminobenzoate, *CQ dl*-camphorquinone

Olympus, Tokyo, Japan) at a magnification of 10× to determine the failure mode. On the basis of the percentage of substrate area (adhesive − resin composite − dentin) observed at the debonded resin composite and tooth-bonding sites, the types of bond failure were recorded as (1) adhesive failure, (2) cohesive failure of the composite, (3) cohesive failure of the dentin, or (4) mixed failure—partially adhesive and partially cohesive.

Scanning electron microscopy observations

The specimens for observing resin/dentin interfaces were prepared as for the bond strength test described above. The bonded specimens were stored at 37 °C in distilled water for 24 h, embedded in epoxy resin, and then longitudinally sectioned with a diamond saw (IsoMet 1000 Precision Sectioning Saw). The sectioned surfaces were polished to a high gloss with abrasive disks (Fuji Star Type DDC) followed by diamond pastes (DP-Paste; Struers, Ballerup, Denmark) with a final particle size of 0.25 μm. Half of the polished specimens were etched with HCl solution (6 mol/L) for 25 s and deproteinized by immersion in a 6% NaOCl solution for 3 min to visualize the internalized resin tags clearly. All 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 from the final 100% bath to a chamber of freeze-drying system (Model ID-3; Elionix, Tokyo, Japan) for 30 min. The resin/dentin 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 perpendicularly to the polished surfaces. Finally, all SEM specimens were coated with a thin film of gold in a vacuum evaporator (Quick Coater, Type SC-701, Sanyu Denchi, Tokyo, Japan) and observed by FE-SEM (ERA-8800FE, Elionix) at an operating voltage of 10 kV. The following aspects of the images were

Table 2 Bonding procedures for the tested adhesives

Universal adhesive	**Adhesive application protocol**
SU in ER mode	Dentin surface was phosphoric acid etched for 15 s. Etched surface was rinsed with water for 15 s (three-way dental syringe). Adhesive was applied to air-dried dentin surface with rubbing motion for 20 s and then medium air pressure applied to surface for 5 s. Light irradiated for 10 s.
ER adhesives	**Adhesive application protocol**
SM (three-step)	Dentin surface was phosphoric acid etched for 15 s. Etched surface was rinsed with water for 15 s. Air-dried gently for 2 s. Left moist. Primer was applied to dentin. Air-dried gently for 5 s. Adhesive was applied to dentin. Light cured for 10 s.
SB (two-step)	Dentin surface was phosphoric acid etched for 15 s. Etched surface was rinsed and blotted dry. Priming adhesive was applied to dentin for 15 s. Air-dried gently for 5 s. Light cured for 10 s.

evaluated: thickness of the adhesive layer (AL), thickness of the HL, lengths of the internalized resin tags, and alterations near the interface between the AL and the dentin substrate.

Statistical analysis

Before analyses of variance (ANOVA), homogeneity of variance (Bartlett's test) and normal distribution (Kolmogorov–Smirnov test) were confirmed for each group. Differences in SBS values among the different groups were analyzed using a two-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference test ($\alpha = 0.05$). Statistical analyses were performed using Sigma Plot software, version 11.0 (SPSS, Chicago, IL, USA).

Results

Shear bond strength of the thermal cycle groups

Obtained SBS values under thermal cycling conditions are shown in Table 3. Two-way ANOVA revealed that the type of adhesive system significantly influenced the SBS values ($P < 0.001$). On the other hand, the number of TCs did not influence the SBS values ($P = 0.071$). The two-way interaction between the type of adhesive system and the number of TCs was significant ($P < 0.001$).

