



# Effect of double-layer application on the early enamel bond strength of universal adhesives

Eizo Hirokane<sup>1</sup> · Toshiki Takamizawa<sup>1</sup> · Yuta Kasahara<sup>1</sup> · Ryo Ishii<sup>1</sup> · Akimasa Tsujimoto<sup>1</sup> · Wayne W. Barkmeier<sup>2</sup> · Mark A. Latta<sup>2</sup> · Masashi Miyazaki<sup>1</sup>

Received: 22 January 2020 / Accepted: 25 May 2020 / Published online: 30 May 2020  
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

## Abstract

**Objectives** The present study aimed to investigate the changes in the enamel bond strengths of universal adhesives in the early phase of specimen creation and evaluate the effect of double-layer application on enamel bond effectiveness using different etching modes.

**Method** Four universal adhesives, namely Clearfil Universal Bond Quick, G-Premio Bond, Scotchbond Universal, and Tokuyama Universal Bond, were used. Clearfil SE Bond two-step self-etch adhesive system was used as a comparison. Fifteen specimens per group were used for determining the shear bond strength (SBS) to bovine enamel in the etch-and-rinse or self-etch mode. The adhesive was applied to specimens in accordance with the manufacturers' instructions (single-layer application), or the adhesive was applied twice (double-layer application). The bonded specimens were stored in distilled water at 37 °C for 5 min or 1, 6, 12, or 24 h before SBS testing. The flexural properties of the resin composite Clearfil AP-X were measured for the same storage periods.

**Results** All universal adhesives exhibited increased SBS values with prolonged storage periods regardless of the application technique or etching mode used. Double-layer application was associated with higher SBS values than single-layer application for most universal adhesives over the same storage period.

**Conclusion** Phosphoric acid pre-etching and double-layer application of universal adhesives resulted in increased enamel bond strength in the early phase of specimen bonding.

**Clinical relevance** Care should be taken when using universal adhesives in the self-etch mode for enamel bonding because the enamel adhesion is sensitive to the duration after filling.

**Keywords** Enamel bond strength · Double application technique · Universal adhesive · Early bonding performance

## Introduction

Functional monomers have been added to the formulations of adhesive materials for facilitating both mechanical interlocking and chemical bonding to tooth structures. At present, two- and three-step etch-and-rinse (ER) and one- and two-step self-etch (SE) adhesive systems are used [1–3].

Even though the durability of bonding is crucial for the long-lasting outcome of resin composite restorations, initial bonding effectiveness is also a critical factor in preventing restorative failure and post-operative sensitivity.

In clinical situations, external and internal forces that arise shortly after completing resin composite restorations may diminish the bonding performance of adhesive systems [4–6]. For instance, considerable external forces are created by removing the matrix after filling the resin composite or finishing and polishing procedures, and internal forces are caused by the contraction stress of the polymerization reaction. Although several laboratory bond strength tests have been performed after storage for 24 h or longer in 37 °C distilled water, previous studies have illustrated that bond strengths in the early phase were lower than those after 24 h of water storage [6–8]. Irie et al. [6] compared the immediate enamel and

✉ Toshiki Takamizawa  
takamizawa.toshiki@nihon-u.ac.jp

<sup>1</sup> Department of Operative Dentistry, Nihon University School of Dentistry, 1-8-13, Kanda-Surugadai, Chiyoda-ku, Tokyo 101-8310, Japan

<sup>2</sup> Department of General Dentistry, Creighton University School of Dentistry, Omaha, NE, USA

dentin bond strengths of eight single-step SE adhesives and one two-step SE adhesive with those after 24 h of water storage. Most of the single-step SE adhesives displayed significantly lower enamel and dentin bond strengths immediately after creating specimens than after 24 h of water storage. Although it is difficult to describe the reason for the increased bond strength over time, post-polymerization effects after light irradiation of the adhesive layer and resin composites might be extremely important [9–13]. For single-step SE adhesives, water is essential to ionize functional monomers [1, 3]. However, water and solvent remaining in the adhesive layer after application and the low pH of the functional monomer might lead to lower C=C double-bond reactions in the adhesives [1, 14–16].

