Influence of application time on dentin bond performance and surface free energy of universal adhesives in different etching modes

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Contents

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Summary

Recent trend in the development of universal adhesives is reducing the adhesive's application time. Some universal adhesives allow immediate air blowing after adhesive is applied to the tooth surface, which may reduce contamination risk and shorten treatment time. Dissolving the hydroxyapatite (HAp) on dentin surface may reduce chemical bonding while in the etch-&-rinse 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 aim of this study was to determine how reduced application time of universal adhesives in different etching modes influenced bonding effectiveness to dentin based on shear bond strength (SBS) tests, morphological observations, and surface free energy (SFE) characteristics.

Six universal adhesives used were, Adhese Universal (AU), Clearfil Universal Bond Quick (CQ), G-Premio Bond (GP), Scotchbond Universal (SU), Scotchbond Universal Plus Adhesive (SP), and Tokuyama Universal Bond (TU). Specimen preparation was performed in accordance with ISO 29022. Extracted mandibular bovine incisors were used as substitutes for human teeth. Ten specimens were used for each test group to determine the SBS to dentin with phosphoric acid pre-etching (ER: etch-&-rinse mode) or not (SE: self-etch mode). For each different etched dentin surface, the adhesives were applied and immediately subjected to air blowing (IA: immediate air blow), or adhesives were applied according to the manufacturer's recommended application time (PA: prolonged application). After the adhesive was applied to the dentin surface, resin composite cylinders were formed on the surfaces by clamping plastic molds in a fixture against the adherent surfaces. The resin composite was placed into the mold and light irradiated for 30 s. The bonded specimens were stored for 24 h in distilled water at 37°C before testing.

The specimens for measuring SFE were prepared the same as for the SBS test. The prepared specimens were used for contact angle measurements, and SFE values were determined by measuring the surface contact angles using the three test liquids. The 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. The SFE of dentin was measured on 10 specimens from each group and the mean values were determined. Representative treated dentin surfaces, restorative–dentin interfaces, and failure sites of the debonded specimens were observed by a scanning electron microscopy (SEM).

Three-way analysis of variance (ANOVA) revealed that dentin SBS values were significantly influenced by the factors of adhesive type and application duration, but the factor of pre-etching was not significant. When comparing the SBS values between IA and PA treatments in SE mode, the AU, SU, and SP 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. The predominant failure mode for all of the adhesives was adhesive failure, regardless of etching mode or application time.

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 the SEM observations of the treated dentin surfaces, 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. 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. For the SEM images of demineralized and deproteinized resin–dentin interfaces, clear differences were observed between specimens in the different etching modes in the vicinity of the adhesive–dentin interface.

The results of the present laboratory study did not reveal any significant differences in dentin SBS values between IA and PA treatments in CQ, GP, and TU, regardless of etching mode. However, AU, SU, and SP, which required active and longer application times, demonstrated lower SBS values in IA than in PA treatment in both SE and ER modes. From the results of SFE measurements, the γ_S was dependent on the adhesive and etching mode. For baseline groups, a significantly lower total free energy (γ_S) value in phosphoric acid etching group was observed when compared with the initial group. 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 with PA compared to IA treatment in SE 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.

A recent study examined how the application time of universal adhesives influenced enamel bond effectiveness in different etching modes through shear bond strength (SBS) test and surface free energy (SFE) measurement (7). 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-&-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.

Evaporating the water is important in order to establish the mechanical properties of the cured adhesive layer (8, 9). Hence, a specific length of application time should allow the residual water and solvents to evaporate, leading to development of a uniform adhesive layer (10). Dissolving HAp on 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, morphological observations, and SFE characteristics. The null hypotheses proposed that neither reducing application time nor changing etching mode affected dentin SBS or SFE.

Materials and methods

Study materials

The materials used in the present study are shown in Table 1. Six universal adhesives used were, Adhese Universal (AU; Ivoclar Vivadent, Schaan, Liechtenstein), Clearfil Universal Bond Quick (CQ; Kuraray Noritake Dental, Tokyo, Japan), G-Premio Bond (GP; GC, Tokyo, Japan), Scotchbond Universal (SU; 3M Oral Care, St. Paul, MN, USA), Scotchbond Universal Plus Adhesive (SP; 3M Oral Care), and 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. A halogen quartz tungsten curing unit was used to avoid any influence from the reported nonuniformity of light-emitting diode curing units (11, 12). 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. Approximately of two-thirds of the apical root structure of each tooth was removed using a diamond-impregnated disk in a low-speed 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, 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 (13). The experimental protocols for the bonding procedures are shown in Table 2 and Fig. 1. For each test group, 10 specimens were used to measure the dentin SBS in ER mode (phosphoric acid was applied for 15 s 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 AU, SU, and SP adhesives were applied according to the manufacturer's recommended application time (20 s), and the CQ, GP, and TU adhesives were applied for 10 s (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 24 groups.