The lowest mean SBS value in SU was 36. 0 (4.0) in the 24-h group, and the highest one was 45.6 (2.4) in the TC 30,000 group. The corresponding values in SM were 20.6 (3.8) in the TC 50,000 group and 36.7 (2.9) in the 24-h group, respectively. The corresponding values in SB were 42.1 (1.4) in the TC 10,000 group and 44.4 (2.4) in the TC 50,000 group, respectively. Defining the baseline (24 h) SBS value for each tested adhesive system as 100%, the SBS values under thermal cycling conditions ranged from 56.1 to 70.3% for SM, from 98.4 to 103.7% for SB, and from 120.3 to 126.7% for SU (Table 3). For SU, the TC groups showed significantly higher SBS values than the control group. For SM, the SBS values decreased with increasing TCs, and 50,000 TCs showed a significantly lower SBS value than both the control and 10,000 TC groups. For SB, no significant differences were found in SBS values in any of the TC groups. In the control group, SB showed significantly higher SBS values than the other adhesives. In the groups subjected to 50,000 TCs, no significant differences in SBS values were found between SB and SU, and SM showed a significantly lower SBS value than SB and SU.

Shear bond strength of the water storage groups

Results for the SBS values under WS conditions are shown in Table 4. Two-way ANOVA revealed that both WS period and the type of adhesive system significantly influenced dentin SBS values ($P < 0.001$). The two-way interaction between these factors was significant ($P < 0.001$).The lowest mean SBS value in SU was 36. 0 (4.0) in the 24-h group, and the highest one was 42.5 (2.1) in the 6-month group. The corresponding values in SM were 24.3 (5.2) in the 1-year group and 36.7 (2.9) in the 24-h group, respectively. The corresponding values in SB were 42.0 (4.6) in the 1-year group and 44.2 (6.9) in the 6-month group, respectively. Defining the baseline (24 h) dentin SBS value for each tested adhesive system as 100%, the SBS values under WS conditions ranged from 102.5 to 118.1% for SU, from 66.2 to 71.4% for SM, and from 98.1 to 103.3% for SB (Table 4). The three tested adhesives showed differences in SBS values under WS conditions over time. SU showed significantly higher SBS values in the 6-month group than those in the 24-h and 1-year groups. However, SM showed lower SBS values with higher WS periods, and SB did not show any significant differences in SBS values over time.

Failure mode analysis of debonded specimens

The frequencies of different failure modes after SBS tests for all groups are shown in Fig. 1. The frequency of the failure modes of each group showed different trends in different adhesive systems, degradation conditions, and length of storage. For the control group, although mixed or cohesive failure of the dentin was found for SM and SB, SU showed only adhesive failure. However, both mixed and cohesive failures were found for SU in the TC and WS groups. For SM, adhesive

Table 3 Influence of thermal cycling on dentin bond strength (MPa)

	24 h	TC 10,000	TC 30,000	TC 50,000
SU	36.0 (4.0)^{bB} [100%]	44.0 (3.1)^{aA} [122.2%]	45.6 (2.4)^{aA} [126.7%]	43.3 (1.8)^{aA} [120.3%]
SM	36.7 (2.9)^{bA} [100%]	25.8 (5.5)^{bB} [70.3%]	23.9 (6.4)^{bBC} [65.1%]	20.6 (3.8)^{bC} [56.1%]
SB	42.8 (2.9)^{aA} [100%]	42.1 (1.4)^{aA} [98.4%]	43.3 (2.8)^{aA} [101.2%]	44.4 (2.4)^{aA} [103.7%]

$N = 15$, 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 indicate standard deviation

Table 4 Influence of water storage on dentin bond strength (MPa)

	24 h	6 months	1 year
SU	36.0 (4.0)^{bB} [100%]	42.5 (2.1)^{aA} [118.1%]	36.9 (3.6)^{bB} [102.5%]
SM	36.7 (2.9)^{bA} [100%]	26.2 (4.7)^{bB} [71.4%]	24.3 (5.2)^{cB} [66.2%]
SB	42.8 (2.9)^{aA} [100%]	44.2 (6.9)^{aA} [103.3%]	42.0 (4.6)^{aA} [98.1%]

N = 15, 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 indicate standard deviation

failure was observed for all TC groups and in the 1-year WS group. SB showed a similar trend under both degradation conditions: both mixed and cohesive failures of the dentin were observed for all TC groups and all storage durations.

