Dentin bond durability of a universal adhesive and two etch-and-rinse adhesive systems under different degradation conditions

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Contents

This thesis is based on the published articles listed below with additional data.

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Summary

Dental adhesive systems can be divided into two categories based on etching strategies: etch-and-rinse (ER) and self-etch (SE) systems. 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 composition but also in the application procedure.

Universal adhesives are fundamentally categorized as SE systems and are similar to single-step SE adhesive systems in 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. Although the use of an etchant prior to the application of SE adhesives is not a standard dentin bonding procedure, previous studies of universal adhesives showed that the use of this approach for dentin bonding yields a bond strength equal to or greater than the use of an SE approach. 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 universal adhesive used was Scotchbond Universal (SU, 3M Oral Care). 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 pre-etching was performed with 35% phosphoric acid using Ultra-Etch (Ultradent Products).

Bond specimen preparation and shear bond strength (SBS) test were conducted according to the ISO 29022 specification. Bovine incisors were used as substitute for human teeth. The dentin surfaces were wet ground using a sequence of silicone carbide (SiC) papers ending with #320-grit. For the prepared dentin surfaces, Ultra-Etch was applied for 15 s prior to the application of the primer or adhesive, and then removed by rinsing with water for 15 s. After phosphoric acid pre-etching, bonding procedures were conducted in accordance with the manufacturer's instructions. An Ultradent bonding assembly was used in this study. Following adhesive application to the dentin adherent surface, the resin composite was condensed into a mold, and light irradiated for 30 s. Fifteen specimens of each test group were prepared, and SBS was measured to determine the bonding durability after different degradation conditions, such as thermal cycling (TC) or long-term water storage (WS). The bonded specimens were divided into three groups: 1) specimens subjected to TC, where the bonded specimens were subjected to 10,000, 30,000 or 50,000 TC between 5°C and 55°C, with a dwell time of 30 s; 2) specimens stored in 37°C distilled water for 6-month, 1-year, or 2-year; and 3) specimens stored in 37°C distilled water for 24 h, serving as a baseline. The SBS tests were conducted using a universal testing machine at a cross-head speed of 1.0 mm/min. After testing, the bonding sites on the tooth surfaces and the resin composite cylinders were observed to determine the failure mode.

Representative resin-dentin interfaces and debonded fracture sites were observed using scanning electron microscopy (SEM). For ultrastructural morphological observations of the resin/dentin interfaces, bonded specimens were embedded in epoxy resin, and then longitudinally sectioned. The sectioned surfaces were polished to a high gloss with SiC papers followed by diamond pastes down to a particle size of 0.25 μm. The polished specimens were dehydrated in ascending grades of *tert*-butyl alcohol and then transferred to the chamber of a freeze-drying system for 30 min. 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. The other sectioned resin-dentin interfaces were subjected to argon-ion beam etching for 40 s with the beam directed perpendicular to the polished surfaces. The d-bonded specimens from each storage condition were prepared directly for SEM. Finally, all SEM specimens were coated with a thin film of gold in a vacuum evaporator, and observations were performed under SEM at an operating voltage of 10 kV.

Defining the baseline (24 h) SBS value for each tested adhesive system as 100%, the SBS values under thermal cycling conditions ranged from 120.3% to 126.7% for SU, from 98.4% to 103.7% for SB, and 56.1% to 70.3% for SM. For SU, the TC groups showed significantly higher SBS values than the control group. For SM, the SBS values decreased with increasing TCs, and TC 50,000 showed a significantly lower SBS value than bo0th the control and TC 10,000 groups. For SB, no significant differences were found in SBS values in any of the TC groups. In the baseline group, SB showed significantly higher SBS values than the other adhesives. In the groups subjected to TC 50,000, no significant differences in SBS values were found between SB and SU, and SM showed a significantly lower SBS value than SB and SU.

Defining the baseline (24 h) dentin SBS value for each tested adhesive system as 100%, the SBS values under WS conditions ranged from 84.4% to 118.1% for SU, from 87.9% to 103.3% for SB, and from 64.3% to 71.4% for SM. 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 in the other WS groups. However, SM showed lower SBS values with higher WS periods, and SB did not show any significant differences in SBS values over time, apart from the 2-year WS group. The frequency of the failure modes of

each group showed different trends in different adhesive systems, degradation conditions, and length of storage.