A bonding assembly (Ultradent Products) 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) in a fixture against the adherent surfaces. The resin composite was placed into the mold and light irradiated for 30 s. After removing the mold, the specimens were stored in distilled water at 37°C for 24 h and loaded to failure at 1.0 mm per min with Ultradent shearing fixture (Ultradent Products) using a universal testing machine (Type 5500R; Instron,

Norwood, 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 optical microscopy (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 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, the uncured adhesive layer was removed 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 or 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 (14). 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. The equilibrium contact angle (θ) of each test liquid was measured using the sessile drop method at 23 ± 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 (W_{SL}), and the SFE of the liquid and solid (W_L and W_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 (15):

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

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

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

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). 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 h were set in epoxy resin and sectioned lengthwise with the saw (IsoMet 1000 Precision Sectioning Saw). 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 min, the polished surface was etched in hydrogen chloride solution (6 mol/L) for 25 s and

deproteinized by immersing in 6% sodium hypochlorite solution for 3 min. 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 min, 75% for 20 min, 95% for 20 min, and 100% for 2 h) and transferred to a freeze-drying system (Model ID-3, Elionix) for 30 min. The resin–dentin interfaces of the specimens were subjected to argon-ion beam etching (EIS-200ER; Elionix) for 20 s with an ion beam (accelerating voltage 1.0 kV, ion current density 0.4 mA/cm^2) directed perpendicular to the polished surfaces. Finally, all the specimens were coated using an automatic ion spatter (Quick Coater Type SC-701; Sanyu Electron, Tokyo, Japan) with a thin film of gold. SEM 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 = 0.25 (medium), $\alpha = 0.05$ (two sided), power = 0.95, and number of groups = 24, a total sample size of 212 was needed. And then, the effect size of more than 0.56 from the F values and df by using three-way ANOVA on the result data were obtained. The results indicated that at least 9.2 specimens per group were needed. 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.

Three-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$) 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 ($\alpha = 0.05$) 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, Chicago, IL, USA).

Results

SBS

The results for dentin SBS using the different bonding procedures are shown in Table 3. Three-way ANOVA revealed that dentin SBS values were significantly influenced by the factors of adhesive type and application duration ($p < 0.001$), but the factor of pre-etching was not significant ($p = 0.634$). 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, SU, and SP 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

The frequency of different failure modes is shown in Fig. 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.

SFE

The SFE values and components of the different application modes are shown in Fig. 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 Figs. 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 Figs. 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 Figs. 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 (Fig. 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 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 (Fig. 9).

Discussion

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, AU, SU, and SP, 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 AU, SU, and SP.

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 (16, 17). 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. 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 (18, 19).

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, it can be speculated 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 (20) 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, it might be inferred that those monomers could penetrate deep into the demineralized dentin and polymerize to develop a stable polymer network producing strong micromechanical interlocking.

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 (15) 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 (21, 22). Although HAp has high SFE due to the concentration of hydroxyl groups, collagen fibrils composed of insoluble fibrous protein have low SFE (23). 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 (24). 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 (25).

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 (26). 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 indicated 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 (27, 28).

In addition, from the perspective of pH values of the tested adhesive, AU, SU, and SP 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 (14). 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, SU, and SP (29). 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.

Conclusions

The results of the present laboratory study did not reveal any significant differences in dentin SBS values between IA and PA treatments in CQ, GP, and TU, regardless of etching mode. However, AU, SU, and SP, which required active and longer application times, demonstrated lower SBS values in IA than in PA treatment in both SE and ER modes. From the results of SFE measurements, the γ_S was dependent on the adhesive and etching mode. For baseline groups, a significantly lower total free energy (γ_S) value in phosphoric acid etching group was observed when compared with the initial group. 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 with PA compared to IA treatment in SE mode.