SEM observations

Representative SEM images of the resin/dentin interfaces are shown in Fig. 2. In the SEM images after argon-ion etching (Fig. 2a, c, e), the thickness of the AL of the SM (40–50 μm) was four to five times greater than that of SU and SB. In addition, SM and SB showed a homogeneous AL, but SU showed a heterogeneous AL due to the inclusion of nanofillers. All tested adhesives had a 2- to 3-μm-thick HL between the AL and the dentin substrate. Although a high-density layer below the HL was not observed clearly in SM and SB, SU showed a thin, high-density layer (Fig. 2a, arrow).

In the SEM images of the demineralized and deproteinized interfaces, no clear differences were found between the adhesive systems in terms of their morphological features near the interface (Fig. 2b, d, f). For all adhesives, dense resin tags longer than 50 μm and the HL were observed. In addition, adhesive penetration into the branches of the dentinal tubules was observed for all the adhesives.

Representative SEM images of the resin side of the debonded specimens are shown in Fig. 3. The appearance of the failure pattern was dependent on the storage conditions and adhesive system. For the 24-h groups, a similar morphological appearance was observed for SM and SB (Fig. 3b, c). SM and SB exhibited more cracks and cleavages in the adhesives and more retained portions of resin tags compared with SU. In addition, attached dentin fragments were more clearly observed for SM and SB. For the groups subjected to 50,000 TCs, SB and SU (Fig. 3d, f) showed complicated failure patterns with cracks and cleavages and clear evidence of resin tags. However, SM showed clean detachment at the adhesive–dentin interface, with resin tags broken off very close to the surface (Fig. 3e). SM had a similar appearance in both the 1-year WS and 50,000 TC groups (Fig. 3h). That is, detachment at the adhesive–dentin interface was observed, and the resin tags were broken off at the interface. However, SB did not exhibit any clear differences between the different storage conditions (Fig. 3c, f, i).

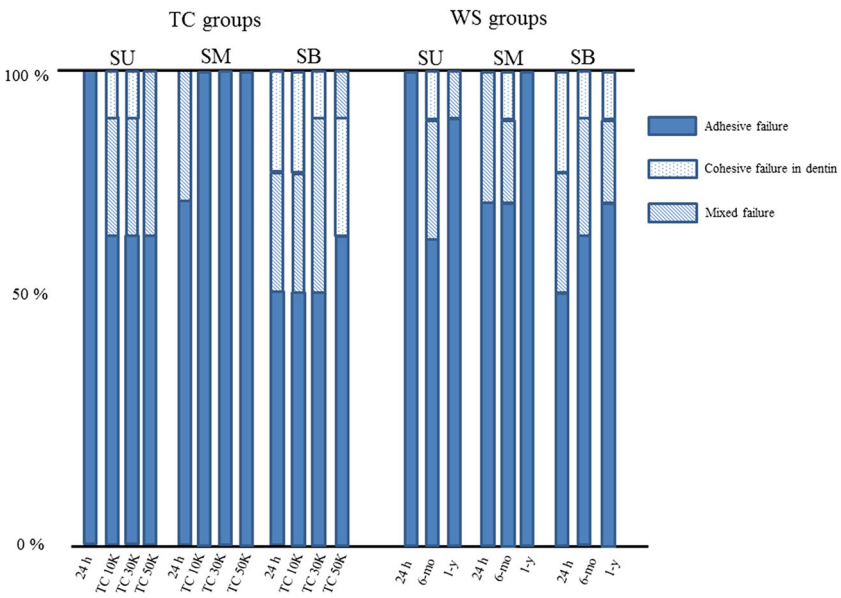

Fig. 1 Failure mode analysis of the de-bonded dentin specimens. Abbreviations: SU: Scotchbond Universal, SM: Scotchbond Multi-purpose plus, SB: Single Bond Plus, TC: thermal cycle, WS: water storage