Although the latest adhesive systems, namely universal adhesives, are based on single-step SE adhesives, they have different characteristics regarding their composition and clinical procedures, as either the ER or SE mode can be used for the tooth substrate [17–20]. In addition, they can be used with resin cement for indirect restorations because of the presence of chemical polymerization initiators [21]. In terms of enamel and dentin bonding, several investigations revealed that universal adhesives have equal or higher bonding effectiveness than the previous generation of single-step SE adhesives [17, 18, 20, 22]. Although universal adhesives are widely used because of their versatility, universal adhesives cannot completely overcome the drawbacks of single-step SE adhesives. To simplify the bonding procedures of universal adhesives, certain amounts of water and solvents are necessary, similarly as required for the conventional single-step SE adhesives [1, 3]. Therefore, similar phenomena might occur at the interface, leading to compromised bonding effectiveness in the early phase and reduced long-term durability of bonding. To improve the long-term durability of restorations using universal adhesives, several techniques such as hydrophobic layer addition, double-layer adhesive application, and hot air blowing have been investigated [23–28]. Double-layer application techniques have proven effective for enhancing the bond durability of universal adhesives in terms of fatigue stress [28]. Conversely, it remains unclear whether double-layer application is also effective for enhancing bonding quality in the early phase. However, there is little information regarding the changes in bonding performance within 24 h or the effect of double-layer application on the bonding effectiveness in the early phase.

This study was designed to determine the changes in the early enamel bond strengths of universal adhesives and clarify whether double-layer application enhanced enamel bonding performance. The null hypotheses to be tested were as follows: (1) the early bonding performance of universal adhesives would not change during the test period, and (2) the double-layer application technique would not increase the enamel bond strength of universal adhesives.

## Materials and methods

### Study materials

The materials used in this study are shown in Table 1. The four universal adhesives used were Clearfil Universal Bond Quick (CU, Kuraray Noritake Dental, Tokyo, Japan), G-Premio Bond (GP, GC, Tokyo, Japan), Scotchbond Universal (SU, 3M Oral Care, St. Paul, MN, USA), and Tokuyama Universal Bond, which is known as Bondmer Lightless in Japan (TU, Tokuyama Dental, Tokyo, Japan). A conventional two-step SE adhesive system, Clearfil SE Bond (CB, Kuraray Noritake Dental) was used as a comparison material. The phosphoric acid etching agent Ultra-Etch (Ultradent Products, South Jordan, UT, USA) and the resin composite Clearfil AP-X (Kuraray Noritake Dental) were used. An Optilux 501 visible-light curing unit (sds Kerr, Danbury, CT, USA) was used, and the light irradiance ( $> 600 \text{ mW/cm}^2$ ) of the curing unit was checked using a dental radiometer (Model 100, Kerr, Orange, CA, USA).

### Specimen preparation

Bovine incisors were used as substitutes for human teeth. Each tooth was cut at the cervical line using a diamond-impregnated disk in a low-speed saw (Isomet Low Speed Saw, Buehler, Lake Bluff, IL, USA) to remove root structures. Pulp tissues were then removed, and the labial surfaces were ground with wet 240-grit silicon carbide (SiC) paper (Fuji Star Type DDC, Sankyo Rikagaku, Saitama, Japan) for 20 s to create a flat enamel surface. Each tooth was then mounted in self-curing acrylic resin (Tray Resin II, Shofu, Kyoto, Japan) to expose the flattened area. The flattened area was polished using 320-grit SiC paper (Fuji Star Type DDC) for 5 s with running water and then dried under oil-free compressed air. Finally, an approximately  $30 \text{ mm}^2$  (6 mm in diameter) flattened enamel surface at the center of the tooth surface was obtained.

