©Operative Dentistry, 2020, **45-2,** 183-195

Influence of Application Time on Dentin Bond Performance in Different Etching Modes of Universal Adhesives

T Saito • T Takamizawa • R Ishii • A Tsujimoto • E Hirokane
WW Barkmeier • MA Latta • M Miyazaki

Clinical Relevance

Although reduced application time of dentin appears to be acceptable for some universal adhesives, care should be taken when using universal adhesives that recommend applying the adhesive with active motion, regardless of the etching mode.

SUMMARY

We attempted to determine the effect of universal adhesive application time on dentin bond performance in different etching modes based on shear bond strength (SBS) tests and surface free energy (SFE) measurements. The five universal adhesives used were Adhese Universal (AU), Clearfil Universal Bond Quick (CQ), G-Premio Bond (GP), Scotchbond Universal (SU), and Tokuyama Universal Bond (TU). Bovine dentin specimens were divided into four groups of 10 for each adhesive. SBS and SFE were determined after applying the following surface treatments: 1) self-etch (SE) mode with immediate air blowing after adhesive application (IA treatment), 2) SE mode with prolonged application time (PA treatment), 3) etch-and-rinse (ER) mode with IA treatment, and 4) ER mode with PA treatment. Bonded specimens were subjected to SBS testing. The SFE of adhesive-treated dentin surfaces was measured after rinsing with acetone and water. Three-way analysis of variance revealed that dentin SBS values ($p<0.001$) were significantly influenced by the factors of adhesive type and application duration, but the factor of pre-etching was not significant

Tatsuro Saito, DDS, Operative Dentistry, Nihon University School of Dentistry, Tokyo, Japan

*Toshiki Takamizawa, DDS. PhD, Operative Dentistry, Nihon University School of Dentistry, Tokyo, Japan

Ryo Ishii, DDS, PhD, Operative Dentistry, Nihon University School of Dentistry, Tokyo, Japan

Akimasa Tsujimoto, DDS, PhD, Operative Dentistry, Nihon University School of Dentistry, Tokyo, Japan

Eizo Hirokane, DDS, Operative Dentistry, Nihon University School of Dentistry, Tokyo, Japan

Wayne W Barkmeier, DDS, MS, General Dentistry, Creighton University School of Dentistry, Omaha, NE, USA

Mark A Latta, DMD, MS, General Dentistry, Creighton University, Omaha, NE, USA

Masashi Miyazaki, DDS, PhD, Operative Dentistry, Nihon University School of Dentistry, Tokyo, Japan

*Corresponding author: 1-8-13, Kanda-Surugadai, Chiyoda-Ku, Tokyo, 101-8310, Japan; e-mail: takamizawa.toshiki@nihon-u.ac.jp

https://doi.org/10.2341/19-028-L

($p=0.985$). The manufacturer's instructions require longer application times for AU and SU, which showed significantly lower SBS values in IA than in PA treatment in both etching modes. However, the difference in the other adhesives was not significant between the IA and PA treatments in either etching mode. The total SFE (γ_S) was dependent on the adhesive and etching mode. The γ_S value of the initial group (SiC paper ground group) at baseline was 69.5 ($mN \cdot m^{-1}$) and that of the pre-etching group at baseline was 30.6 ($mN \cdot m^{-1}$). For all the adhesives, γ_S in SE mode showed significantly higher values than in ER mode, regardless of the application time. In SE mode, almost all universal adhesives tested showed lower γ_S values in PA treatment than in IA treatment. For ER mode, all the adhesives showed significantly higher γ_S values than those of the pre-etching baseline, regardless of the application time. Most adhesives did not show any significant differences in γ_S values between IA and PA treatments, regardless of etching mode.

INTRODUCTION

Several years have passed since universal adhesives were introduced as the latest adhesive systems for use in clinical situations.[1-4] Although these adhesive systems were similar to conventional single-step self-etch (SE) adhesives, they may benefit practitioners who utilize multi-etching modes and apply adhesives to different types of indirect restorations.[5,6] Manufacturers continue to develop new universal adhesive products to meet different clinical requirements.

Another trend in the development of universal adhesives is reducing the adhesive's application time.[7-9] Some universal adhesives allow immediate air blowing after adhesive is applied to the tooth surface, which may reduce contamination risk and shorten treatment time.

On the other hand, a previous study investigated how the reduced application time of universal adhesives influenced enamel bonding from the perspective of bond strength and surface free energy (SFE) changes. The findings indicated that the quality and quantity of chemical interaction might be higher with prolonged application time, regardless of the etching mode.[8] However, the influence of these changes on bond strength was unclear.

A recent study examined how the application time of universal adhesives influenced enamel bond effectiveness in different etching modes through a shear bond strength (SBS) test and SFE measurement.[8] For the SE mode, although all the tested adhesives tended to show increased enamel bond strengths with increased application time, three of five universal adhesives did not show any significant differences between the group with air blown immediately after adhesive application and the group with prolonged application time. Conversely, adhesives with recommendations to apply by rubbing exhibited decreased SBS values with increased application time in etch-and-rinse (ER) mode. However, from the perspective of SFE, chemical bonding tended to increase with increased application time, regardless of the etching mode, suggesting that although prolonged application time of universal adhesives might enhance the chemical reaction with hydroxyapatite (HAp), enamel bond strength values might be influenced by etching mode and adhesive type.

Structurally, enamel is homogeneous in nature and is mainly composed of HAp. On the contrary, dentin is heterogeneous, consisting of collagen and HAp, and the water content is significantly higher than that of enamel. As with conventional single-step SE adhesives, the high levels of water and solvents contained in some universal adhesives allow ionization of the included acidic functional monomers and induce resin monomer infiltration.[10,11] However, residual water inhibits polymerization of resin monomers; thus, evaporating the water is important in order to establish the mechanical properties of the cured adhesive layer.[12,13] Hence, a specific length of application time should allow the residual water and solvents to evaporate, leading to development of a uniform adhesive layer.[7,14] Dissolving the HAp on the dentin surface may reduce chemical bonding while in the ER mode with dentin because HAp has a higher affinity with functional monomers compared with dentin collagen. Therefore, it is important to understand the efficacy of dentin bonding and the characteristics of calcium salt formation in different etching modes using different adhesive application times.

The present study attempted to determine how reduced application time of universal adhesives in different etching modes influenced bonding effectiveness to dentin based on SBS tests, morphologic observations, and SFE characteristics. The null hypotheses proposed that neither reducing application time nor changing etching mode affected dentin SBS or SFE.