In the SEM images after argon-ion etching, the thicknesses of the adhesive layer (AL) were material dependent, and the three-step ER adhesive SM formed a thicker adhesive layer than the other adhesives. 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 hybrid layer (HL) between the AL and the dentin substrate. Although a high-density reaction layer (RL) below the HL was not observed clearly in SM and SB, SU showed a thin, high-density RL. 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 and dense resin tags longer than 50 μm and the HL was observed in all the adhesives. After the SBS test, the appearance of the failure pattern was dependent on the storage conditions and adhesive system.

This laboratory study clearly indicated that SBS was adhesive and degradation period dependent. Although the two-step ER adhesive SB showed relatively stable dentin bond performance under two different degradation conditions, the three-step ER adhesive SM showed decreased dentin SBS with prolonged storage periods in both degradation conditions. The universal adhesive SU did not show any significant decrease in SBS from the baseline under any degradation condition, apart from the 2-year WS group. Therefore, the universal adhesive showed comparable adhesive performance with the two-step ER adhesive. In the SEM images, all tested adhesives had a 2- to 3-μm-thick hybrid layer (HL) between the AL and the dentin substrate, and a high-density reaction layer (RL) below the HL was observed clearly in SU.

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 composition but also in the application procedure. 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 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 etchant 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 hypothesis to be tested was 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. The phosphoric acid pre-etching agent (Ultra-Etch, Ultradent Products) was applied for 15 s prior to the application of the primer or adhesive, and then removed by rinsing with water 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, the manufacturer's instructions were followed and surface moisture was monitored. 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°C and 55°C, with a dwell time of 30 s. Bonded specimens in the WS groups were stored in distilled water at 37°C for 6-month, 1-year, or 2-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, Norwood, 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; 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 (SEM) observation

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 the low-speed saw. The sectioned surfaces were polished to a high gloss with abrasive discs (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 a 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 Denshi, Tokyo, Japan) and observed by FE-SEM (ERA-8800FE; Elionix) at an operating voltage of 10 kV. The following aspects of the images were 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 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 (SBS) of the thermal cycle (TC) groups

The SBS values obtained 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) MPa in the 24 h group, and the highest one was 45.6 (2.4) MPa in the TC 30,000 group. The corresponding values in SM were 20.6 (3.8) MPa in the TC 50,000 group and 36.7 (2.9) MPa in the 24 h group. The corresponding values in SB were 42.1 (1.4) MPa in the TC 10,000 group and 44.4 (2.4) MPa in the TC 50,000 group. Defining the baseline (24 h) SBS value for each tested adhesive system as 100%, the SBS values under thermal cycling conditions ranged from 120.3% to 126.7% for SU, from 98.4% to 103.7% for SB, and 56.1% to 70.3% for SM (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 TC 50,000 showed a significantly lower SBS value than both the control and TC 10,000 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 TC 50,000, 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 (SBS) of the water storage (WS) 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 30.4 (4.6) MPa in the 2-year group, and the highest one was 42.5 (2.1) MPa in the 6-month group. The corresponding values in SM were 23.6 (4.7) MPa in the 2-year group and 36.7 (2.9) MPa in the 24 h group. The corresponding values in SB were 37.6 (2.6) MPa in the 2-year group and 44.2 (6.9) MPa in the 6-month group. Defining the baseline (24 h) dentin SBS value for each tested adhesive system as 100%, the SBS values under WS conditions ranged from 84.4% to 118.1% for SU, from 87.9% to 103.3% for SB, and from 64.3% to 71.4% for SM (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 other WS groups. SM showed lower SBS values with higher WS periods. Although SB did not show any significant differences in SBS values until the 1-year period, the 2-year group showed a significantly lower SBS value than the other groups. In the groups subjected to 2-year water storage period, SM showed a significantly lower SBS value than SB and SU, as for the TC 50,000 group.