### Adhesive application protocol

The adhesive application protocols are shown in Table 2. Fifteen specimens were used for each test group to determine the shear bond strength (SBS) to enamel in the SE (without phosphoric acid pre-etching) or ER mode (phosphoric acid pre-etching for 15 s). For single-layer application, the adhesives were used in accordance with the manufacturers' instructions. For double-layer application, the adhesives were applied twice, each time in accordance with the duration recommended by the manufacturers' instructions. For the two-step SE mode, the second application of adhesive resin was performed without priming, followed by exposure to light irradiation. Regarding the adhesive application, only SU was

**Table 1** Materials used in this study

Code	Adhesive (lot. no)	Main components	pH	Manufacturer
CU	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
TU	Tokuyama Universal Bond (004067)	Liquid A: phosphate monomer, bis-GMA, TEGDMA, HEMA, MTU-6, others Liquid B: acetone, isopropanol, water, acryl borate catalyst, $\gamma$ -MPTES, peroxide, others	2.2	Tokuyama Dental, Tokyo, Japan
CB	Clearfil SE Bond (Primer: 5852494) (Adhesive: 5847004)	Primer: MDP, HEMA, water, initiators Adhesive: MDP, HEMA, bis-GMA, initiators, microfiller	2.0 (Primer)	Kuraray Noritake Dental, Tokyo, Japan
<b>Pre-etching agent</b>		<b>Main components</b>		<b>Manufacturer</b>
	Ultra-Etch (G017)	35% phosphoric acid		Ultradent Products, South Jordan, UT, USA
<b>Resin composite</b>		<b>Main components</b>		<b>Manufacturer</b>
	Clearfil AP-X (380094)	bis-GMA, TEGDMA, silane barium glass filler, silane silica filler, silanated colloidal silica, CQ, pigments, others		Kuraray Noritake Dental

*bis-GMA* 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy) phenyl] propane, *MDP* 10-methacryloyloxydecyl dihydrogen phosphate, *HEMA* 2-hydroxyethyl methacrylate, *4-MET* 4-methacryloyloxyethyl trimellitate, *MEPS* methacryloyloxyalkyl thiophosphate methylmethacrylate, *BHT* butylated hydroxytoluene, *MCAP* methacrylated carboxylic acid polymer, *TEGDMA* triethyleneglycol dimethacrylate, *MTU-6* 6-methacryloyloxyhexyl-2-thiouracil-5-carboxylate,  $\gamma$ -*MPTES*  $\gamma$ -methacryloyloxypropyltriethoxysilane

applied with a rubbing motion, in accordance with the manufacturers' recommendations.

### SBS tests

Specimens were clamped in an Ultradent Bonding Jig (Ultradent Products), and resin composites were placed on the enamel surfaces using plastic molds with an internal diameter and height of 2.38 and 2.0 mm, respectively, followed by light irradiation for 30 s. The molds were removed and the excessive adhesive around the assembly was removed with a sharp scalpel, and then the bonded specimens were stored in distilled water at 37 °C in the dark for 5 min or 1, 6, 12, or 24 h before SBS testing.

After each storage period, the SBS to enamel was measured using the notched-edge SBS test according to the ISO 29022 specification [29]. An Ultradent bonding assembly (Ultradent Products) was used to measure SBS. The specimens were loaded to failure at a crosshead speed of 1.0 mm/min with an Ultradent shearing fixture using a universal testing machine (Type 5500R, Instron, Canton, MA, USA). The SBS values (MPa) were calculated as the peak load at failure divided by the bonded surface area. After testing, the bonded tooth surfaces and resin composite cylinders were observed under an optical microscope (SZH-131, Olympus Ltd., Tokyo, Japan) at  $\times 10$  magnification to determine the bond failure mode. Based on the percentage of substrate area (adhesive-resin

composite-tooth) observed on the debonded cylinders and tooth bonding sites, the types of bond failure were recorded as (1) adhesive failure, (2) cohesive failure in composite, (3) cohesive failure in enamel, or (4) mixed failure (partially adhesive and partially cohesive).