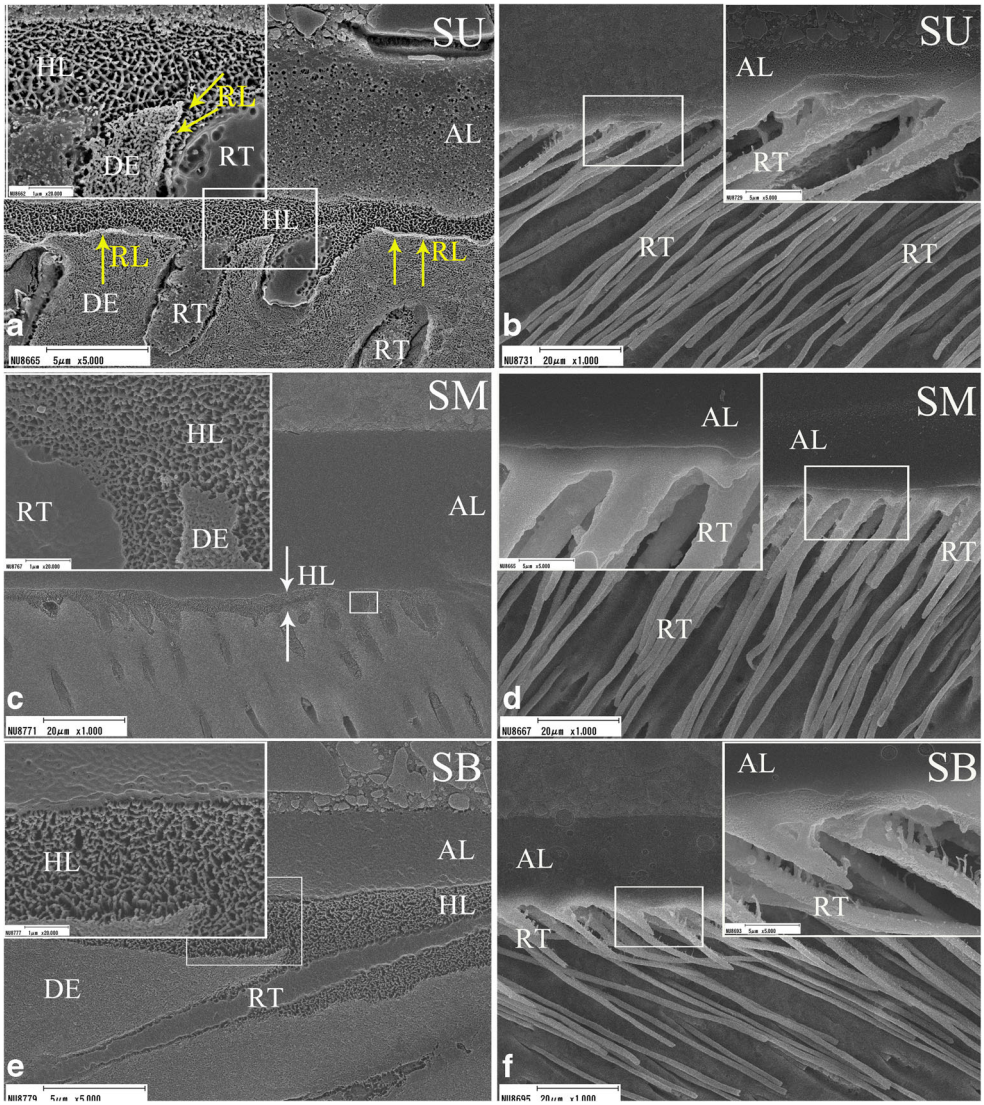

Fig. 2 Representative SEM images of resin dentin interface. The visible material is indicated by abbreviations: SU: Scotchbond Universal, SM: Scotchbond Multi-purpose plus, SB: Single Bond Plus, AL: adhesive layer, HL: hybrid layer, RL: reaction layer, DE: dentin, RT: resin tag. **a** Resin dentin interface with SU after argon-ion etching (5000× and 20,000×). **b** Resin dentin interface with SU after demineralized and deproteinized (1000× and 5000×). **c** Resin dentin interface with SM after argon-ion etching (1000× and 20,000×). **d** Resin dentin interface with SM after demineralized and deproteinized (1,000× and 5000×). **e** Resin dentin interface with SB after argon-ion etching (5000× and 20,000×). **f** Resin dentin interface with SB after demineralized and deproteinized (1000× and 5000×)