Table 1: *Materials Used in the Study*

Material	Main Components	pH	Manufacturer
Adhesive (Lot No.)			
AU: Adhese Universal (U49302)	MDP, bis-GMA, HEMA, MCAP, D3MA, ethanol, water, initiator, stabilizers, silicon dioxide	2.5-3.0	Ivoclar Vivadent, Schaan, Lichtenstein
CQ: Clearfil Universal Bond Quick (9T0050)	bis-GMA, MDP, HEMA, hydrophilic amide monomer, filler, ethanol, water, NaF, photoinitiators, chemical polymerization, accelerator, silane coupling agent, others	2.3	Kuraray Noritake Dental, Tokyo, Japan
GP: G-Premio Bond (4G0011)	MDP, 4-MET, MEPS, BHT, acetone, dimethacrylate resins, initiators, filler, water	1.5	GC, Tokyo, Japan
SU: Scotchbond Universal (41256)	MDP, HEMA, dimethacrylate resins, Vitrebond copolymer, filler, ethanol, water, initiators, silane	2.7	3M ESPE, St Paul, MN, USA
TU: Tokuyama Universal Bond (004067)	Liquid A: phosphate monomer, bis-GMA, TEGDMA, HEMA, MTU-6, others Liquid B: acetone, isopropanol, water, acryl borate catalyst, γ-MPTES, peroxide, others	2.2	Tokuyama Dental, Tokyo, Japan
Pre-Etching Agent			
Ultra-Etch (G017)	35% phosphoric acid		Ultradent Products, Inc, South Jordan, UT, USA
Resin Composite			
Clearfil AP-X (N416713)	bis-GMA, TEGDMA, silane barium glass filler, silane silica filler, silanated colloidal silica, *dl*-camphorquinone, pigments, others		Kuraray Noritake Dental

Abbreviations: BHT, butylated hydroxytoluene; bis-GMA, 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy) phenyl] propane; D3MA, decandiol dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; MCAP, methacrylated carboxylic acid polymer, MDP, 10-methacryloyloxydecyl dihydrogen phosphate; MEPS, methacryloyloxyalkyl thiophosphate methylmethacrylate; 4-MET, 4-methacryloyloxyethyl trimellitate; γ-MPTES. γ-methacryloyloxypropyltriethoxysilane; NaF, sodium fluoride; TEGDMA, triethyleneglycol dimethacrylate.

METHODS AND MATERIALS

Study Materials

The materials used in the present study are shown in Table 1. Briefly, we used the following universal adhesives: 1) Adhese Universal (AU; Ivoclar Vivadent, Schaan, Liechtenstein), 2) Clearfil Universal Bond Quick (CQ; Kuraray Noritake Dental, Tokyo, Japan), 3) G-Premio Bond (GP; GC Corp, Tokyo, Japan), 4) Scotchbond Universal (SU; 3M ESPE, St Paul, MN, USA), and 5) Tokuyama Universal Bond (TU; Tokuyama Dental, Tokyo, Japan). Pre-etching with phosphoric acid was performed using Ultra-Etch (Ultradent Products, South Jordan, UT, USA). Clearfil AP-X (Kuraray Noritake Dental) was used as a resin composite to bond to dentin. We used a halogen quartz tungsten curing unit to avoid any influence from the reported nonuniformity of light-emitting diode curing units.[15,16] A visible light curing unit (Optilux 501; SDS Kerr, Danbury, CT, USA) was used, and light irradiance (average 600 mW/cm^2) was checked during the course of the experiment.

Specimen Preparation

Extracted mandibular bovine incisors stored frozen for up to 2 weeks were substituted for human teeth. We removed approximately two-thirds of the apical root structure of each tooth using a low-speed diamond saw (IsoMet 1000 Precision Sectioning Saw, Buehler, Lake Bluff, IL, USA). The labial surfaces were ground on wet 240-grit silicon carbide (SiC) paper (Fuji Star Type DDC, Sankyo Rikagaku, Saitama, Japan) to create a flat dentin surface. Next, each tooth was mounted in self-curing acrylic resin (Tray Resin II, Shofu Inc, Kyoto, Japan) to expose the flattened area. A water coolant and a sequence of SiC papers ending with a 320-grit SiC paper were used to polish the dentin surfaces (Fuji Star Type DDC).

SBS Tests

The SBS to dentin was measured according to ISO 29022.[17] The experimental protocols for the bonding procedures are shown in Table 2 and Figure 1. For each test group, 10 specimens were used to measure

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

Method		Pre-etching Protocol
ER	Dentin surface was etched with phosphoric acid for 15 seconds. Etched surface was rinsed with water for 15 seconds. (three-way dental syringe) and air-dried.	
SE	Phosphoric acid pre-etching was not performed.	
Adhesive	**Application Method**	**Adhesive Application Protocol**
AU	IA	Adhesive was applied to the air-dried dentin surface and immediately medium air pressure was applied over the liquid adhesive for 5 seconds. Light irradiation was done for 10 seconds.
	PA[a]	Adhesive was applied to the air-dried dentin surface with rubbing motion for 20 seconds, and then medium air pressure was applied to surface for 5 seconds. Light irradiation was done for 10 seconds.
CQ	IA[a]	Adhesive was applied to air-dried dentin surface and immediately medium air pressure was applied over the liquid adhesive for 5 seconds or until the adhesive no longer moved and the solvent had completely evaporated. Light irradiation was done for 10 seconds.
	PA	Adhesive was applied to air-dried dentin surface for 10 seconds, and then medium air pressure was applied over the liquid adhesive for 5 seconds or until the adhesive was no longer moved and the solvent had completely evaporated. Light irradiation was done for 10 seconds.
GP	IA[a]	Adhesive was applied to air-dried dentin surface and immediately a strong stream of air was applied over the liquid adhesive for 5 seconds or until the adhesive was no longer moving and the solvent had completely evaporated. Light irradiation was done for 10 seconds.
	PA	Adhesive was applied to air-dried dentin surface for 10 seconds and then a strong stream of air was applied over the liquid adhesive for 5 seconds or until the adhesive no longer moved and the solvent had completely evaporated. Light irradiation was done for 10 seconds.
SU	IA	Adhesive was applied to air-dried dentin surface and immediately medium air pressure was applied over the liquid adhesive for 5 seconds. Light irradiation was done for 10 seconds.
	PA[a]	Adhesive was applied to air-dried dentin surface with rubbing motion for 20 seconds and then medium air pressure was applied to surface for 5 seconds. Light irradiation was done for 10 seconds.
TU	IA[a]	Adhesive was applied to the air-dried dentin surface and immediately medium air pressure was applied over the liquid adhesive for 5 seconds. No light irradiation was done.
	PA	Adhesive was applied to the air-dried dentin surface for 10 seconds and then medium air pressure was applied over the liquid adhesive for 5 seconds. No light irradiation was done.

Abbreviations: ER, Etch-&-rinse; SE, Self-etch; AU, Adhese Universal; CQ, Clearfil Universal Bond Quick; GP, G-Premio Bond; SU, Scotchbond Universal; TU, Tokuyama Universal Bond; IA, immediately air-blow after application of adhesive; PA, application of adhesive according to each manufacturer's instructions (AU and SU) or applied adhesive for 10 seconds (CQ and TU)
[a] *Manufacturer's instructions*

the dentin SBS in ER mode (phosphoric acid was applied for 15 seconds before applying the adhesive) or SE mode (without phosphoric acid etching). For each different etched dentin surface, the adhesives were applied and immediately subjected to air blowing (IA: immediate air blow), or the GP, SU, and AU adhesives were applied according to the manufacturer's recommended application time, and the CQ and TU adhesives were applied for 10 seconds (PA: prolonged application). Air blowing was always performed as stated in each manufacturer's instructions (Table 2). The experimental groups included four combinations of IA or PA treatment in ER and SE modes for each adhesive, for a total of 20 groups.