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Tables and Figures

Table 1: Materials used in this study

Code	Adhesive	Main components	pH	Manufacturer
Universal adhesives				
AU	Adhese Universal (U49302)	MDP, bis-GMA, HEMA, MCAP, D3MA, ethanol, water, intiator, stabilizers, silicon dioxide	2.5─3.0	Ivoclar Vivadent, Schaan, Lichtenstaein
CQ	Clearfil Universal Bond Quick (9T0050)	bis-GMA, MDP, HEMA, hydrophilic amide monomer, filler, ethanol, water, NaF, photo initiators, 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 Oral Care, St. Paul, MN, USA
SP	Scothchbond Universal Plus Adhesive (7279357)	MDP, HEMA, Vitrebond copolymer, dimethacrylate resins (BPA derivative-free), ethanol, water, initiators, dual-cure accelerator, optimized mixture of silane, filler	2.7	3M Oral Care
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, South Jordan, UT, USA
Resin composite				
	Clearfil AP-X (N416713)	bis-GMA, TEGDMA, silane barium glass filler, silane silica filler, silanated colloidal silica, CQ, pigments, others		Kuraray Noritake Dental

MDP: 10-methacryloyloxydecyl dihydrogen phosphate, bis-GMA: 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy) phenyl) propane, HEMA: 2-hydroxyethyl methacrylate, MCAP: methacrylated carboxylic acid polymer, D3MA: decandiol dimethacrylate, NaF: sodium fluoride, 4-MET: 4-methacryloxyethyl trimellitate, MEPS: methacryloyloxyalkyl thiophosphate methylmethacrylate, BHT: butylated hydroxytoluene, BPA: bisphenol A, TEGDMA: triethyleneglycol dimethacrylate, MTU-6: 6-methacryloyloxyhexyl-2-thiouracil-5-carboxylate, γ-MPTES: γ-methacryloyloxypropyltriethoxysilane

Table 2: Application protocol for pre-etching and universal adhesives

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

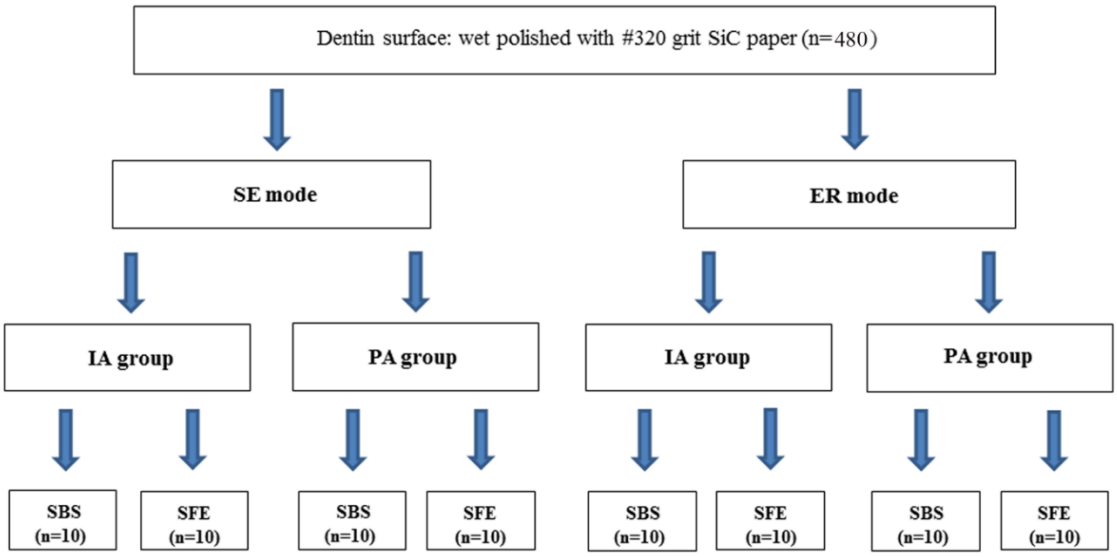
SE: Self-etch, ER: Etch-&-rinse, IA: immediately air-blow after application of adhesive, PA: application of adhesive according to each manufacturer's instructions (AU, SU, and SP) or applied adhesive for 10 s (CQ, GP, and TU)

* Manufacturer's instructions

Table 3: Influence of application time on dentin bond strength (MPa)

	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}
SP	30.6 (4.3)^{abB}	36.4 (4.3)^{aA}	32.4 (4.0)^{abAB}	36.6 (3.8)^{aA}
TU	30.3 (4.7)^{abA}	29.4 (4.2)^{bA}	29.5 (3.1)^{bA}	33.9 (4.5)^{aA}

N=10, mean (SD) in MPa
Same lower case letter in vertical columns indicates no difference at 5% significance level.
Same capital letter in horizontal rows indicates no difference at 5% significance level.
Values in parentheses indicate standard deviation.



SE mode: Phosphoric acid pre-etching was not performed
ER mode: Phosphoric acid etching for 15 s
IA group: Immediately air-blow after application of adhesive
PA group: Application of adhesive according to each manufacturer's instructions (AU, SU, and SP) or applied adhesive for 10 s (CQ, GP, and TU)
SBS: Shear bond strength test
SFE: Surface free energy measurement

Fig. 1 The flow diagram of this study protocol.