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 baseline 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 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 (Figs. 2A, 2C, and 2E), the thickness of the AL of the SM (40–50 μm) was four to five times greater than that of SU and SB (note that 2C is shown at a different scale from 2A and 2E, to include the entire thickness of the AL in the image). 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 (Figs. 2B, 2D, and 2F). 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 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 (Figs. 3B and 3C). 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 TC 50,000, SB and SU (Figs. 3D and 3F) 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 TC 50,000 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 (Figs. 3C, 3F, and 3I).

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.

Thermal cycling followed by bond strength testing is considered a simulation of oral conditions in terms of changes in temperature (13), and a previous report by Gale *et al.* (14) stated that approximately 10,000 TCs were 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 TC 50,000 group showed a significantly lower SBS value than the baseline and TC 10,000 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 (13). Discrepancies in thermal expansion between the dentin and the adhesives might lead to cracks at bonded interfaces due to mechanical stress from temperature changes (15). 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 the hydrophilic AL found in two-step ER adhesives, single-step SE adhesives, and universal adhesives (16–19). However, SM, a three-step ER adhesive, showed decreased SBS values with increased numbers of TCs. It was speculated 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 (20). On the other hand, the universal adhesive SU showed a significantly higher SBS value in the TC 10,000 group than in 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 10-methacryloyloxydecyl dihydrogen phosphate (MDP), which may lead to poorer polymerization at the early stage used to determine baseline values (21). However, the mechanical properties of the AL appear to increase over time due to post-curing effects, resulting in SBS values increasing by 22% in the TC 10,000 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 2-year WS group for SM showed the highest reduction in SBS values compared with the baseline group, despite separate bonding procedures. Among the tested adhesives, SM contains by far the highest level of HEMA (30–40 wt%). 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 TC 50,000 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 wt%). 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 ionizing 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 a 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.* (30) investigated the chemical interaction between synthetic HAp and vitrebond-copolymer-containing adhesives using FTIR and $^{13}C/^{31}P$ NMR spectroscopy. 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 MDP (29). It is unclear how pendant Vitrebond copolymer would behave 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). This 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.

Conclusions

Within the limitations of the present study, the SBS tests under different degradation conditions indicated that SBS was adhesive and degradation period dependent. Although the two-step ER adhesive SB showed relatively stable dentin bond performance under two different degradation conditions, the three-step ER adhesive SM showed decreased dentin SBS with prolonged storage periods in both degradation conditions. The universal adhesive SU did not show any significant decrease in SBS from the baseline under any degradation condition apart from the 2-year WS group. Therefore, the universal adhesive showed comparable adhesive performance with the two-step ER adhesive. In the SEM observations, all the tested adhesives had a 2- to 3-μm-thick hybrid layer (HL) between the AL and the dentin substrate, but a high-density reaction layer (RL) below the HL was observed clearly only in SU.

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Table 1: Materials used in this study

Code	Adhesive	Main components	pH	Manufacturer
SU	Scotchbond Universal (Universal adhesive) 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
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. N89889	ethanol (25−35 wt%), bis-GMA (10−20 wt%), silane treated silica (nanofiller; 10−20 wt%), Vitrebond copolymer (5−10 wt%), HEMA (5−15 wt%), GDMA (5−10 wt%), UDMA (< 5 wt%), water (< 5 wt%),	4.7	3M Oral Care

	diphenyliodonium hexafluorophosphate (<1 wt%), EDMAB (<1 wt%), CQ	
Pre-Etching agent		
Ultra-Etch Lot No. G017	35% phosphoric acid	Ultradent Products, South Jordan, UT, USA

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

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), then the surface was air-dried with medium air pressure for 5 s. Adhesive was applied to air-dried dentin surface with rubbing motion for 20 s and then air medium 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 5s. 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.

Table 3: Influence of thermal cycling on dentin bond strength

	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.