A bonding assembly (Ultradent Products, Inc) was used to measure the SBS. After the adhesive was applied to the dentin surface, resin composite cylinders were formed on the surfaces by clamping plastic molds (2.4-mm internal diameter, approximately 2.5-mm height; Ultradent Products Inc) in a fixture against the adherent surfaces. The resin composite was placed into the mold and light irradiated for 30 seconds. After removing the mold, the specimens were stored in distilled water at 37°C for 24 hours and loaded to failure at 1.0 mm per minute with an Ultradent shearing fixture (Ultradent Products, Inc) using a universal testing machine (Type 5500R, Instron, Canton, MA, USA). The SBS values (MPa) were calculated from the peak load at failure divided by the bonded surface area. After testing, we evaluated the failure mode by viewing the bonding tooth surfaces and debonded resin composite cylinders under an optical microscope (SZH-131, Olympus, Tokyo, Japan) at 10× magnification. On the basis of the percentage of substrate area (adhesive – resin composite – dentin) seen on the debonded cylinders and tooth

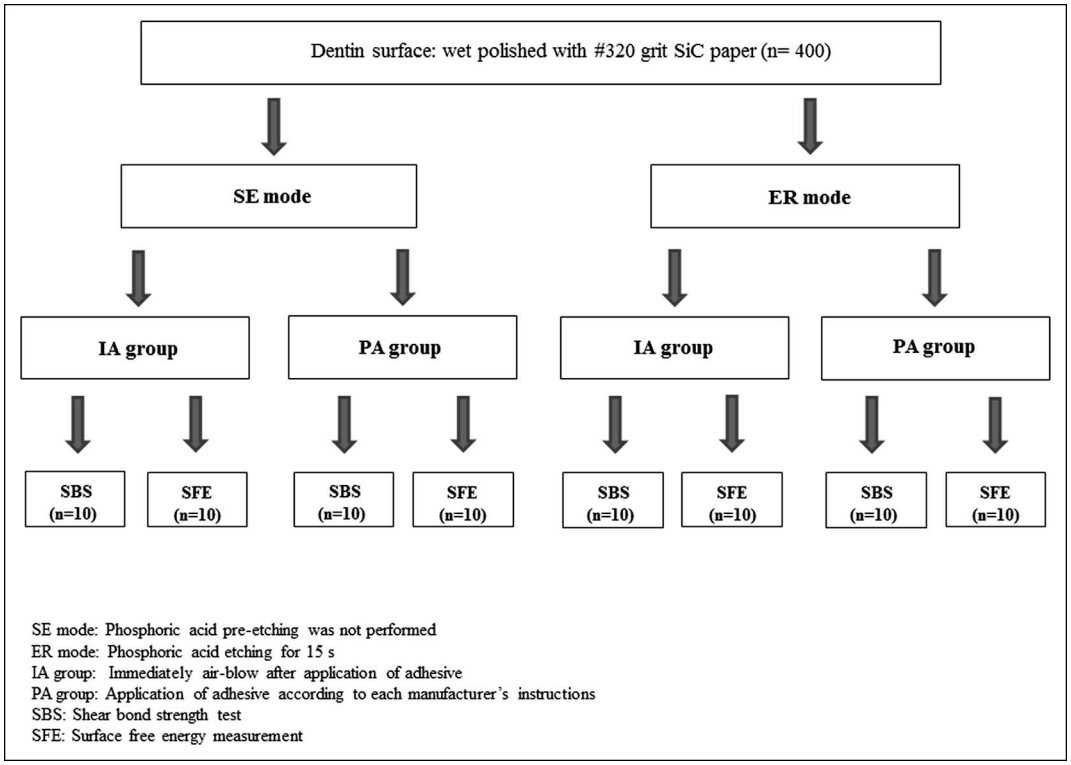


Figure 1. *The flow diagram of this study protocol.*

surface sites, the types of failure were recorded as 1) adhesive failure, 2) cohesive failure in composite, 3) cohesive failure in dentin, or 4) mixed failure (partially adhesive and partially cohesive).

SFE Measurements

The specimens for measuring SFE were prepared the same as for the SBS test described earlier. After the dentin surface was treated, we removed the uncured adhesive layer by three alternating rinses with acetone and water. Next, oil-free compressed air was used to dry the dentin surface. Specimens polished with wet 320-grit SiC paper with and without phosphoric acid pre-etching were also measured as a baseline, although they were only rinsed with water. The prepared specimens were used for contact angle measurements, and SFE values were determined by measuring the surface contact angles using the following three test liquids: 1) 1-bromonaphthalene, 2) diiodomethane, and 3) distilled water, each with known SFE parameters as previously reported[8,18] (Table 3). The contact angle meter (Drop Master DM500, Kyowa Interface Science, Saitama, Japan) was connected to a charge-coupled device camera, allowing automatic contact angle measurements.

Table 3: *Influence of Application Duration on Dentin Bond Strength (MPa) Shown as Mean (Standard Deviation (n=10)[a,b]*

Code	SE Mode		ER Mode	
	IA Group	PA Group	IA Group	PA Group
AU	26.4 (4.0)^{bcB}	*31.9 (3.7)^{bA}	18.5 (3.5)^{cC}	*34.7 (5.3)^{aA}
CQ	*34.5 (1.9)^{aA}	34.3 (4.8)^{abA}	*34.4 (4.3)^{aA}	34.5 (5.9)^{aA}
GP	*27.4 (3.4)^{bcA}	29.9 (4.6)^{bA}	*29.1 (4.8)^{bA}	31.6 (3.3)^{aA}
SU	25.8 (3.1)^{cB}	*37.7 (4.8)^{aA}	29.6 (2.7)^{bB}	*35.2 (4.5)^{aA}
TU	*30.3 (4.7)^{abA}	29.4 (4.2)^{bA}	*29.5 (3.1)^{bA}	33.9 (4.5)^{aA}

[a] Asterisk indicates manufacturer's recommended application time.
[b] Same lowercase letter in vertical columns indicates no difference at 5% significance level. Same capital letter in horizontal rows indicates no difference at 5% significance level.
Abbreviations: ER, Etch-&-rinse; SE, Self-etch; AU, Adhese Universal; CQ, Clearfil Universal Bond Quick; GP, G-Premio Bond; SU, Scotchbond Universal; TU, Tokuyama Universal Bond;

The equilibrium contact angle (θ) of each test liquid was measured using the sessile drop method at 23°C ± 1°C in 10 dentin specimens for each condition. Sessile drops of each liquid were dispensed at a volume of 1.0 μL using a micropipette. The fundamental concepts of wetting were used to determine the SFE parameters of the solids. The Young–Dupré equation describes the work of adhesion for a solid (S) in contact (W_{SL}) with a liquid (L),

the interfacial free energy between the solid and the liquid (γ_{SL}), and the SFE of the liquid and solid (γ_L and γ_S, respectively), as follows:

$$W_{SL} = \gamma_L + \gamma_S - \gamma_{SL} = \gamma_L(1 + \cos\theta).$$

The Fowkes equation was extended as follows, using the Kitazaki-Hata method[19]:

$$\gamma_{SL} = \gamma_L + \gamma_S - 2(\gamma_L^d\gamma_S^d)^{1/2} - 2(\gamma_L^p\gamma_S^p)^{1/2} - 2(\gamma_L^h\gamma_S^h)^{1/2}$$

$$\gamma_L = \gamma_L^d + \gamma_L^p + \gamma_L^h, \gamma_S = \gamma_S^d + \gamma_S^p + \gamma_S^h,$$

where γ^d, γ^p, and γ^h are SFE (γ) components arising from the dispersion force, the polar (permanent and induced) force, and the hydrogen-bonding force, respectively. The θ values were determined for the three test liquids, and SFE parameters of the treated dentin surfaces were calculated on the basis of the extended Fowkes equation following the Kitazaki-Hata method using add-on software and the included interface measurement and analysis system (FAMAS; Kyowa Interface Science Co). The SFE of dentin was measured on 10 specimens from each group and the mean determined.