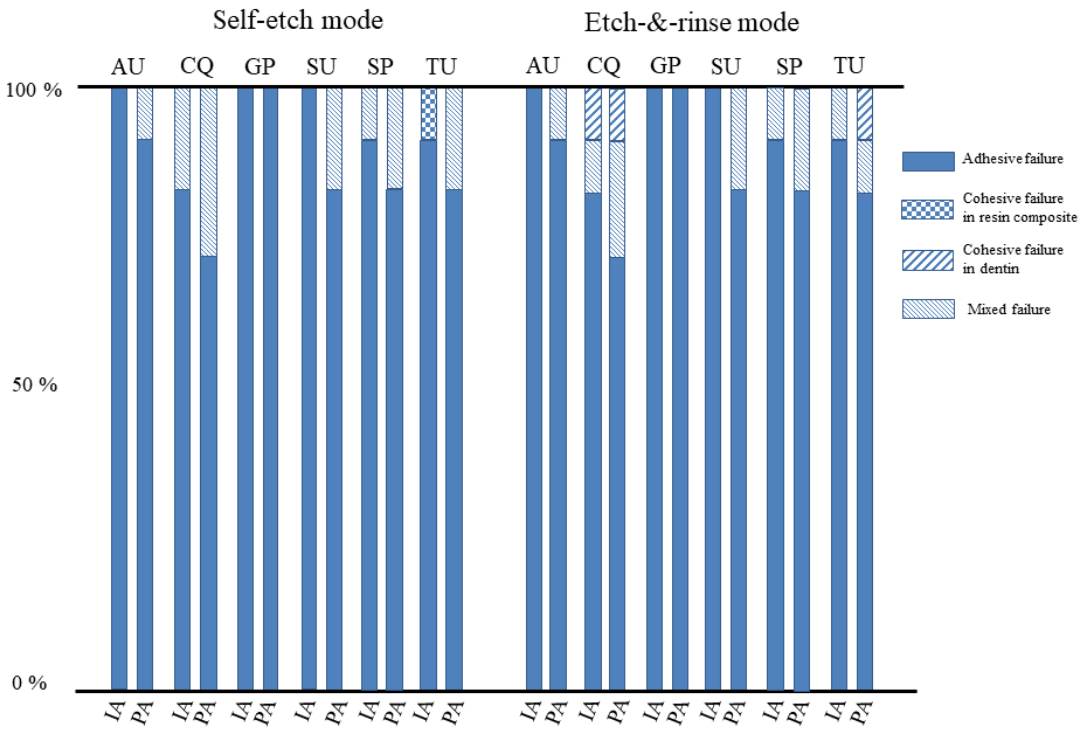


Fig. 2. Failure mode analysis of the de-bonded dentin specimens.