Discussion

Bovine teeth were used in this study. Although conflicting data exist regarding whether bovine teeth can be considered an appropriate substitute for human teeth in dental research, there have been many studies that showed no significant differences in shear dentin bond strength between human teeth and bovine teeth [12]. The advantage of using bovine teeth instead of human teeth is that they are easy to obtain in large quantities in good condition and have a less variable composition than human teeth. Further, bovine teeth have large flat surfaces and have not had prior caries challenges that might affect the test results. Therefore, bovine dentin was used as a substitute for human dentin in this study, as in previous studies [12]. Although the three adhesives used in this study were produced by the same manufacturer, their dentin-bonding mechanisms and adhesive application procedures are completely different. The main purpose of this study was to investigate these different bonding mechanisms and their influence on dentin bond durability based on SBS tests under different degradative storage conditions. In addition, SEM was performed to identify the bonding mechanism from the perspective of an adhesive's distinct morphological features.

Other bond testing methods are also used. μ-TBS also provides useful data, but it is sensitive to localized degradation of the bond, while SBS testing averages over the whole bonding surface. For SBS testing, mold-enclosed SBS has many advantages when measuring immediate bond strength or conducting fatigue testing [13]. However, as the mold cannot be removed and replaced, it would need to be left in place for the degradation procedures. The presence of a stainless-steel mold would have a large influence on degradation, so this technique is not appropriate for this study.

Thermal cycling followed by bond strength testing is considered a simulation of oral conditions in terms of changes in temperature [14], and a previous report by Gale et al. [15],