### Flexural strength test

To understand the changes in the mechanical properties of the used resin composite in the early phase after light irradiation, flexural properties were determined following the ISO 4049 specifications [30]. The resin composite was filled into a stainless-steel split mold with the dimensions of  $25 \times 2 \times 2$  mm. The mold was then positioned on a glass slide. The middle third of the specimen was irradiated for 30 s, after which the other thirds were irradiated for 30 s each. The hardened specimen was removed from the mold, and all six sides were wet-polished with 1200-grit SiC (Fuji Star type DDC) under running water. The dimensions of the polished specimen were checked with a digital caliper (500-151, CD-15C, Mitutoyo, Tokyo, Japan) during the polishing procedures to obtain standard specimens ( $25 \times 2 \times 2$  mm). Specimens were divided into five groups to measure the flexural strength at 5 min or 1, 6, 12, or 24 h after light irradiation. The 5-min group was tested immediately after the completion of polishing. The other groups were stored in distilled water at 37 °C in the dark for the specified times. Twelve specimens per test group were subjected to

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

Etching mode	Pre-etching protocol
SE (self-etch)	Phosphoric acid pre-etching was not performed.
ER (etch-and-rinse)	Enamel surface was phosphoric acid etched for 15 s. Etched surface was rinsed with water for 15 s (three-way dental syringe) and air-dried until white turbidity was visible.
<b>Adhesive</b>	<b>Adhesive application protocol</b>
CU	<p>Single layer</p> <p>Adhesive was applied to air-dried enamel surface for 10 s and then 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 irradiated for 10 s.</p> <p>Double layer</p> <p>The above application procedure was performed twice, applying the second coat of adhesive immediately after completing light irradiation.</p>
GP	<p>Single layer</p> <p>Adhesive was applied to air-dried enamel surface for 10 s and then a strong stream of air applied over the liquid adhesive for 5 s or until the adhesive no longer moving and the solvent had completely evaporated. Light irradiated for 10 s.</p> <p>Double layer</p> <p>The above application procedure was performed twice, applying the second coat of adhesive immediately after completing light irradiation.</p>
SU	<p>Single layer</p> <p>Adhesive was applied to air-dried enamel surface with rubbing motion for 20 s and then medium air pressure was applied over the liquid adhesive for 5 s. Light irradiated for 10 s.</p> <p>Double layer</p> <p>The above application procedure was performed twice, applying the second coat of adhesive immediately after completing light irradiation.</p>
TU	<p>Single layer</p> <p>Adhesive was applied to the air-dried enamel surface for 10 s and then medium air pressure was applied over the liquid adhesive for 5 s. No light irradiation.</p> <p>Double layer</p> <p>The above application procedure was performed twice. After air blowing the first layer, the second layer of adhesive was applied for 10 s. No light irradiation.</p>
CB	<p>Single layer</p> <p>Primer was applied to air-dried enamel surfaces for 20 s followed by medium air pressure was applied to surfaces for 5 s. Adhesive was then applied to primed surfaces and was air thinned gently. Adhesive was light irradiated for 10 s.</p> <p>Double layer</p> <p>The primer was applied once, followed by two applications of adhesive, as above. Applying the second coat of adhesive immediately after completing light irradiation.</p>

the three-point bending test (span length = 20 mm) using a universal testing machine (model 5500R; Instron) at a crosshead speed of 1.0 mm/min until the breaking point was reached. The flexural strength ( $\sigma_f$ ) and elastic modulus ( $E$ ) were determined from the stress–strain curve using the built-in computer software (Bluehill version 2.5; Instron) connected to the testing machine.

### SEM observation

Resin composite/enamel interfaces from each group and representative fracture sites after the SBS test were observed via

field-emission microscopy (FE-SEM, ERA-8800FE, Elionix, Tokyo, Japan). For ultrastructural observations of the resin composite/enamel interface, specimens bonded using single- or double-layer application were embedded in epoxy resin (Epon 812, Nisshin EM, Tokyo, Japan) and then longitudinally sectioned using a low-speed saw (Isomet Low Speed Saw, Buehler). The sectioned surfaces were polished to a high gloss with abrasive discs (Fuji Star Type DDC) followed by diamond pastes down to a particle size of 0.25  $\mu\text{m}$  (DP-Paste, Struers, Ballerup, Denmark). All scanning electron microscopy (SEM) specimens were dehydrated in ascending grades of *tert*-butyl alcohol (50% for 20 min, 75% for 20 min, 95% for