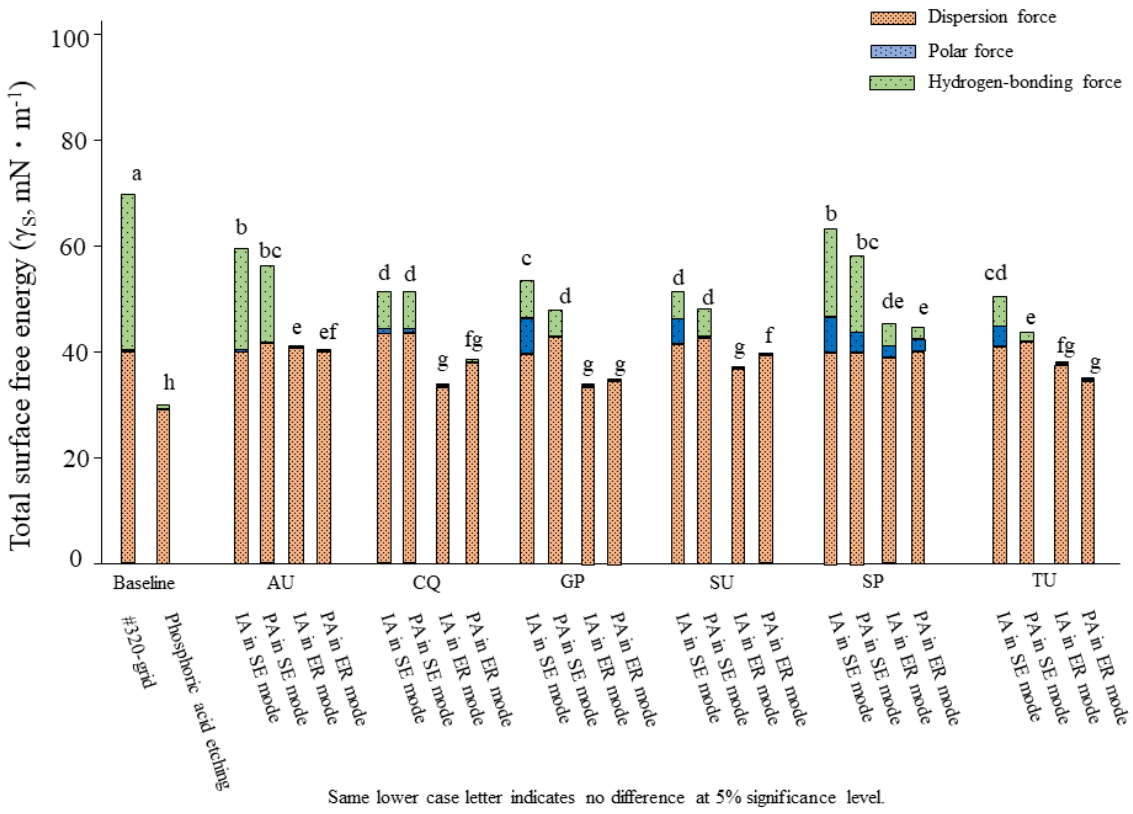


Fig. 3. Total SFE results from different application times in different etching modes.

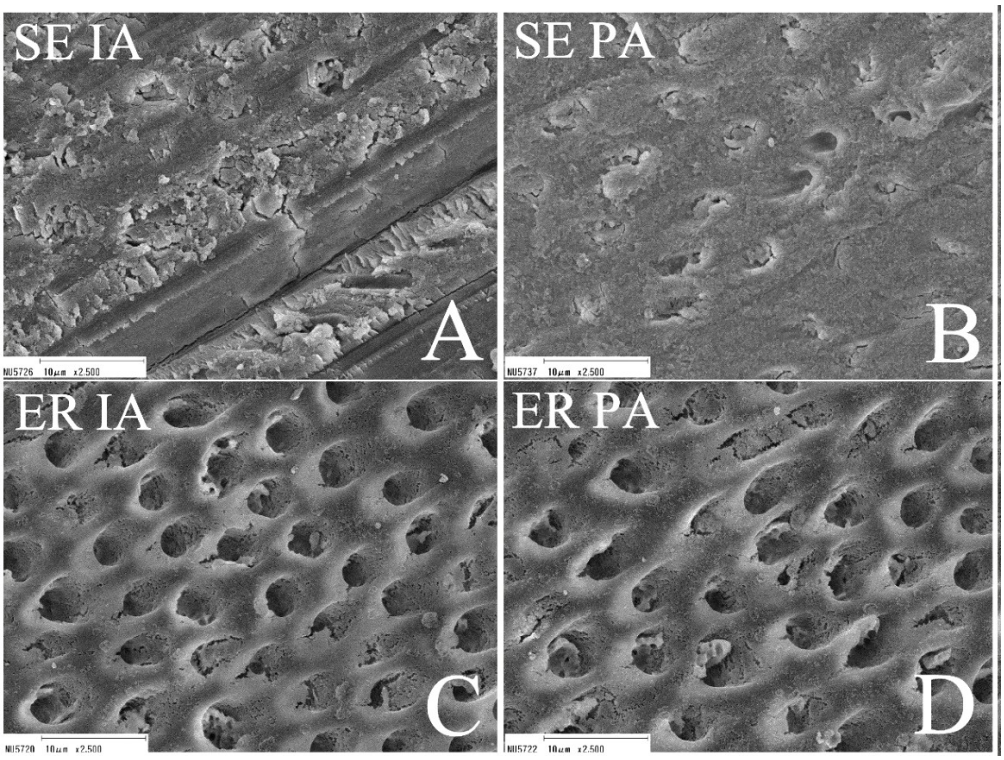


Fig. 4. Representative SEM images of treated dentin surface from different bonding procedures (×5,000).
(A):AU with IA treatment in SE mode. (B): AU with PA treatment in SE mode.
(C): AU with IA treatment in ER mode. (D): AU with PA treatment in ER mode.