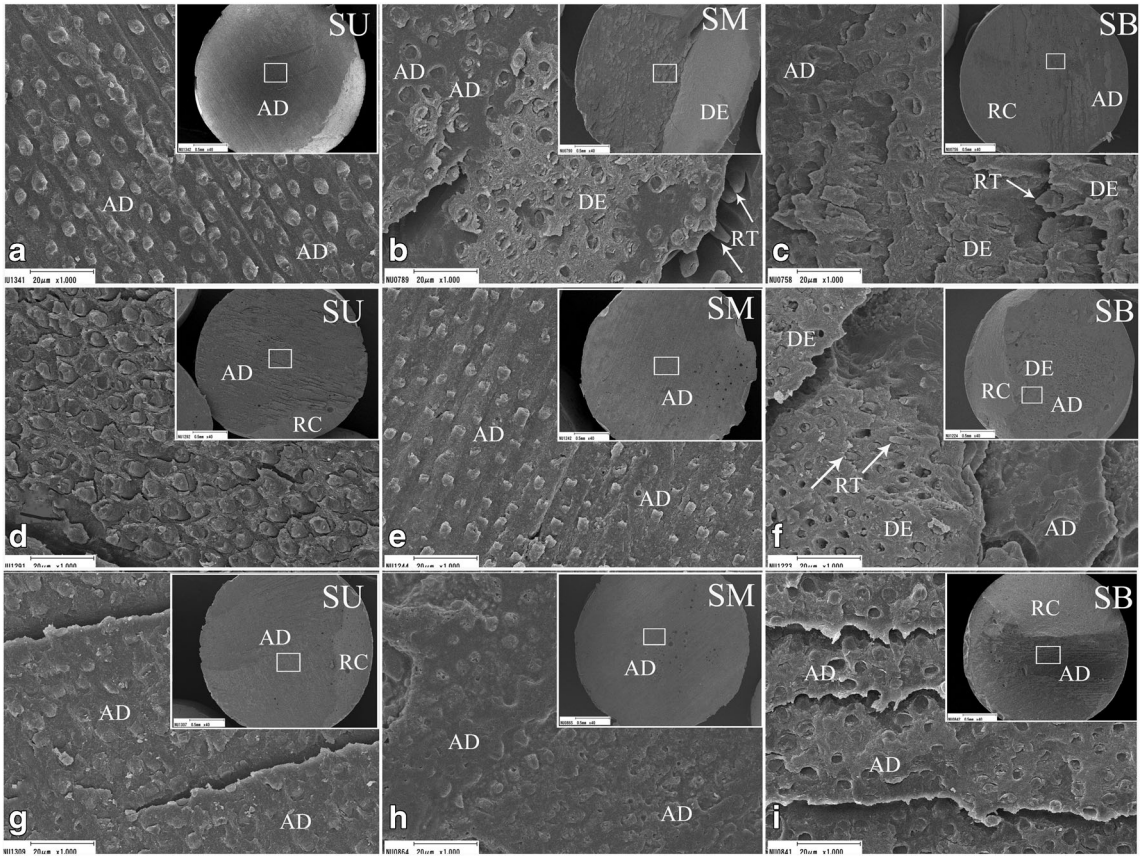

Fig. 3 Representative SEM images of the failure site after different degradation conditions. The visible material is indicated by abbreviations: AD: adhesive, DE: dentin, RC: resin composite, RT: resin tag. **a** SU at 24-h water storage (40× and 1000×). **b** SM at 24-h water storage (40× and 1000×). **c** SB at 24-h water storage (40× and 1000×). **d** SU at 50,000 TC cycles (40× and 1000×). **e** SM at 50,000 TC cycles (40× and 1000×). **f** SB at 50,000 TC cycles (40× and 1000×). **g** SU at 1-year water storage (40× and 1000×). **h** SM at 1-year water storage (40× and 1000×). **i** SB at 1-year water storage (40× and 1000×)

approximately 10,000 thermal cycles are equivalent to 1 year in intraoral conditions. The results of SBS tests under TC conditions indicate that SBS is adhesive dependent. The bond strength of SM decreased with increasing numbers of TCs: the 50,000 TC group showed a significantly lower SBS value than the control and 10,000 TC groups. On the other hand, SB did not show any significant differences in SBS values among the tested periods, and the TC groups for SU showed significantly higher SBS values than those at baseline. Under TC conditions, deterioration at the resin/dentin interface was accelerated by differences in the thermal expansion of the materials composing the bonded interfaces [14]. The discrepancies in thermal expansion between the dentin and the adhesives might lead to cracks at bonded interfaces due to mechanical stress from temperature changes [16]. Considering the bonding procedures of the tested adhesives, SM requires separate priming and bonding procedures, but the other adhesives do not. In theory, a thick hydrophobic AL might have more resistance to hydrolytic degradation and mechanical stress than a hydrophilic AL found in a two-step ER adhesive, a single-step SE adhesive, and a universal adhesive [17–20]. However, SM, a three-step ER adhesive, showed decreased SBS values with increased numbers of TCs. We speculate that although a thicker and more hydrophobic AL might effectively prevent degradation from mechanical forces and water absorption, a thicker AL might induce greater dimensional alterations due to expansion and contraction from temperature changes, resulting in the deterioration of the bonded interface [21]. On the other hand, the universal adhesive SU showed a significantly higher SBS value in the 10,000 TC group compared with that of the baseline group, and the SBS values were unchanged following any number of TCs. This phenomenon might be explained by post-curing effects on the AL and chemical reactions with HAp. In particular, the post-curing effects on SU may be greater than those on the other adhesives. SU has the lowest pH value among the tested adhesives due to inclusion of the functional monomer MDP, which may lead to poor polymerization at the early stage used to determine baselines values. However, the mechanical properties of the AL appear to increase over time due to the post-curing effect, resulting in SBS values increasing by 22% in the 10,000 TC group compared with those in the baseline group.

The pattern of SBS changes in SM and SB under WS degradation conditions was similar to those under TC degradation conditions. Although no significant reduction in SBS was observed for SU in the 1-year WS group compared with that in the

baseline group, the 1-year WS group showed a significantly lower SBS value than the 6-month WS group. Therefore, the null hypothesis that the universal adhesive in ER mode would not differ from conventional three- and two-step ER systems in terms of dentin bond durability was rejected.