Scanning Electron Microscopy (SEM) Observations

Representative treated dentin surfaces, restorative–dentin interfaces, and debonded fracture sites were observed on field emission SEM (ERA-8800FE, Elionix, Tokyo, Japan). Dentin surfaces were initially treated according to the experimental protocol for bonding procedures and rinsed with acetone and water. For ultrastructural morphologic observations of the restorative–dentin interfaces to determine adhesive penetration, bonded specimens stored in distilled water at 37°C for 24 hours were set in epoxy resin and sectioned lengthwise with a low-speed saw (IsoMet 1000). The sectioned surfaces were polished to a high gloss using 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 3 minutes, the polished surface was etched in hydrogen chloride solution (6 mol/L) for 25 seconds and deproteinized by immersing in 6% sodium hypochlorite solution for 3 minutes. Treated surfaces and debonded fracture sites were prepared directly for the 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 2 hours) and 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 with an ion beam (accelerating voltage 1.0 kV, ion current density 0.4 mA/cm^2) directed perpendicular to the polished surfaces. Finally, a vacuum evaporator (Quick Coater, SC-701, Sanyu Electron, Tokyo, Japan) coated all SEM specimens with a thin film of gold. Observations were performed at an operating voltage of 10 kV.

Statistical Analysis

Before testing, the sample size was determined from the G Power calculator. With an effect size of d = 0.5 (medium), α = 0.05 (two sided), power = 0.95, and number of groups = 20, a total sample size of 210 was needed. And then, we obtained the effect size of more than 0.56 from the F values and df by using three-way ANOVA on the result data. The results indicated that at least 8.8 specimens per group were needed. Therefore, this experiment was initially performed with sample sizes of ten. After gathering the data, post hoc power tests were performed, and these tests indicated that the sample size was adequate.

Three-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test (α=0.05) was used to analyze the full data set. Factors included 1) etching mode, 2) application time, and 3) adhesive system. One-way ANOVA followed by Tukey's HSD test (α=0.05) was used for making comparisons within subsets of the data, as described later. Statistical analysis was performed using Sigma Plot software, version 11.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

Shear Bond Strength

The results for dentin SBS using the different bonding procedures are shown in Table 3. Three-way ANOVA revealed that dentin SBS values ($p<0.001$) were significantly influenced by the factors of adhesive type and application duration, but the factor of pre-etching was not significant ($p=0.985$). The three-way interaction among the factors ($p<0.001$) and all pairwise interactions were significant ($p<0.05$).

When comparing the SBS values between IA and PA treatments in SE mode, the AU and SU values were significantly higher for PA compared with IA treatment. However, no significant difference was

seen between the IA and PA treatments with the other adhesives. Among the tested adhesives in SE mode, SU with IA treatment exhibited the lowest SBS value, and the highest SBS value was observed for SU with PA treatment.

When comparing the SBS values between IA and PA treatments in ER mode, AU and SU showed significantly higher SBS values with PA compared with IA treatment; however, no significant difference in SBS was observed between IA and PA treatments with the other adhesives. Among the tested adhesives in ER mode, AU with IA treatment had a significantly lower SBS value compared with the other adhesives. However, no significant difference was seen among the tested adhesives for PA treatment.

Failure Mode Analysis of Debonded Specimens

The frequency of different failure modes is shown in Figure 2. The predominant failure mode for all of the adhesives was adhesive failure, regardless of etching mode or application time. However, for all of the adhesives except GP, mixed failure increased in both etching modes with PA treatment.

Surface Free Energy

The SFE values and components of the different application modes are shown in Figure 3. The total SFE (γ_S) was dependent on the adhesive and etching mode. The γ_S value of the initial group (320 grit) at baseline was 69.5 ($mN{\cdot}m^{-1}$) and that of the pre-etching group at baseline was 30.6 ($mN{\cdot}m^{-1}$). The pre-etching group demonstrated a significantly lower baseline γ_S value compared with the initial group due to significantly lower values for dispersion (γ_S^d) and hydrogen-bonding forces (γ_S^h) in the pre-etching group.

For all the adhesives, γ_S in SE mode showed significantly higher values than in ER mode, regardless of the application time. Further, all the adhesives showed significantly lower γ_S values than the initial baseline. For ER mode, all the adhesives showed significantly higher γ_S values than those of the pre-etching baseline, regardless of the application time. Most adhesives did not show any significant differences in γ_S values between IA and PA treatments, regardless of etching mode.

For all the groups, dispersion force (γ_S^d) in SE mode showed similar values of approximately 40 ($mN{\cdot}m^{-1}$) and higher values than in ER mode, irrespective of the application time. Apart from CQ, all the adhesives with IA treatment in SE mode showed higher polar force (γ_S^p) values than with PA treatment in SE. On the other hand, none of the adhesives in ER mode showed much difference between IA and PA treatments. Regarding the hydrogen-bonding forces (γ_S^h), all the adhesives in SE mode showed higher γ_S^h values than in ER mode. In SE mode, most adhesives showed higher γ_S^h values in IA treatment than in PA treatment.

SEM Observations

Representative SEM images of the treated dentin surfaces are shown in Figures 4 and 5. Remaining scratch marks and smear layer were clearly observed for the specimens with IA treatment in SE mode. Although the specimens with PA treatment in SE mode had a morphologic trend similar to that of IA treatment, part of the smear layer and smear plugs were dissolved. On the other hand, for the specimens in ER mode, the smear layer was completely dissolved and open dentinal tubules were observed, regardless of the application time or type of adhesive.

Representative SEM images of demineralized and deproteinized resin–dentin interfaces are shown in Figures 6 and 7. Clear differences were observed between specimens in the different etching modes in the vicinity of the adhesive–dentin interface. In SE mode, infiltrated resin tags were sparse in both IA and PA treatment; however, the length of resin tags with PA treatment was slightly longer than with IA treatment. This trend was particularly evident in SU and AU compared with the other adhesives. In ER mode, dense resin tags longer than 50 μm and approximately 1 μm of hybrid layer were detected, regardless of the application time or type of adhesive. In addition, adhesive penetration into the branches of dentinal tubules was more apparent in ER mode. Resin tag density did not vary between IA and PA treatments; however, those in PA treatment appeared to be longer.

Representative SEM images of the failure sites after the SBS test are shown in Figures 8 and 9. The appearance of the failure pattern was dependent on etching mode and adhesive material. The failure pattern of CQ in different etching modes identified similar morphologic etching patterns. However, PA treatment showed more cracks in the adhesives and clearer evidence of resin tags compared with the debonded specimens with IA treatment, regardless of the etching mode (Figure 8). IA treatment of SU in SE mode showed detached areas at the adhesive–resin composite interface. On the other hand, SU in SE mode with PA treatment showed either detached

