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Effect of Active Application on Bond Durability of Universal Adhesives

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Clinical Relevance

Active application may be helpful for enhancing the dentin bond durability of universal adhesives independent of etching mode.

SUMMARY

The purpose of this study was to determine the effect of different adhesive application methods and etching modes on the dentin bond durability of universal adhesives under thermal cycling (TC). All-Bond Universal (Bisco), Adhese Universal (Ivoclar Vivadent), and Scotchbond Universal (3M ESPE) were used as adhesives. In total, 600 bovine teeth with exposed dentin were divided into 12 groups according to the type of adhesive and subjected to the following bonding procedures: 1) etch-and-rinse mode with active application; 2) etch-and-rinse mode with inactive application; 3) self-etch mode with active application; and 4) self-etch mode with inactive application. Bonded specimens were stored in distilled water at 37°C for 24 hours and then subjected to 5000, 10,000, 30,000, or 50,000 TC between 5°C and 55°C before shear bond strength (SBS) testing, creating a division into a total of five different storage conditions. Baseline specimens were stored in distilled water at 37°C for 24 hours. The SBS test was performed at a cross-head speed of 1.0 mm/min. Three-way analysis of variance revealed that all the factors of application mode, adhesive, and thermal cycle period significantly influenced the SBS values ($p<0.001$), regardless of the etching mode. In the baseline groups, all of the tested adhesives with active application had higher SBS values than those with inactive application, regardless of etching mode. In the TC groups, significantly lower SBS values were observed at 50,000 TC with inactive application compared to those with active application, regardless of the etching mode. From the scanning electron microscopy observation of demineralized and deproteinized resin/dentin interfaces, dense resin tags longer than 50 μm were observed in the etch-and-rinse

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with active application group. On the other hand, the resin tags in self-etch mode were sparse, thin, and much shorter than those in etch-and-rinse mode. Comparing the penetration status of the resin tags with active and inactive application in self-etch mode, the resin tag penetration with inactive application was much lower than that with active application. Active application is effective in enhancing the dentin bond durability of universal adhesives. When using universal adhesives with different etching modes, practitioners should select the optimal etching mode and appropriate application method in accordance with the cavity conditions.

INTRODUCTION

A prepared tooth consists of complex structures with more than one component and unique morphologies. It is essential for restorations to bond effectively to the external and internal walls of a prepared tooth despite the cavity complexity. One of the most significant problems with restorations is that enamel and dentin respond differently to various treatments. Universal adhesives are versatile and capable of sensitive adaptation to a variety of cavity conditions.[1-4] However, work on the best techniques to use with universal adhesive is still at an early stage.

Phosphoric acid etching of dentin before self-etch adhesive application is believed to have a negative impact on bonding.[5-7] The procedure results in an incompletely hybridized region in the vicinity of the resin-dentin interface, which may be a critical factor in the bond degradation of self-etch adhesives.[8-10] Phosphoric acid dissolves the hydroxyapatite (HAp) surrounding the collagen fibrils, causing the chemical bonds between HAp and functional monomers to decline both in quality and quantity.[7,8] Therefore, when using self-etch adhesives, avoiding phosphoric acid pre-etching or selectively etching only the enamel region are recommended.[11,12] In the particular case of enamel bonding when using self-etch adhesives, selective etching of enamel is effective because it creates stronger micromechanical interlocking and increases surface wettability.[13,14] However, it is difficult to precisely etch only the enamel region, particularly with small tooth preparations, complex configurations, or proximal surface area. Hence, concerns remain that the phosphoric acid agent may attack the dentin region of the cavity.

Universal adhesives have distinctive characteristics compared to the previous generations of adhesive systems. These adhesives can be used with different types of substrates, such as enamel, dentin, silica-based glass ceramics, zirconia ceramics, and metal alloys.[15-18] Therefore, universal adhesives can be used not only for direct resin composite restorations but also for indirect restorations. In addition, universal adhesives are expected to help simplify the bonding procedure for patch restorations used to repair aged restorations with flaws on the surface. Another important characteristic of universal adhesives is that they can be used with either etch-and-rinse or self-etch approaches.[2,3] Universal adhesives enable the practitioner to select the optimal etching approach based on the cavity conditions, including depth, size, location, and the proportion of enamel and dentin. In general, universal adhesives may be classified as single-step self-etch adhesives as a result of their usage and broadly similar composition to that of single-step self-etch adhesives.

Previous studies[2,3,19,20] showed that although the enamel and dentin bond strengths of universal adhesives were not higher and indeed were often lower than those of two-step self-etch adhesives, they were equal to or greater than those of single-step self-etch adhesives. However, there is concern that the technology of universal adhesives may not offer a genuine advantage when compared to previous generations of single-step self-etch adhesives.[15,21] For example, when considering the dentin bonding of universal adhesives in etch-and-rinse mode, concerns are raised that the chemical bonds between HAp and functional monomers may be reduced as a result of lack of HAp, as is the case with single-step self-etch adhesives.