Table 4: Influence of water storage on dentin bond strength

	24 h	6-month	1-year	2-year
SU	36.0 (4.0)^{bB} [100%]	42.5 (2.1)^{aA} [118.1%]	36.9 (3.6)^{bB} [102.5%]	30.4 (4.6)^{bC} [84.4%]
SM	36.7 (2.9)^{bA} [100%]	26.2 (4.7)^{bB} [71.4%]	24.3 (5.2)^{cB} [66.2%]	23.6 (4.7)^{cB} [64.3%]
SB	42.8 (2.9)^{aA} [100%]	44.2 (6.9)^{aA} [103.3%]	42.0 (4.6)^{aAB} [98.1%]	37.6 (2.6)^{aB} [87.9%]

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.

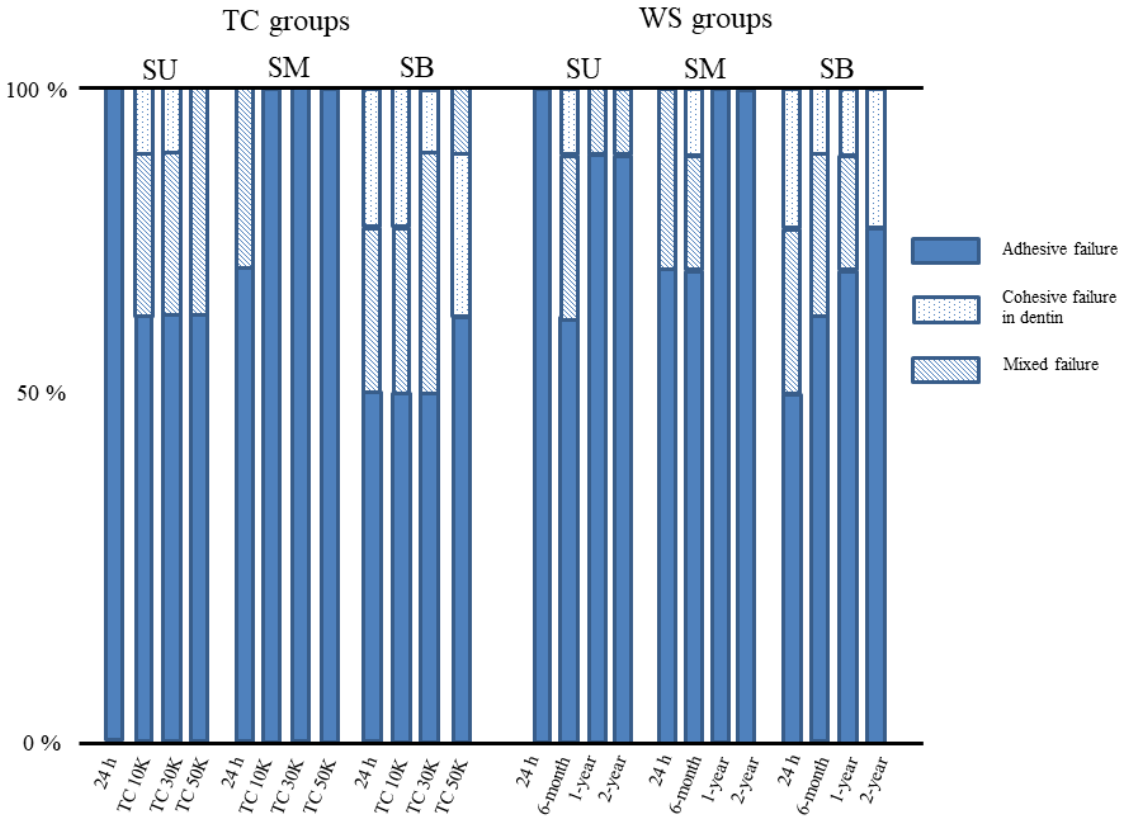

Fig. 1: Failure mode analysis of the debonded dentin specimens.
Abbreviations: SU: Scotchbond Universal, SM: Scotchbond Multi-Purpose Plus, SB: Single Bond Plus, TC: thermal cycle, WS: water storage