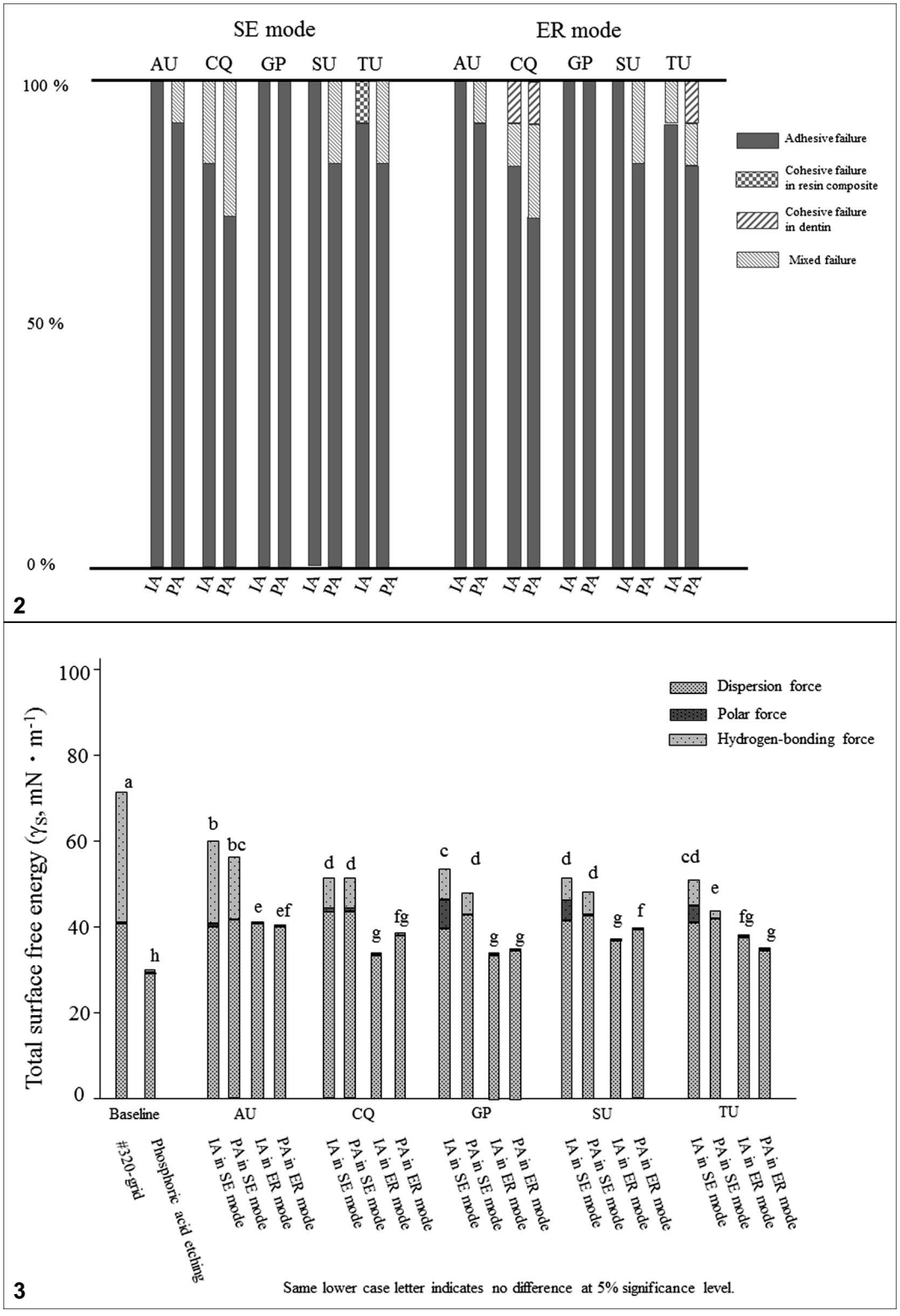
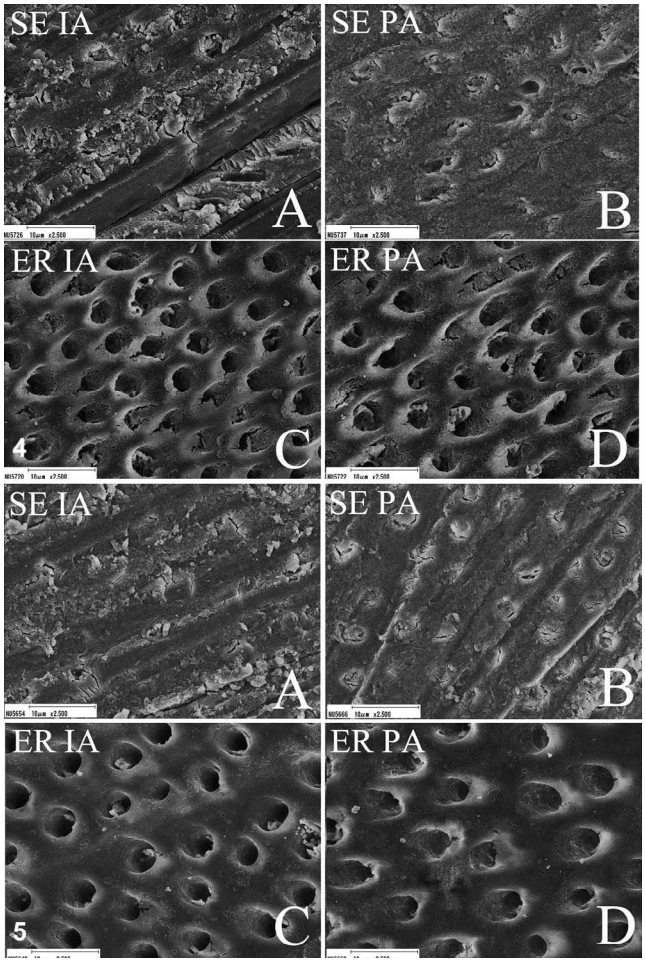


Figure 2. *Failure mode analysis of the de-bonded dentin specimens.*

Figure 3. *Total SFE results from different application times in different etching modes.*



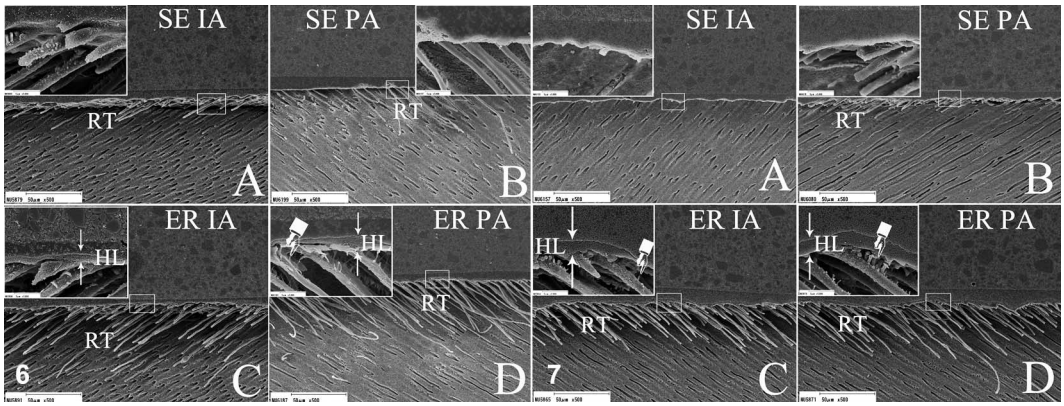
Figures 4 and 5. *Representative SEM images of treated dentin surface from different bonding procedures. ×5000 magnification. (4A): AU with IA treatment in SE mode. (4B): AU with PA treatment in SE mode. (4C): AU with IA treatment in ER mode. (4D): AU with PA treatment in ER mode. (5A): CQ with IA treatment in SE mode. (5B): CQ with PA treatment in SE mode. (5C): CQ with IA treatment in ER mode. (5D): CQ with PA treatment in ER mode.*

areas at the adhesive–dentin interface or cohesive failure in dentin. Failure sites for SU in ER mode showed detachment mostly at the adhesive–dentin interface, and evidence of resin tags was observed regardless of the application time (Figure 9).