To enhance the bonding effectiveness of universal adhesives, an active adhesive application technique is recommended.[1,4] Active application of adhesives may lead to micromechanical interactions with the underlying mineralized tissue and penetration into the tooth substrate.[22] Stirring the adhesives can also accelerate chemical reactions between HAp and functional monomers.[23] That is, active application may mobilize a greater number of H^+ ions to react with mineral components.[4] Imai and others[4] evaluated the influence of adhesive application methods on enamel bond strength and surface free energy using four commercially available universal adhesives under different etching modes and concluded that although active application was effective in self-etch mode, it had a negative impact on enamel bond strength in etch-and-rinse mode. Although active application may enhance enamel bonding performance with the self-etch approach, little information is available on the effect of active application on the

Table 1: *Materials Used in the Study*

Code	Adhesive (Lot No.)	Main Components	Classification According to pH	Manufacturer
AB	All-Bond Universal (1300008503)	MDP phosphate monomer, Bis-GMA, HEMA, ethanol, water, initiators	Ultra-mild (pH=3.2)[a]	Bisco Schaumburg, IL USA
AU	Adhese Universal (U49302)	MDP, Bis-GMA, HEMA, MCAP D3MA, ethanol, water, initiator, silicon dioxide, stabilizers	Ultra-mild (pH=2.5-3.0)[a]	Ivoclar Vivadent Schaan, Lichtenstein
SU	Scotchbond Universal (41256)	MDP phosphate monomer, HEMA, dimethacrylate resins, Vitrebond copolymer, filler, ethanol, water, initiators, silane	Ultra-mild (pH=2.7)[a]	3M ESPE Dental Products St Paul, MN, USA
	Etching agent Ultra-Etch (G017)	35% phosphoric acid		Ultradent Products South Jordan, UT, USA
	Resin composite Clearfil AP-X (N416713)	Bis-GMA, TEGDMA, silane barium glass filler, silane silica filler, CQ, pigments, others		Kuraray Noritake Dental Tokyo, Japan

Abbreviations: Bis-GMA, 2,2-bis [4-(2-hydroxy-3-methacryloyloxypropoxy) phenyl] propane; CQ, dl-*camphorquinone; D3MA, decandiol dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; MCAP, methacrylated carboxylic acid polymer; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; TEGDMA, triethyleneglycol dimethacrylate;*
[a] *References 1, 19.*

long-term dentin bond performance of universal adhesives in different conditions.

The purpose of the present study was to determine the effect of active application on bovine dentin bond durability when using different etching modes with universal adhesives by measuring the bond strength after thermal cycling, coupled with morphological observations of adherent surfaces and the resin-dentin interfaces. The null hypothesis was that active application does not affect dentin bond durability, regardless of the etching mode used.

METHODS AND MATERIALS

Study Materials

The materials used in this study are shown in Table 1. The three universal adhesives used were 1) All Bond Universal (AB; Bisco, Schaumburg, IL, USA), 2) Adhese Universal (AU; Ivoclar Vivadent, Schaan, Liechtenstein), and 3) Scotchbond Universal (SU; 3M ESPE, St Paul, MN, USA). These products are recommended for use with active application. The phosphoric acid pre-etching agent used was Ultra-Etch (Ultradent Products, South Jordan, UT, USA). Clearfil AP-X (Kuraray Noritake Dental, Tokyo, Japan) was used as a restorative material for bonding to dentin. A visible light-curing unit (Optilux 501, sds Kerr, Danbury, CT, USA) was used, and the light irradiance (average 600 mW/cm^2) of the curing unit was checked using a dental radiometer (Model 100, Demetoron, Lincoln, NE, USA). During the course of this experiment, we checked the light irradiance of the quartz-tungsten halogen light-curing unit before use in each experimental group. When the light irradiance of the curing unit measured below 600 mW/cm^2, we changed to a new halogen lamp and confirmed its irradiance.

Specimen Preparation

Extracted mandibular bovine incisors stored frozen for up to two weeks were used. Approximately two-thirds of the apical root structure of each tooth was removed using a diamond-impregnated disc in a low-speed saw (IsoMet 1000, Precision Sectioning Saw, Buehler, Lake Bluff, IL, USA). The labial surface of each tooth was 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 mounted in self-curing acrylic resin (Tray Resin II, Shofu Inc, Kyoto, Japan) to expose the flattened area. Dentin adherent surfaces were polished using a water coolant and a series of SiC polishing papers, ending with 320-grit SiC paper (Fuji Star Type DDC). This grit was chosen for consistency with the ISO 29022[24] Standard for the shear bond strength (SBS) tests, and with clinical conditions.

Thermal Cycling (TC) and SBS Tests

The prepared dentin adherent surfaces were treated in accordance with the experimental protocol for the bonding procedures (Table 2). Six hundred specimens in total were divided into 12 groups according to the type of adhesive and subjected to the following surface treatments (10 specimens for each group): 1) etch-and-rinse mode (phosphoric acid applied for 15 seconds prior to application of the adhesives) with active application, 2) etch-and-rinse mode with inactive application, 3) self-etch mode (without phosphoric acid etching) with active application,

Table 2: *Application Protocol for Pre-etching and Self-etching Adhesives*

Method		Pre-etching Protocol
Etch-and-rinse		Dentin surface was conditioned with phosphoric acid for 15 s. Conditioned surface was rinsed with water for 15 s (three-way dental syringe) and air-dried.
Self-etch		Phosphoric acid pre-etching was not performed.
Code	**Application Method**	**Adhesive Application Protocol**
AB	Active application	Adhesive was applied to dentin surface (not desiccated) with rubbing action for 10-15 s per coat. No light cure between coats. Gentle stream of air applied over the liquid for at least 10 s. Light irradiation performed for 10 s.
	Inactive application	Adhesive was applied to dentin surface (not desiccated) without rubbing action for 10-15 s per coat. No light cure between coats. Gentle stream of air applied over the liquid for at least 10 s. Light irradiation performed for 10 s.
AU	Active application	Adhesive was applied to the dentin surface with rubbing action for 20 s, followed by application of medium air pressure for 5 s. Light irradiation performed for 10 s.
	Inactive application	Adhesive was applied to the dentin surface with rubbing action for 20 s, followed by application of medium air pressure for 5 s. Light irradiation performed for 10 s.
SU	Active application	Adhesive was applied to the dentin surface using rubbing action for 20 s, followed by application of medium air pressure for 5 s. Adhesive light cured for 10 s.
	Inactive application	Adhesive was applied to the dentin surface without rubbing action for 20 s, followed by application of medium air pressure for 5 s. Adhesive light cured for 10 s.