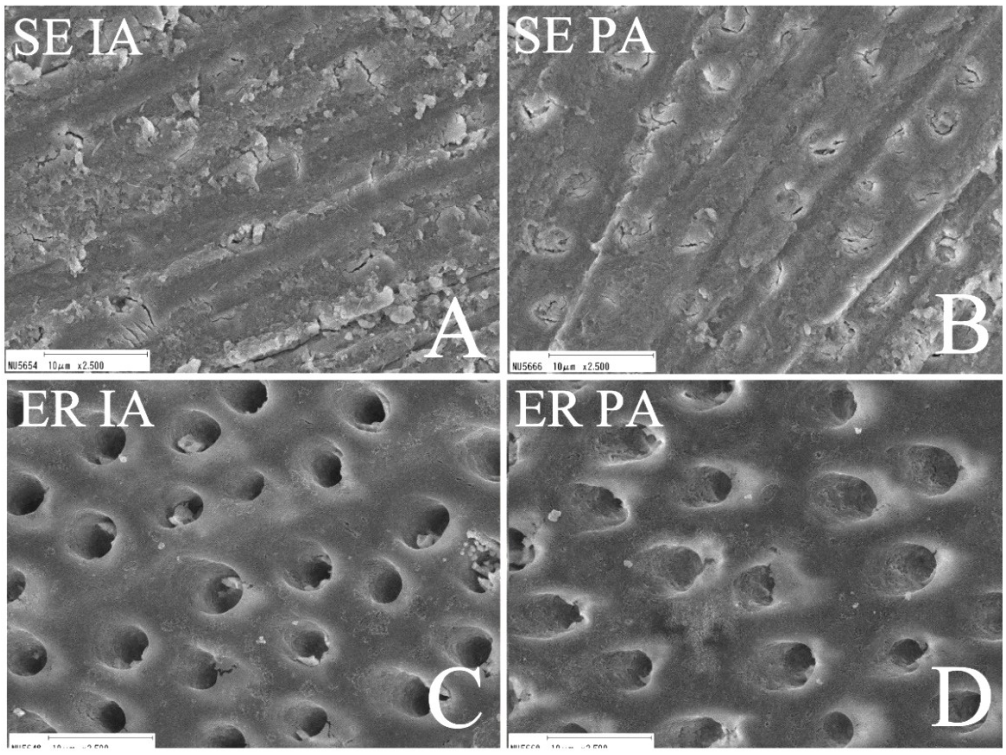


Fig. 5. Representative SEM images of treated dentin surface from different bonding procedures (×5,000).
(A): CQ with IA treatment in SE mode. (B): CQ with PA treatment in SE mode.
(C): CQ with IA treatment in ER mode. (D): CQ with PA treatment in ER mode.

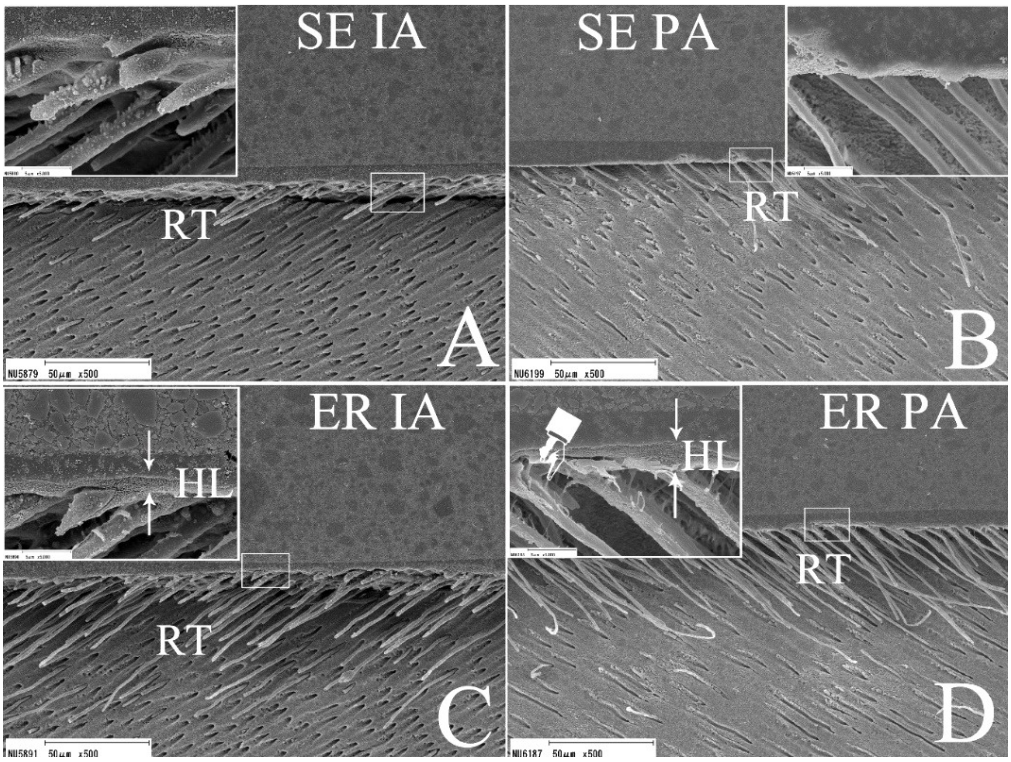


Fig. 6. Representative SEM micrographs of the resin–dentin interfaces. The main images are at ×500.
The smaller white rectangle indicates the location in the main image of the enlarged area, at ×5,000, in the upper right or left corner. The visible material is indicated by abbreviations: HL: hybrid layer, RT: resin tag.
(A): AU with IA treatment in SE mode. (B): AU with PA treatment in SE mode. (C): AU with IA treatment in ER mode. (D): AU with PA treatment in ER mode.