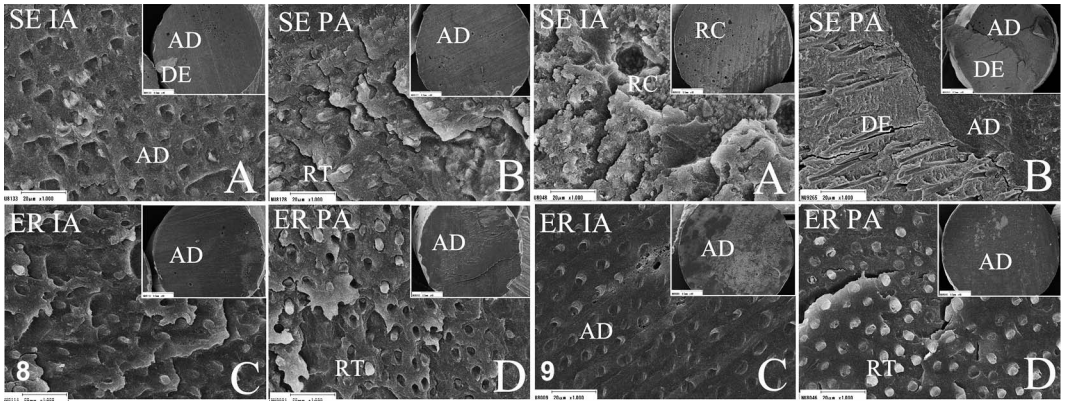
DISCUSSION

Some of the new universal adhesives feature shortened application times, and this could prove to be a clinically appealing feature for clinicians.[7-9] The present study focused on the influence of different application times for universal adhesives on dentin SBS and SFE values. In the SBS test results, SU and AU, for which the instructions require active motion and longer application times, revealed significantly lower SBS values in IA treatment than in PA treatment. On the other hand, CQ, GP, and TU, which require air blowing immediately after adhesive application, were not significantly different between IA and PA treatments in either etching mode. Therefore, the null hypothesis that reducing application time or changing etching mode did not affect dentin SBS was not rejected for CQ, GP, and TU but was rejected for SU and AU.

The bonding mechanisms in the ER and SE modes are completely different when considering the dentin SBS. For ER systems, phosphoric acid etching performs dentin demineralization with a depth of 5–8 μm, exposing collagen fibrils without Hap.[20,21] To prevent hydrolysis of collagen fibrils, resin monomers should offset and reinforce the spaces formerly occupied by HAp crystals. Micromechanical retention of resin tags within the hybrid layer is considered the primary contribution of phosphoric acid etching to adhesion.[20,21] However, one of the



Figures 6 and 7. *Representative SEM micrographs of the resin-dentin interfaces. The main images are at ×5000 magnification. The smaller white rectangle indicates the location in the main image of the enlarged area, at ×20,000, in the upper right or left corner. The visible material is indicated by abbreviations: HL indicates hybrid layer; RT, resin tag. (6A): AU with IA treatment in SE mode. (6B): AU with PA treatment in SE mode. (6C): AU with IA treatment in ER mode. (6D): AU with PA treatment in ER mode. (7A): CQ with IA treatment in SE mode. (7B): CQ with PA treatment in SE mode. (7C): CQ with IA treatment in ER mode. (7D): CQ with PA treatment in ER mode.*



Figures 8 nd 9. *Representative SEM images of the fractured resin surface in SE mode and in ER mode with different application times. The visible material is indicated by abbreviations: AD indicates adhesive; DE, dentin; RC, resin composite; RT, resin tag. (8A): CQ with IA treatment in SE mode (50× and 1000×). (8B): CQ with PA treatment in SE mode (50× and 1000×). (8C): CQ with IA treatment in ER mode (50× and 1000×). (8D): CQ with PA treatment in ER mode (50× and 1000×). (9A): SU with IA treatment in SE mode (50× and 1000×). (9B): SU with PA treatment in SE mode (50× and 1000×). (9C): SU with IA treatment in ER mode (50× and 1000×). (9D): SU with PA treatment in ER mode (50× and 1000×).*

concerns regarding the ER mode is incomplete resin monomer penetration into the full depth of demineralized dentin, resulting in insufficient hybridization and unprotected collagen fibrils.[22,23] For the SE mode, the key components are acidic functional monomers, which partially demineralize the tooth surface and achieve chemical bonding with HAp.[10,11] In particular, chemical bonding is more important for dentin compared with enamel because the smaller crystals and plate-like structure of dentin HAp are considered more accessible to chemical reaction compared with enamel HAp.[24,25]

Although the dentin bonding mechanism of universal adhesives in SE mode is similar to conventional single-step SE adhesives, that of universal adhesives in ER mode may not be exactly the same as those of three-step or two-step systems. On the basis of these results, we can speculate that the role of functional monomers of universal adhesives is important for achieving a chemical interaction not only with HAp but also with exposed collagen fibrils. Hiraishi and others[26] proposed that 10-methacryloyloxydecyl dihydrogen phosphate (MDP) has a relatively stable interaction with collagen due to the hydrophobic interactions between the MDP moieties and the collagen surface, as measured by saturation transfer difference nuclear magnetic resonance spectroscopy. Five of the six tested universal adhesives contained MDP, and functional monomers might penetrate the intact dentin substrate through naked collagen fibrils after pre-etching. In particular, no significant difference was seen between the IA and PA treatments in ER mode using CQ, GP, or TU, suggesting that their resin monomers should have a higher penetration ability. CU contains 2-hydroxyethyl methacrylate (HEMA) and a newly developed amide monomer, both of which are highly hydrophilic and mobile as monomers, and the amide monomer can penetrate deeper into dentin and polymerize to form a stable bond. Therefore, we could suppose that monomers could penetrate deep into the demineralized dentin and polymerize to develop a stable polymer network producing strong micromechanical interlocking.

In order to better understand the dentin bonding mechanism of universal adhesives, we also examined the surface chemistry. In the SFE measurements, the baseline group demonstrated a significantly lower total free energy (γ_S) value than did the initial group (SiC paper ground group) in ER mode. In particular, the dispersion force (γ_S^d) and hydrogen-bonding force (γ_S^h) values were significantly lower for the demineralized dentin surfaces than for the initial group. The γ_S value was expressed as the sum of three parameters, γ_S^d, γ_S^p, and γ_S^h,[19] indicating that dentin wettability after phosphoric acid etching was lower than for dentin surfaces covered by a smear layer. Because it is not easy to standardize dentin moisture conditions, wettability and SFE measurement of demineralized dentin remain controversial.[27-30] Although HAp has high SFE due to the concentration of hydroxyl groups, collagen fibrils composed of insoluble fibrous protein have low SFE.[31] The reason for the decrease in γ_S and γ_S^h values of dentin for phosphoric acid–etched dentin might be related to a decreased mineral/organic ratio due to the loss of HAp.[32] In addition, it can be assumed that changes in surface morphology, including exposed collagen fibrils and dentinal tubules, lead to a decrease in γ_S^d. In contrast to a

smear layer–covered dentin surface, the morphology of demineralized dentin, with a mesh structure of exposed collagen fibrils and opened dentin tubules, is more complex and might trap air, resulting in decreased γ_S^d values.[33]

For ER mode, all the adhesives showed significantly higher γ_S values than the pre-etching baseline. In particular, the γ_S^d in all adhesive treated groups was significantly higher than that of the pre-etching baseline, regardless of the application time. The increased γ_S values in adhesive treated groups after pre-etching may be attributed to the increased γ_S^d. The reason for increased γ_S^d values in the adhesive treated surfaces after phosphoric acid pre-etching is unclear. However, it can be speculated that chemical and physical interaction between surface collagen fibrils and adhesive components influence the γ_S^d. In particular, these interactions may change morphologic features of a demineralized dentin surface in spite of washing with acetone and water.