and 4) self-etch mode with inactive application. The adhesive agents were applied using a microbrush either with (active) or without (inactive) a rubbing motion. In the active application group, the adhesive was rubbed for the duration indicated by the manufacturer, whereas in the inactive application group, the adhesive was allowed to stand for the same period of time. An Ultradent bonding assembly (Ultradent Products) was used in this study. Following adhesive application to the dentin adherent surface, bonded resin composite cylinders were built on dentin surfaces with plastic molds (Bonding Mold Insert, 2.4 mm in internal diameter, approximately 2.5 mm in height, Ultradent Products) in the fixture (Bonding Clamp, Ultradent Products) against the dentin surfaces. The resin composite was condensed into the mold, and light irradiation was applied for 30 seconds with a curing unit. The bonded specimens were stored in distilled water at 37°C for 24 hours and then treated with 5000, 10,000, 30,000, or 50,000 thermal cycles (TC) between 5°C and 55°C with a dwell time of 30 seconds, creating a division into a total of five different storage conditions. Baseline specimens were stored in distilled water at 37°C for 24 hours before the SBS tests (baseline group). The SBS of the three universal adhesives to dentin was measured using the notched-edge SBS test, as described in ISO 29022.[24] The bonded specimens were loaded to failure at 1.0 mm/min with a shearing fixture (Test Base Clamp, Ultradent Products) using a universal testing machine (Type 5500R, Instron Corp, Canton, MA, USA). SBS values (MPa) were calculated by dividing the peak load at failure by the bonded surface area. After testing, the bonding sites on the tooth surfaces and the resin composite cylinders were observed under an optical microscope (SZH-131, Olympus, Tokyo, Japan) at a magnification of 10× to determine the bond failure mode. Based on the percentage of substrate area (adhesive–resin composite–dentin) observed in the debonded resin composites and tooth bonding sites, bond failure was classified into 1) adhesive failure, 2) cohesive failure in the composite, 3) cohesive failure in the dentin, or 4) mixed failure, defined as partially adhesive and partially cohesive.

Scanning Electron Microscopy (SEM) Observation

Representative treated dentin surfaces, resin-dentin interfaces, and debonded fracture sites were observed using SEM (ERA-8800FE, Elionix, Tokyo, Japan). Dentin surfaces were first treated in accordance with the experimental protocol for bonding procedures, then rinsed with acetone and water. For ultrastructural morphological observations of the resin-dentin interfaces to determine the penetration of the adhesives, the bonded specimens stored in 37°C distilled water for 24 hours were embedded in epoxy resin and longitudinally sectioned using a low-speed saw (IsoMet 1000). The sectioned surfaces were polished to a high gloss with SiC papers (Fuji Star Type DDC) followed by diamond pastes down to a particle size of 0.25 μm (DP-Paste, Struers, Ballerup, Denmark). After ultrasonic cleaning for three min-

Table 3: *Influence of Thermal Cycling (TC) on Shear Bond Strength (SBS)— Etch-and-rinse Mode*[a]

	24-h	5000 TC	10,000 TC	30,000 TC	50,000 TC
AB					
Active	41.5 (6.4)aA	41.9 (4.8)aA	38.6 (3.7)bA	39.6 (3.5)bA	39.8 (5.0)abA
Inactive	36.2 (4.6)abA	38.6 (4.6)abA	33.5 (3.9)bcAB	35.1 (5.5)bAB	32.1 (3.5)cB
AU					
Active	32.7 (6.6)bA	37.0 (4.7)abA	35.9 (7.4)bcA	36.6 (6.6)bA	40.0 (4.0)aA
Inactive	29.3 (5.3)bA	30.6 (4.8)cA	30.4 (5.2)cA	27.8 (5.1)cA	15.3 (5.0)dB
SU					
Active	35.2 (4.3)abC	41.0 (3.7)aB	45.3 (2.7)aA	47.5 (1.8)aA	43.8 (1.8)aAB
Inactive	33.5 (1.9)bB	34.0 (5.6)bB	40.2 (2.6)abA	37.6 (3.6)bAB	34.6 (3.9)bcB

[a] *Same lowercase letters in vertical columns indicate no significant difference. Same capital letters in horizontal rows indicate no significant difference;* $p > 0.05$.

utes, the polished surface was etched with HCl solution (6 mol/L) for 25 seconds and deproteinized by immersion in 6% NaOCl solution for three minutes. The debonded specimens from each storage condition were prepared directly for SEM. All SEM specimens were dehydrated in ascending grades of *tert*-butyl alcohol (50% for 20 minutes, 75% for 20 minutes, 95% for 20 minutes, and 100% for two hours) and then transferred to a critical-point dryer (Model ID-3, Elionix) for 30 minutes. The resin-dentin interfaces of the specimens were subjected to argon-ion beam etching (EIS-200ER, Elionix) for 20 seconds using an ion beam (accelerating voltage 1.0 kV, ion current density 0.4 mA/cm^2) directed perpendicular 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). Observations were performed under SEM at an operating voltage of 10 kV.