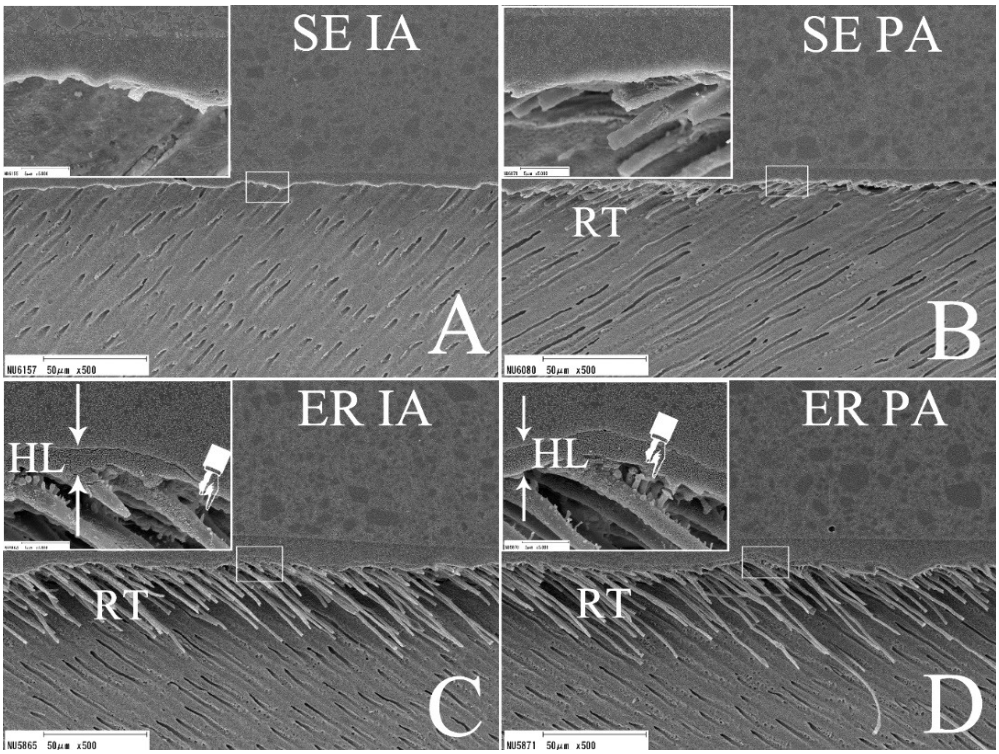


Fig. 7. Representative SEM micrographs of the resin–dentin interfaces. The main images are at ×500.
The smaller white rectangle indicates the location in the main image of the enlarged area, at ×5,000, in the upper left corner. The visible material is indicated by abbreviations: HL: hybrid layer, RT resin tag.
(A): CQ with IA treatment in SE mode. (B): CQ with PA treatment in SE mode. (C): CQ with IA treatment in ER mode. (D): CQ with PA treatment in ER mode.