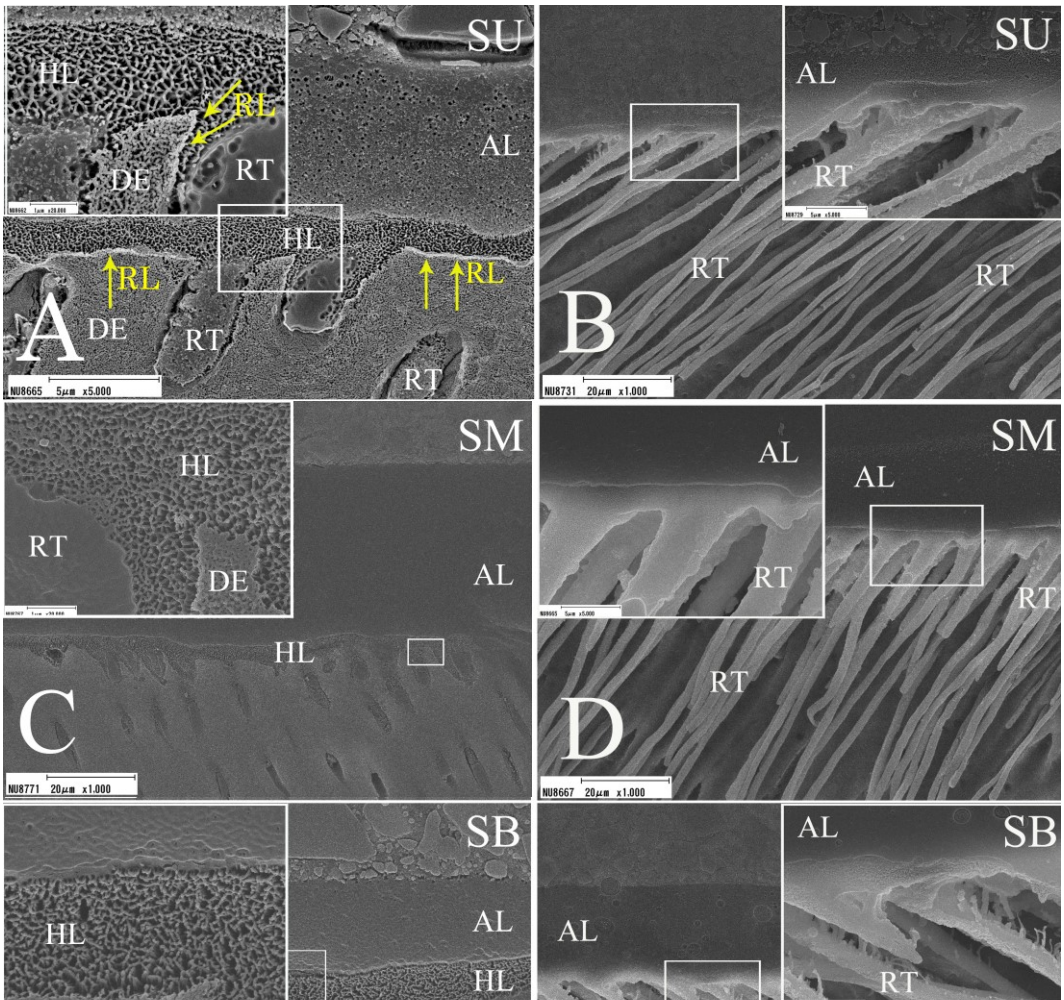

Fig. 2: Representative SEM images of resin dentin interface. The visible material is indicated by abbreviations: SU: Scotchbond Universal, SM: Scotchbond Multi-Purpose Plus, SB: Single Bond Plus, AL: adhesive layer, HL: hybrid layer, RL: reaction layer, DE: dentin, RT: resin tag

Fig. 2A: Resin dentin interface with SU after argon-ion etching (5,000× and 20,000×)
Fig. 2B: Resin dentin interface with SU after demineralised and deproteinised (1,000× and 5,000×).
Fig. 2C: Resin dentin interface with SM after argon-ion etching (1,000 × and 20,000×).
Fig. 2D: Resin dentin interface with SM after demineralised and deproteinised (1,000× and 5,000×).
Fig. 2E: Resin dentin interface with SB after argon-ion etching (5,000× and 20,000×).
Fig. 2F: Resin dentin interface with SB after demineralised and deproteinised (1,000× and 5,000×).