Statistical Analysis

A statistical power analysis indicated that at least nine samples were necessary for effective measurement of bond strength. Therefore, this experiment was initially performed with sample sizes of 10. After gathering the data, post hoc power tests were performed, and these tests indicated that the sample size was adequate. Because of their homogeneity of variance (Bartlett test) and normal distribution (Kolmogorov-Smirnov test), the data obtained from each adhesive were subjected to analysis of variance (ANOVA) followed by Tukey honestly significant difference test at a significance level of 0.05. Three-way ANOVA was used for statistical analysis of the etch-and-rinse and self-etch mode SBS data separately. All statistical analyses were performed using the Sigma Plot software (ver 11.0; SPSS Inc, Chicago, IL, USA).

RESULTS

SBS Measurements

Three-way ANOVAs for SBS in etch-and-rinse mode revealed that all the factors evaluated significantly influenced the SBS values ($p<0.001$), and the three-way interactions between the evaluated factors and all the interactions were significant ($p<0.005$). Three-way ANOVA for the SBS values in self-etch mode revealed that all the factors significantly influenced the SBS ($p<0.001$), similar to the results for the etch-and-rinse mode. Although the three-way interaction between the factors and the interactions between the adhesives and thermal cycles significantly influenced the SBS values ($p<0.05$), other pairwise interactions were not significant (application method vs adhesive; $p=0.302$, and application method vs thermal cycle: $p=0.851$).

The SBS values in etch-and-rinse mode under different numbers of TC are shown in Table 3. For all the adhesives in the baseline groups (24-hour water storage), no significant differences were observed in the SBS values between the active and inactive application groups. For the TC groups, all the adhesives in the inactive application group showed lower SBS values as the number of thermal cycles increased, and significant differences were found between the active and inactive application groups at 50,000 TC cycles. On the other hand, all of the universal adhesives in the active application group were observed to have reliable SBS values, in that there were no significant SBS reductions in the 50,000 TC groups when compared to those of the baseline groups.

The SBS results in self-etch mode under different numbers of TC are shown in Table 4. For the baseline groups, similar to the etch-and-rinse mode, all the adhesives showed higher SBS values with the

Table 4: *Influence of Thermal Cycling (TC) on Shear Bond Strength (SBS)—Self-etch Mode*[a]

	24-h	5000 TC	10,000 TC	30,000 TC	50,000 TC
AB					
Active	40.2 (2.0)^{aA}	37.8 (3.0)^{abA}	39.9 (3.9)^{abA}	41.0 (2.7)^{aA}	41.7 (2.2)^{aA}
Inactive	37.9 (2.0)^{abA}	32.9 (3.2)^{cB}	33.3 (2.9)^{cB}	35.7 (3.8)^{bcAB}	35.5 (4.3)^{bcAB}
AU					
Active	34.1 (4.4)^{bC}	41.8 (5.1)^{aAB}	43.2 (3.3)^{aA}	37.0 (2.8)^{bcBC}	31.3 (3.3)^{cdC}
Inactive	28.2 (4.1)^{cB}	38.0 (2.6)^{abA}	37.2 (2.1)^{bcA}	34.5 (3.4)^{cdA}	29.9 (2.6)^{dB}
SU					
Active	37.7 (5.7)^{abA}	38.2 (3.5)^{abA}	40.2 (2.6)^{abA}	38.8 (2.5)^{abA}	39.2(2.2)^{abA}
Inactive	34.6 (3.7)^{bAB}	34.9 (4.8)^{bcAB}	38.1 (2.8)^{bA}	32.8 (1.5)^{dB}	33.4 (4.6)^{cdB}

[a] *Same lowercase letters in vertical columns indicate no significant difference. Same capital letters in horizontal rows indicate no significant difference;* $p > 0.05$.

active application method, and the difference was significant for AU. For the TC groups, all the adhesives in the active application group showed higher SBS values than with inactive application at all TC conditions. However, neither application method showed significant differences in SBS between the 50,000 TC and the baseline groups. When observing the SBS values under different TC conditions, all the adhesives showed similar, but not identical, patterns of change. The SBS value for AU in etch-and-rinse mode at 50,000 TCs was markedly lower than that for the other adhesives (15.3 compared to 30 to 40).

Failure Mode Analysis

The frequencies of different failure modes are shown in Figures 1 and 2. Adhesive failure was most commonly observed in all debonded baseline specimens, regardless of the etching mode, type of adhesive, or application method. When TC was applied, although mixed failure and cohesive failure in dentin were observed frequently for all the adhesives at 5000 TC, the adhesive failure increased with higher TC numbers. In particular, this trend was obvious in self-etch mode with active application, regardless of the type of adhesive. When comparing the failure pattern between active and inactive application, adhesive failure was more common in the inactive application group, regardless of the TC condition.

SEM Observations

Representative SEM images of the treated dentin surfaces are shown Figures 3 and 4. In etch-and-rinse mode, complete removal of the smear layer, smear plugs, and open dentin tubules was clearly observed for all the adhesives tested, regardless of the application method (Figures 3 and 4). For AU, greater amounts of deposit on the etched surface were observed in the active application group than in the inactive group (Figure 4). On the other hand, for the specimens in self-etch mode, the smear layer and the scratch marks caused by the SiC paper were clearly visible regardless of the adhesive and application method used. However, the smear layer and smear plugs were dissolved in some locations with the active application method (Figures 3 and 4), in contrast to the inactive method (Figures 3and 4).