The reason for the different outcomes in different adhesives is thought to be related to their component properties. In particular, the amount of 2-hydroxyethyl methacrylate (HEMA) and retained water in the AL might contribute to hydrolytic degradation over time [22–24]. It is notable that the 1-year WS group for SM showed the highest reduction in SBS values (~ 35%) compared with the baseline group, despite separate bonding procedures. Among the tested adhesives, SM contains much more HEMA (30–40 wt%) than the other adhesives. In addition, the bonding procedure makes it likely that water and HEMA might remain at the interface between the primed dentin and the AL. Although hydrophilic HEMA helps the resin monomer penetrate the demineralized dentin due to better compatibility with water-rich conditions, it is thought to be susceptible to hydrolytic degradation over time [22, 25]. This speculation is supported by SEM observations of the failure mode. When comparing the failure patterns of SM in the 50,000 TC and 1-year WS groups with those of the other adhesives, detachment at the adhesive–dentin interface was observed and the resin tags were broken off at the interface.

In this study, the bonding procedures for SU were the same as for SB; that is, the application of adhesive was performed after phosphoric acid pre-etching. However, SB showed more stability under both TC and WS degradation conditions than SU. SB contains a lower percentage of HEMA (5–15 wt%) than the other tested adhesives and has a low water content (< 5%). Furthermore, a higher ethanol percentage (25–35 wt%) might induce the evaporation of the retained water in the AL. On the other hand, SU contains 10–15% water, which helps ionize the functional resin monomer, and it is difficult to completely remove water from the AL. The remaining water may jeopardize bond durability during long-term water storage [22]. All tested adhesives contain the polyalkenoic acid copolymer, namely Vitrebond copolymer. This copolymer is thought to bond chemically with Ca^{2+} in dentin HAp and contribute to long-term bond durability [26–29]. Sezinando et al. investigated the chemical interaction between synthetic HAp and vitrebond-copolymer-containing adhesives using FTIR and $^{13}C/^{31}P$ NMR spectroscopy [30]. These authors did not detect any chemical interactions between Vitrebond copolymer and HAp in SU, in contrast to SB. In addition, they argued that Vitrebond in SU did not function effectively because of its lower concentration and competition with 10-methacryloyloxydecyl dihydrogen phosphate (MDP) [29]. It is unclear how free (unbound) Vitrebond copolymer behaves in the AL over time, but such a component might elicit negative effects during long-term water storage because of its hydrophilicity.

In general, decalcified dentin is thought to prevent the establishment of strong chemical interactions in SE systems due to reduced HAp on its surface [31]. However, many in vitro studies have shown little to no difference in the dentin bond strengths of universal adhesives between SE mode and ER mode [8–10]. Our study indicated that universal adhesive in ER mode had better dentin bond durability than the three-step ER adhesive SM.

SEM results showed no clear differences between the adhesive systems in terms of their morphological appearance near the interface after demineralization and deproteinization. The tested adhesives showed similar internalized resin tags. However, morphological appearances below the HL differed between SU and other ER adhesives. SU showed a thin high-density layer, the so-called reaction layer, below the HL. Creation of the reaction layer might be a key phenomenon in the mechanism by which dentin binds to the universal adhesive in ER mode. This layer might be evidence of a chemical interaction between the functional resin monomer and the intact dentin substrate below the decalcified dentin.

Another explanation for the better results of SU might be the interaction between MDP and collagen fibrils. Although the HL plays an important role in micromechanical interlocking, there are concerns about scaffold stability due to hydrolysis and enzymatic action because the collagen fibrils are unprotected by resin monomers [5, 6]. It is well known that MDP stably interacts with collagen because of its hydrophobic interactions with the collagen surface [32]. However, further research is needed to clarify the contributions of the reaction layer and the interaction between the functional resin monomer and naked collagen to dentin bond durability.

The results of this experiment suggest that high levels of HEMA may make an adhesive more susceptible to hydrolytic degradation and that MDP may form a strongly bonded layer that increases durability. However, further work is required to investigate these possible mechanisms.

Compliance with ethical standards

Conflict of interest The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service and/or company that is presented in this article.

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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.

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