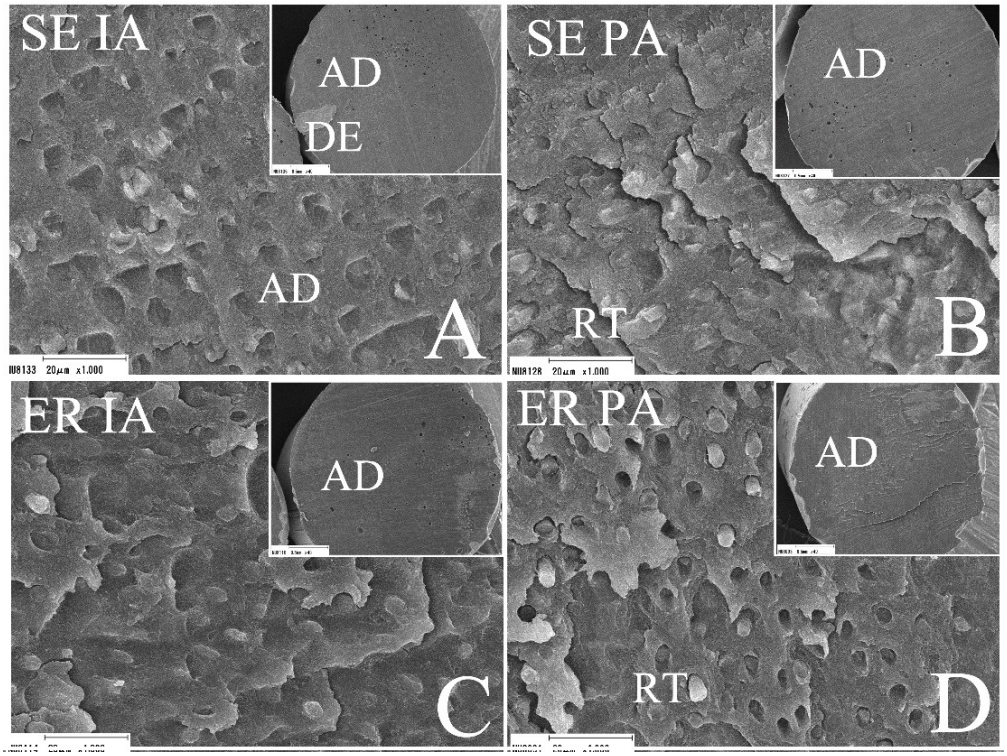


Fig. 8. Representative SEM images of the fractured resin surface in SE mode and in ER mode with different application times. The visible material is indicated by abbreviations: AD: adhesive, DE: dentin, RC: resin composite, RT: resin tag. (A): CQ with IA treatment in SE mode (×50 and ×1,000). (B): CQ with PA treatment in SE mode (×50 and ×1,000). (C): CQ with IA treatment in ER mode (×50 and ×1,000). (D): CQ with PA treatment in ER mode (×50 and ×1,000).

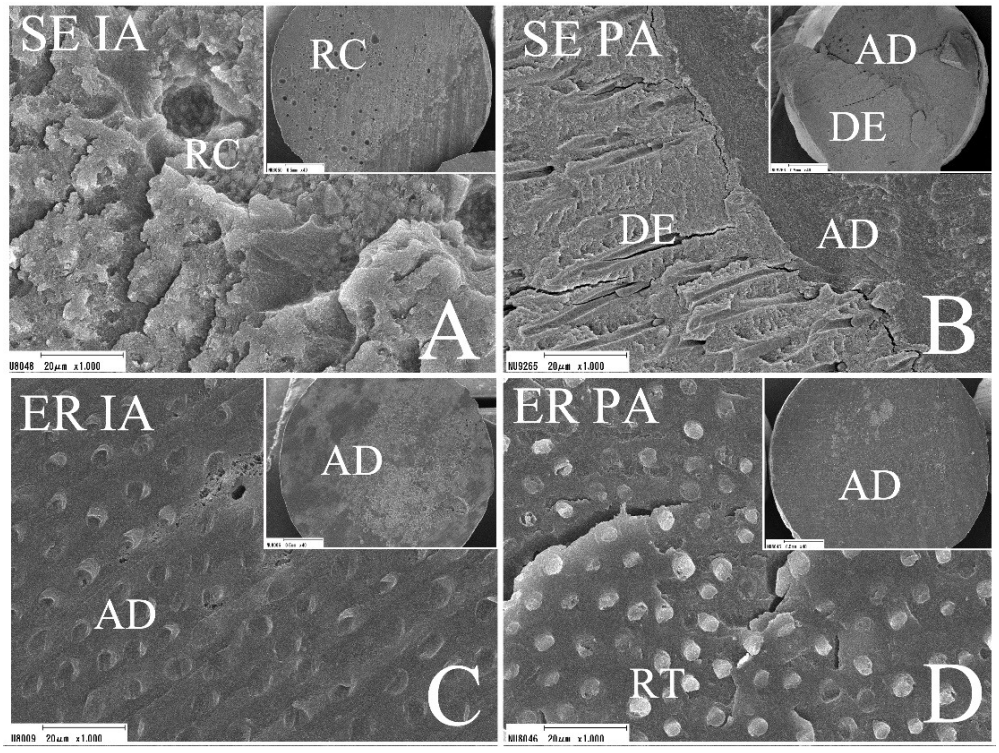


Fig. 9. Representative SEM images of the fractured resin surface in SE mode and in ER mode with different application times. The visible material is indicated by abbreviations: AD: adhesive, DE: dentin, RC: resin composite, RT: resin tag. (A): SU with IA treatment in SE mode (×50 and ×1,000). (B): SU with PA treatment in SE mode (×50 and ×1,000). (C): SU with IA treatment in ER mode (×50 and ×1,000). (D): SU with PA treatment in ER mode (×50 and ×1,000).