Representative SEM images of demineralized and deproteinized resin/dentin interfaces are shown in Figures 5 and 6. For all the adhesives, dense resin tags longer than 50 μm were observed in the etch-and-rinse with active application group. Although resin tags with a similar length were observed in both the active and inactive application groups for AB, the resin tags for SU and AU in the inactive application group were obviously shorter than those in the active application group. A hybrid layer of approximately 1 to 2 μm was observed in etch-and-rinse mode, regardless of the adhesive or application method used. On the other hand, the resin tags in self-etch mode were sparse, thin, and much shorter than those in etch-and-rinse mode, and a hybrid layer was not observed, regardless of the adhesive or application method used. Comparing the penetration status of the resin tags with active and inactive application in self-etch mode, the resin tag penetration with inactive application was much lower than that with active application.

Representative SEM images of the failure sites after the bond strength test are shown in Figures 7 and 8. The appearance of the failure patterns was dependent on the etching mode, application method, and storage condition. For the baseline groups in etch-and-rinse mode, the failure sites primarily showed detachment at the adhesive-dentin interface,

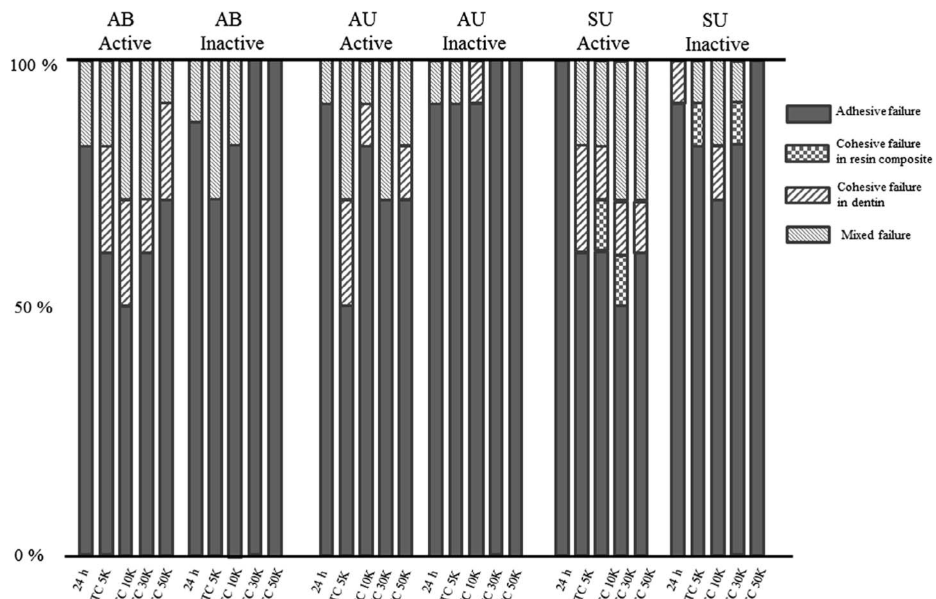


Fig 1

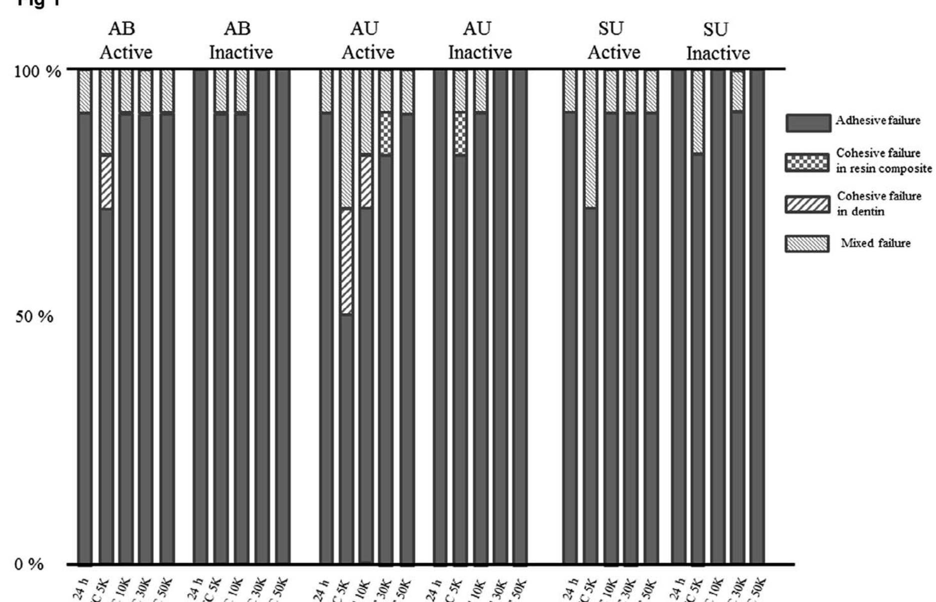


Fig 2

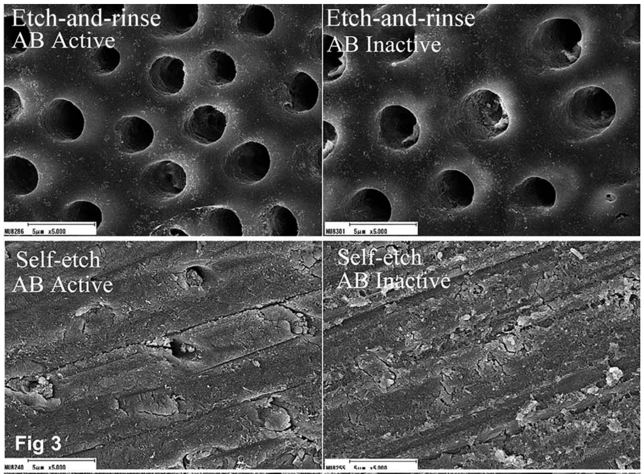
Figure 1. *The frequencies of different failure modes in etch-and-rinse mode.*