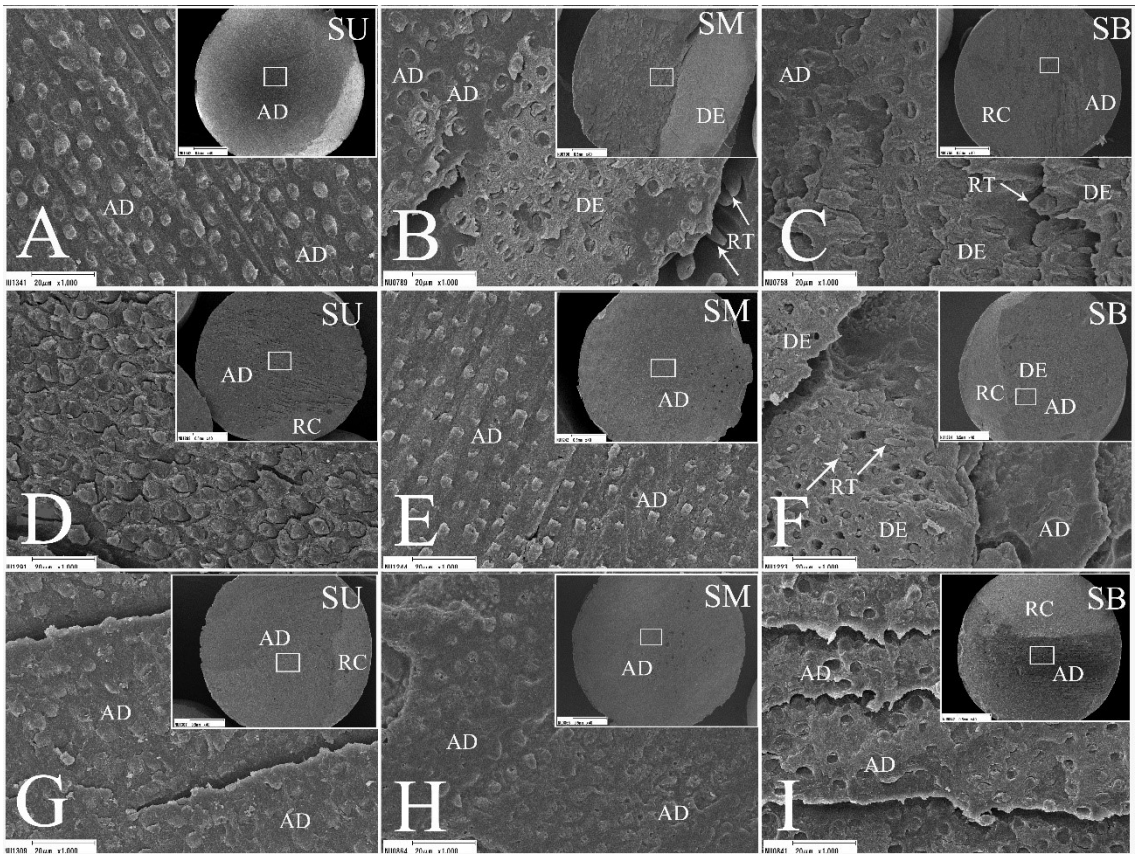

Fig. 3: Representative SEM images of the failure site after different degradation conditions.
The visible material is indicated by abbreviations: SU: Scotchbond Universal,
SM: Scotchbond Multi-Purpose Plus, SB: Single Bond Plus, AD: adhesive, DE: dentin,
RC: resin composite, RT: resin tag

Fig. 3A: SU at 24 h water storage (40× and 1,000×).

Fig. 3B: SM at 24 h water storage (40× and 1,000×).
Fig. 3C: SB at 24 h water storage (40× and 1,000×).

Fig. 3D: SU at TC 50,000 (40× and 1,000×).
Fig. 3E: SM at TC 50,000 TC (40× and 1,000×).
Fig. 3F: SB at TC 50,000 TC (40× and 1,000×).

Fig. 3G: SU at 1-year water storage (40× and 1,000×).
Fig. 3H: SM at 1-year water storage (40× and 1,000×).
Fig. 3I: SB at 1-year water storage (40× and 1,000×).