All of the adhesives in SE mode exhibited significantly lower γ_S values compared with the initial baseline group (320 grit), regardless of the application time. Adhesive-treated surfaces in both IA and PA treatments demonstrated significantly lower γ_S^h values compared with the initial baseline group. The γ_S^h value represents the water and hydroxyl components of the substrate. The γ_S^p value is thought to be associated with electronic and metallic interactions as well as dipolar interactions.[34] Thus, γ_S^h and γ_S^p parameters might be helpful for identifying whether the surface characteristics are hydrophilic or hydrophobic. Substrates with higher γ_S^h and γ_S^p values are typically water soluble, whereas substrates with lower values tend to be soluble in organic solvents. These results indicate that adhesive-treated dentin surfaces lean from hydrophilic to hydrophobic due to chemical interactions and functional monomers forming calcium salts. When comparing different application times in SE mode, lower γ_S values in PA than in IA treatment were seen in almost all of the universal adhesives, although most differences were not statistically significant. Most universal adhesives presented lower γ_S^p and γ_S^h values in PA treatment than in IA treatment; hence, a longer application time may promote chemical interactions between HAp for most universal adhesives in SE mode.

Therefore, the null hypothesis that reduced application time or different etching mode did not affect dentin SFE was rejected for all the adhesives in terms of etching mode but was not rejected in terms of application time.

Moreover, evidence of a consistent effect of increased application time on SFE was seen in ER mode compared with SE mode. However, AU and SU exhibited significantly lower SBS values in IA treatment than in PA treatment and lower SFE values in PA treatment than in IA treatment in SE mode. In contrast to the enamel smear layer, the gel-like collagen in the dentin smear on sound tissue is thought to interfere with the penetration of resin monomers.[35,36] In addition, from the perspective of pH values of the tested adhesive, AU and SU have relatively higher pH values than the other universal adhesives. Although lower pH of adhesive is thought to be inferior in decalcifying mineralized tissue, longer application time and stirring of adhesive might compensate for lower etching capability due to supplying unreacted H^+ ions.[18] Furthermore, active and longer application time might encourage water and solvent evaporation, creating a hydrophobic layer, promoting the penetration of resin monomers, and inducing a chemical interaction for AU and SU.[37] A trend in SBS values was clearly observed in ER mode, as most tested materials demonstrated increased SBS values with increased application time. Although the same trend was seen for all of the materials, only two of the individual differences were statistically significant.

Saikaew and others[7] examined how reduced application time of three universal adhesives in SE mode influenced dentin bond performance and found that the universal adhesives, which required application times of 10 or 20 seconds, showed significantly lower μ-TBS (tensile bond strength) values in the adhesive application groups with immediate air blowing than in the groups following the manufacturer's instructions for bur-cut dentin surfaces. Furthermore, the same research facility investigated the effect of long-term water storage on dentin bond durability, concluding that adhesive application time might compromise bond strength.[9] Although they did not investigate the influence of reduced application time of universal adhesives in ER mode, the present study was in line with their results in terms of immediate bond strength in SE mode. Therefore, based on previous studies as well as the present study, although reduced application time appears to be acceptable for CQ, GP, and TU, at least for immediate dentin bond strength, regardless of etching mode, it has a negative effect on AU and SU.

CONCLUSION

The results of the present laboratory study did not reveal any significant differences in dentin SBS values between IA and PA treatments in three of five universal adhesives, regardless of etching mode. However, the other universal adhesives, which required active and longer application times, demonstrated significantly lower SBS values in IA than in PA treatment in both etching modes. In the results of SFE measurements, the baseline group demonstrated a significantly lower total free energy (γ_S) value than did the initial group (SiC paper ground group) in ER mode. The adhesive-treated dentin surfaces exhibited lower γ_S values for all the adhesives in SE mode than did the initial dentin surfaces, and most adhesives showed lower γ_S values in PA compared with IA treatment.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research, No. 16K11565, 16K20465, 17K11716, and 17K17141 from the Japan Society for the Promotion of Science. This project was also supported in part by the Sato Fund and by a grant from the Dental Research Center of the Nihon University School of Dentistry, Japan.

Regulatory Statement

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

(Accepted 29 April 2019)

REFERENCES

1. Wagner A, Wendler M, Petschelt A, Belli R, & Lohbauer U (2014) Bonding performance of universal adhesives in different etching modes *Journal of Dentistry* **42(7)** 800-807. https://doi: 10.1016/j.jdent.2014.04.012
2. Muñoz MA, Luque-Martinez I, Malaquias P, Hass V, Reis A, Campanha NH, & Loguercio AD (2015) In vitro longevity of bonding properties of universal adhesives to dentin *Operative Dentistry* **40(3)** 282-292. https://doi: 10.2341/14-055-L
3. Da Rosa WL, Piva E, & Da Silva AF (2015) Bond strength of universal adhesives: A systematic review and meta-analysis *Journal of Dentistry* **43(7)** 765-776. https: //doi: 10.1016/j.jdent.2015.04.003
4. Nagarkar S, Theis-Mahon N, & Perdigão J (2019) Universal dental adhesives: Current status, laboratory testing, and clinical performance *Journal of Biomedical Materials Research. Part B, Applied Biomaterials* (in press). https://doi: 10.1002/jbm.b.34305 [Epub ahead of print]
5. Suzuki T, Takamizawa T, Barkmeier WW, Tsujimoto A, Endo H, Erickson RL, Latta MA, & Miyazaki M (2016) Influence of etching mode on enamel bond durability of universal adhesive systems *Operative Dentistry* **41(5)** 520-530. https://doi: 10.2341/15-347-L
6. Takamizawa T, Barkmeier WW, Tsujimoto A, Berry TP, Watanabe H, Erickson RL, Latta MA, & Miyazaki M (2016) Influence of different etching modes on bond strength and fatigue strength to dentin using universal adhesive systems *Dental Materials* **32(2)** e9-e21. https:// doi: 10.1016/j.dental.2015.11.005
7. Saikaew P, Chowdhury AFMA, Fukuyama M, Kakuda S, Carvalho RM, & Sano H (2016) The effect of dentine surface preparation and reduced application time of adhesive on bonding strength *Journal of Dentistry* **47** 63-70. https://doi: 10.1016/j.jdent.2016.02.001
8. Sai K, Takamizawa T, Imai A, Tsujimoto A, Ishii R, Barkmeier WW, Latta MA, & Miyazaki M (2018) Influence of application time and etching mode of universal adhesives on enamel adhesion *Journal of Adhesive Dentistry* **20(1)** 65-77. https://doi: 10.3290/j.jad.a39913
9. Saikaew P, Matsumoto M, Chowdhury AFMA, Carvalho RM, & Sano H (2018) Does shortened application time affect long-term bond strength of universal adhesives to dentin? *Operative Dentistry* **43(5)** 549-558. https://doi: 10.2341/17-205-L
10. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, & De Munck J (2011) State of the art of self-etch adhesives *Dental Materials* **27(1)** 17-28. https://doi: 10.1016/j.dental.2010.10.023
11. Miyazaki M, Tsujimoto A, Tsubota K, Takamizawa T, Kurokawa H, & Platt JA (2014) Important compositional characteristics in the clinical use of adhesive systems *Journal of Oral Science* **56(1)** 1-9. https://doi.org/10.2334/josnusd.56.1
12. Van Landuyt KL, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, Inoue S, Peumans M, Suzuki K, Lambrechts P, & Van Meerbeek B (2005) Monomer-solvent phase separation in one-step self-etch adhesives *Journal of Dental Research* **84(2)** 183-188. https://doi: 10.1177/154405910508400214
13. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, & De Stefano Dorigo E (2008) Dental adhesion review: Aging and stability of the bonded interface *Dental Materials* **24(1)** 90-101. https://doi: 10.1016/j.dental.2007.02.009
14. Cardoso PC, Loguercio AD, Vieira LC, Baratieri LN, & Reis A (2005) Effect of prolonged application times on resin-dentin bond strengths *Journal of Adhesive Dentistry* **7(2)** 143-149.
15. Price RBT, Ruggeberg FA, Labrie D, & Felix CM (2010) Irradiance uniformity and distribution from dental light curing units *Journal of Esthetic and Restorative Dentistry* **22(2)** 86-103. https://doi: 10.1111/j.1708-8240.2010.00318.x