Figure 2. *The frequencies of different failure modes in self-etch mode.*

and evidence of resin tags was clearly visible, regardless of the application method (Figure 7). For the 50,000 TC specimens in etch-and-rinse mode, although evidence of resin tags was observed with both application methods, they were less clear than in the baseline specimens (Figure 7). In particular, a flat and smooth failure site was observed with inactive application (Figure 7). For debonded specimens in self-etch mode, the failure site included detachment at both the adhesive-dentin and adhesive-resin composite interfaces at lower magnification, regardless of the storage condition (Figure 8). At higher magnification, although evidence of resin tags was barely observable with both application methods in the baseline groups (Figure 8), it was difficult to observe any evidence of resin tags after thermal cycling (Figure 8).

DISCUSSION

In this study, no significant differences in dentin SBS were found between active and inactive application in the baseline groups, with the exception of AB in self-etch mode. However, all the adhesives showed significantly higher dentin SBS values in the active application groups than in the inactive



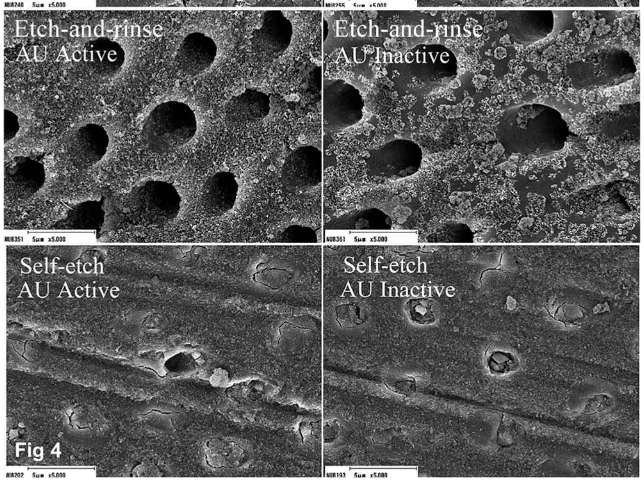


Figure 3. *Representative SEM images of treated dentin surfaces (AB). Upper left image: AB in etch-and-rinse mode with active application method (,000×). Upper right image: AB in etch-and-rinse mode with inactive application method (5000×). Lower left image: AB in self-etch mode with active application method (5000×). Lower right image: AB in self-etch mode with inactive application method (5000×).*

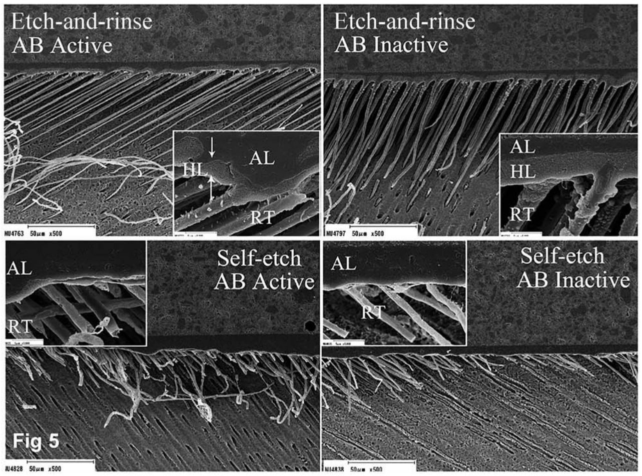
Figure 4. *Representative SEM images of treated dentin surfaces (AU). Upper left image: AU in etch-and-rinse mode with active application method (,000×). Upper right image: AU in etch-and-rinse mode with inactive application method (5000×). Lower left image: AU in self-etch mode with active application method (5000×). Lower right image: AU in self-etch mode with inactive application method (5000×).*

application groups at 50,000 TC, regardless of the etching mode used. Therefore, the null hypothesis that active application would not affect the dentin bond durability, regardless of etching mode, was rejected. Active application of universal adhesives may enhance dentin bond durability as a result of the potential of tooth demineralization, penetration, and chemical bonding with the dentin substrate.

In self-etch mode, the gel-like collagen in the dentin smear on the sound tissue can interfere with the penetration of resin monomers contained in adhesives.[25,26] SEM observations of treated dentin surfaces in self-etch mode showed that actively applied adhesives can dissolve a certain amount of the smear layer, compared to those applied inactively (Figures 3 and 4). It can be speculated that unreacted H^+ ions were supplied from functional monomers in the adhesive, resulting in the progression of the demineralization process.[4,22] Previous studies[27-29] have reported the benefits of active application for optimal dentin bond performance and durability with self-etch adhesives. Increased dentin bond strength with active application has been suggested[28,29] to be due to the stirring of adhesive-inducing solvent evaporation, resulting in a higher rate of resin monomer incorporation inside the smear layer. Furthermore, the nanolayering of calcium-salt formed from HAp and the functional monomer is significantly greater with active application than with inactive application.[23] The induction of a chemical reaction between functional monomers and HAp by active application may also reduce the levels of the acidic monomer, which would contribute to amine co-initiators and enhance the photopolymerization of adhesives containing acidic functional monomers.[30,31] Therefore, active application in self-etch mode may enhance the dentin bond durability of universal adhesives as a result of the same mechanisms seen with conventional self-etch adhesives.