16. Michaud PL, Price RBT, Labrie D, Rueggeberg FA, & Sullivan B (2014) Localised irradiance distribution found in dental light curing units *Journal of Dentistry* **42(2)** 129-139. https://doi: 10.1016/j.jdent.2013.11.014

17. ISO (2013) *ISO 29022: 2013 Dentistry-Adhesion-Notched-edge Shear Bond Strength Test* International Organization for Standardization, Geneva, Switzerland, 1-12.

18. Imai A, Takamizawa T, Sai K, Tsujimoto A, Nojiri K, Barkmeier WW, Latta MA, & Miyazaki M (2017) Influence of application method on surface free-energy and bond strength of universal adhesive systems to enamel *European Journal of Oral Science* **125(5)** 385-395. https://doi: 10.1111/eos.12361

19. Hata T, Kitazato Y, & Saito T (1987) Estimation of the surface energy of polymer solids *Journal of Adhesion* **21(3-4)** 177-194.

20. Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, & Tezvergil- Mutluay A. (2011) State of the art etch-and-rinse adhesives *Dental Materials* **27(1)** 1-16. https://doi: 10.1016/j.dental.2010.10.016

21. Miyazaki M, Tsubota K, Takamizawa T, Kurokawa H, Rikuta A, & Ando S (2012) Factors affecting the *in vitro* performance of dentin-bonding systems *Journal of Dental Science Review* **48(1)** 53-60. https://doi.org/10.1016/j.jdsr.2011.11.002

22. Hashimoto M, Ohno H, Kaga M, Sano H, Tay FR, Oguchi H, Araki Y, & Kubota M (2002) Over-etching effects on micro-tensile bond strength and failure patterns for two dentin bonding systems *Journal of Dentistry* **30(2-3)** 99-105. https://doi.org/10.1016/S0300-5712(02)00004-0

23. Hashimoto M, Ohno H, Sano H, Kaga M, & Oguchi H (2003) In vitro degradation of resin-dentin bonds analyzed by microtensile bond test, scanning and transmission electron microscopy *Biomaterials* **24(21)** 3795-3803. https://doi.org/10.1016/S0142-9612(03)00262-X

24. Yoshihara K, Yoshida Y, Hayakawa S, Nagaoka N, Irie M, Ogawa T, Van Landuyt KL, Osaka A, Suzuki K, Minagi S, & Van Meerbeek B (2011) Nanolayering of phosphoric acid ester monomer on enamel and dentin *Acta Biomaterialia* **7(8)** 3187-3195. https://doi: 10.1016/j.actbio.2011.04.026

25. Yaguchi T (2017) Layering mechanism of MDP-Ca salt produced in demiralization of enamel and dentin apatite *Dental Materials* **33(1)** 23-32. https://doi: 10.1016/j.dental.2016.09.037

26. Hiraishi N, Tochio N, Kigawa T, Otsuki M, & Tagami J (2013) Monomer-collagen interactions studied by saturation transfer difference NMR *Journal of Dental Research* **92(3)** 284-288. https://doi: 10.1177/0022034512474310

27. Aguilar-Mendoza JA, Rosales-Leal JI, Rodriguez-Valverde MA, & Cabrerizo-Vilchez MA (2008) Effect of acid etching on dentin wettability and roughness: Self-etching primers versus phosphoric acid *Journal of Biomedical Materials Research. Part B, Applied Biomaterials* **84(1)** 277-285. https://doi: 10.1002/jbm.b.30871

28. Yilmaz Z, Basbag B, Buzoglu HD, & Gümüsderelioglu M (2011) Effect of low-surface-tension EDTA solutions on the wettability of root canal dentin *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **111(1)** 109-114. https://doi: 10.1016/j.tripleo.2010.08.008

29. Erickson RL (1992) Surface interactions of dentin adhesive materials *Operative Dentistry* (Supplement 5) 81-94.

30. Pelin IM, Trunfio-Sfarghiu AM, Farge P, Piednoir A, Pirat C, & Ramos SM (2013) Multiscale characterization of partially demineralized superficial and deep dentin surfaces *European Journal of Oral Science* **121(4)** 341-348. https://doi: 10.1111/eos.12058

31. Akinmade AO & Nicholson JW (1993) Glass-ionomer cements as adhesives. Part I. Fundamental aspects and their clinical relevance *Journal of Materials Science: Materials in Medicine* **4(2)** 95-101.

32. Besinis A, Van Noort R, & Martin N (2016) The use of acetone to enhance the infiltration of HA nanoparticles into a demineralized dentin collagen matrix *Dental Materials* **32(3)** 385-393. https://doi: 10.1016/j.dental.2015.11.010

33. Farge P, Alderete L, & Ramos SM (2010) Dentin wetting by three adhesive systems: influence of etching time, temperature and relative humidity *Journal of Dentistry* **38(9)** 698-706. https://doi: 10.1016/j.jdent.2010.03.013

34. Tsujimoto A, Iwasa M, Shiimamura Y, Murayama R, Takamizawa T, & Miyazaki M (2010) Enamel bonding of single-step self-etch adhesives: Influence of surface energy characteristics *Journal of Dentistry* **38(2)** 123-130. https://doi: 10.1016/j.jdent.2009.09.011

35. Thanatvarakorn O, Nakajima M, Prasansuttiporn T, Ichinose S, Foxton RM, & Tagami J (2014) Effect of smear layer deproteinizing on resin-dentine interface with self-etch adhesive *Journal of Dentistry* **42(3)** 298-304. https://doi: 10.1016/j.jdent.2013.11.026

36. Takamizawa T, Barkmeier WW, Sai K, Tsujimoto A, Imai A, Erickson RL, Latta MA, & Miyazaki M (2018) Influence of different smear layers on bond durability of self-etch adhesives *Dental Materials* **34(2)** 246-259. https://doi: 10.1016/j.dental.2017.11.002

37. Moritake N, Takamizawa T, Ishii R, Tsujimoto A, Barkmeier WW, Latta MA, & Miyazaki M (2019) Effect of active application on bond durability of universal adhesives *Operative Dentistry* **44(2)** 188-199. https://doi: 10.2341/17-384-L