On the other hand, in etch-and-rinse mode, phosphoric acid pre-etching solubilizes not only the surface debris but also the subsurface of the dentin substrate.[32] A previous investigation[33] of dentin bond durability after four-year water storage showed that a three-step etch-and-rinse system resulted in higher microtensile bond strength than did a two-step etch-and-rinse system, and this tendency did not change over four years. The primary bonding mechanism underlying three-step etch-and-rinse systems is thought to be micromechanical interlocking between demineralized exposed collagen fibrils and resin monomers, leading to hybrid layer and resin tag formation.[32] With phosphoric acid pre-etching performed prior to the application of a self-etch adhesive, concerns remain that demineralized dentin without resin impregnation may persist at the bottom of the hybrid layer, which will act as a weaker region in the vicinity of the resin/dentin interface.[5-7] This unstable region increases the risk of biodegradation and biomechanical influences for not only self-etch adhesives but also for universal adhesives.[34,35]

For all the adhesives subjected to different numbers of TC with active application, SBS values



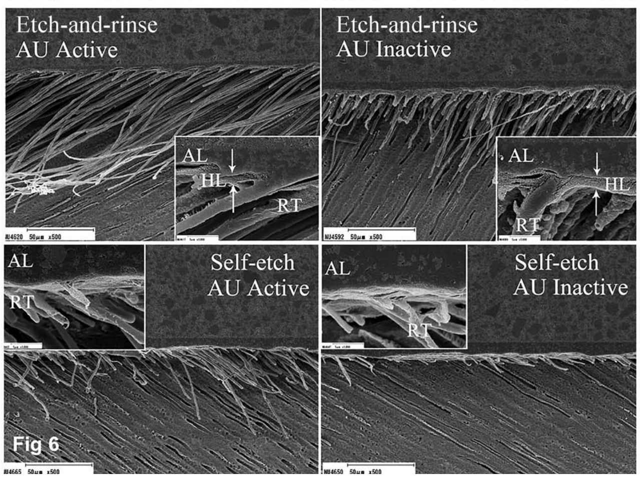


Figure 5. *Representative SEM images of the resin-dentin interfaces. The visible material is indicated by abbreviations: AL, adhesive layer; HL, hybrid layer; RT, resin tag. Upper left image: AB in etch-and-rinse mode with active application method (5000× and 20,000×). Upper right image: AB in etch-and-rinse mode with inactive application method (5000× and 20,000×). Lower left image: AB in self-etch mode with active application method (5000× and 20,000×). Lower right image: AB in self-etch mode with inactive application method (5000× and 20,000×).*

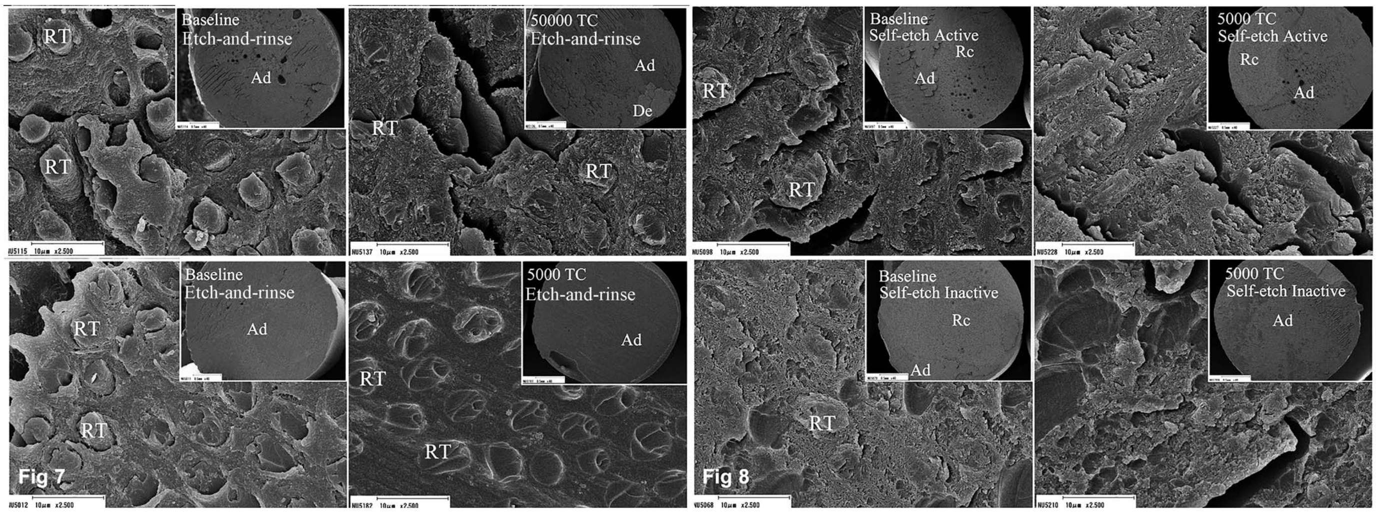
Figure 6. *Representative SEM images of the resin-dentin interfaces. The visible material is indicated by abbreviations: AL, adhesive layer; HL, hybrid layer; RT, resin tag. Horizontal arrows indicate aggregated fillers. Upper left image: AU in etch-and-rinse mode with active application method (5000× and 20,000×). Upper right image: AU in etch-and-rinse mode with inactive application method (5000× and 20,000×). Lower left image: AU in self-etch mode with active application method (5000× and 20,000×). Lower right image: AU in self-etch mode with inactive application method (5000× and 20,000×).*

in etch-and-rinse mode were equal to or greater than those in self-etch mode, with the exception of AU in early cycles. This result was consistent with those of previous studies[2,36] investigating the effects of fatigue stress on the dentin bond durability of universal adhesives in different etching modes. However, SBS values in etch-and-rinse mode with inactive application were lower than those in self-etch mode with active application regardless of the type of adhesive or cycles. Hence, active application is thought to be helpful for the dentin bond durability of universal adhesives in etch-and-rinse mode, similar to the self-etch mode.

The role of resin tag formation in enhancing dentin bond performance is still controversial. SEM images of resin/dentin interfaces in etch-and-rinse mode revealed higher penetration for adhesives with active application (Figure 6). In addition, the penetration of adhesives into the branches of dentin tubules was observed in the active application group, in contrast to behavior in the inactive application group. From the results of the present study, long, thick, and dense resin tags with active application contribute to the higher bond strength due to mechanical retention. Some universal adhesives were designed to have lower hydrophilicity than conventional single-step self-etch adhesives, which may explain the greater resistance of resin tags to hydrolytic degradation. In addition, deep penetration into dentin tubules and creating branches might enhance chemical bonding between functional monomers and the HAp of internal dentin tubules.

The penetration capability of adhesives may be closely dependent on their composition, such as the amount of water, type of solvent, presence or absence of inorganic fillers, and hydrophilic or hydrophobic resin monomers.[37] Although the purity and quantity of each ingredient in the adhesives vary, all the tested adhesives in this study contained the same functional monomer, 10-methacryloyloxydecyl dihydrogen phosphate, the hydrophilic resin monomer 2-hydroxyethyl methacrylate, water, and ethanol as a solvent. However, SEM observations of treated dentin surfaces and resin/dentin interfaces were adhesive dependent. In contrast to AB and SU, greater amounts of deposit were observed on the etched surface in AU (Figure 4).

With regard to resin tag formation, AB showed noticeably longer resin tags than did the other adhesives. This is probably due to its application method, in which the adhesive is applied twice without irradiation between the applications. Thus, the second application may push resin deeper into the substrate. For all the adhesives, no significant SBS reduction was found compared to the baseline until 30,000 TC, regardless of the etching mode or application method used. However, the SBS value for AU in etch-and-rinse mode with inactive application after 50,000 TC was markedly lower than that



Figures 7 and 8. *Representative SEM images of the fractured resin surface in etch-and-rinse and self-etch modes under different storage conditions. The visible material is indicated by abbreviations: Ad, adhesive; De, dentin; Rc, resin composite; RT, resin tag. Figure 7: Upper left image: AU in etch-and-rinse mode with active application method at baseline (40× and 2500×). Upper right image: AU in etch-and-rinse mode with active application method at 50,000 TC (40× and 2500×). Lower left image: AU in total-etch mode with inactive application method at baseline (40× and 2500×). Lower right image: AU in etch-and-rinse mode with inactive application method at 50,000 TC (40× and 2500×). Figure 8: Upper left image: AU in self-etch mode with active application method at baseline (40× and 2500×). Upper right image: AU in self-etch mode with active application method at 50,000 TC (40× and 2500×). Lower left image: AU in self-etch mode with inactive application method at baseline (40× and 2500×). Lower right image: AU in self-etch mode with inactive application method at 50,000 TC (40× and 2500×).*

for all the other conditions. The long tags may explain AB's durability, and SU contains Vitrebond copolymers that utilize glass ionomer technology that may be biocompatible with exposed collagen fibrils.[38] SEM observations revealed that resin monomer penetration in AU was lower than in the other adhesives. This observation is attributed to the presence of aggregated inorganic fillers in AU (Figure 4). Although the incorporation of inorganic fillers enhances the mechanical properties of the adhesive layer, the ability of the adhesive to flow into tubules might decrease.

In terms of clinical practice, this study suggests that active application may enhance the dentin bond durability of universal adhesives regardless of etching mode. However, active application may have a negative impact on immediate enamel bonds in etch-and-rinse mode.[4] Therefore, when using universal adhesives with a combination of different etching modes and application methods, practitioners should take care to select optimal bonding procedures in accordance with the cavity size, depth, and configuration. For instance, when the prepared surface is predominantly enamel, the tested universal adhesives should be applied in etch-and-rinse mode without active application. However, active application should be performed in both etch-and-rinse and self-etch approaches when dentin substrate accounts for a large portion of the prepared surface.

CONCLUSIONS

Within the limitations of this laboratory study, all the factors evaluated, namely application method, adhesive, and thermal cycle period, significantly influenced the SBS values ($p<0.001$), regardless of the etching mode used. For immediate dentin bond results, application method did not strongly influence the dentin bond strengths, regardless of the etching mode. However, for dentin bond durability under thermal cycling stress, application method was a significant influence on dentin bond strengths in both etch-and-rinse and self-etch modes. In conclusion, active application is effective at enhancing the dentin bond durability of universal adhesives.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of approval of the Nihon University School Dentistry.

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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