20 min, and 100% for 2 h) and then transferred from the final 100% bath to a freeze dryer (Model ID-3, Elionix, Tokyo, Japan) for 30 min. Resin composite/enamel interface specimens were then subjected to argon-ion beam etching (EIS-200ER, Elionix, Tokyo, Japan) for 40 s with the ion beam (accelerating voltage 1.0 kV, ion current density 0.4 mA/cm<sup>2</sup>) directed perpendicular to the polished surfaces. Finally, fracture sites and interface specimens were coated in a vacuum evaporator (Quick Coater, Type SC-701, Sanyu Denchi, Tokyo, Japan) with a thin film of Au. SEM observations were conducted at an operating voltage of 10 kV.

### Statistical analysis

The SBS data for each group were tested for homogeneity of variance (Bartlett's test) and normal distribution (Kolmogorov–Smirnov test). To analyze the SBS data, three-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test ( $\alpha = 0.05$ ) was used. The SBS data in the SE and ER modes were analyzed separately. The analysis factors included the (1) application technique, (2) storage time, and (3) adhesive system. One-way ANOVA followed by Tukey's HSD test ( $\alpha = 0.05$ ) was used for comparisons within subsets of the data.

To analyze the flexural properties data, one-way ANOVA followed by Tukey's HSD test ( $\alpha = 0.05$ ) was used. The statistical analysis was performed using the Sigma Plot software system (Ver. 11.0; SPSS, Chicago, IL, USA).

## Results

### SBS

The SBS results are presented in Tables 3 and 4. Three-way ANOVA illustrated that the application technique, storage period, and adhesive system all significantly influenced the SBS values ( $p < 0.001$ ) regardless of the etching mode. In addition, all of the interactions between the factors were significant ( $p < 0.05$ ) in both etching modes.

The results for the SBS in SE mode are presented in Table 3. For the single-layer application, the mean SBS values ranged from 16.6 (1.1) to 26.9 (2.2) MPa in CU, ranged from 21.4 (2.5) to 28.9 (1.9) MPa in GP, ranged from 14.8 (1.4) to 28.6 (1.7) MPa in SU, ranged from 15.4 (1.7) to 29.6 (4.1) MPa in TU, and ranged from 30.5 (1.4) to 41.6 (3.4) MPa in CB, respectively. For the double-layer application, the mean SBS values ranged from 22.6 (2.4) to 34.1 (4.0) MPa in CU, ranged from 25.4 (1.1) to 34.4 (4.5) MPa in GP, ranged from 25.0 (2.7) to 36.4 (4.5) MPa in SU, ranged from 21.8 (2.4) to 36.1 (1.1) MPa in TU, and ranged from 32.3 (1.5) to 40.9 (3.9) MPa in CB, respectively. Although the SBS values were higher for double-layer application than for

single-layer application for all universal adhesives and storage periods excluding GP at 6 h, no differences were observed between single- and double-layer application for CB for any storage period. The SBS values of all adhesives increased with prolonged storage regardless of the application technique. Conversely, no differences in SBS were noted for CB between 6 and 24 h of storage. Setting the SBS values of single-layer application in the 5-min storage group for each tested adhesive at 100%, we found that the SBS values ranged from 99.4 to 245.9% for prolonged storage. In particular, the SBS values for CU, SU, and TU after double-layer application and 24 h of storage were higher than those of GP and CB (Fig. 1).

The results for the SBS in ER mode are presented in Table 4. For the single-layer application, the mean SBS values ranged from 28.0 (2.1) to 46.8 (4.5) MPa in CU, ranged from 26.4 (2.0) to 35.0 (2.5) MPa in GP, ranged from 27.2 (2.7) to 39.1 (4.0) MPa in SU, ranged from 26.6 (2.1) to 42.8 (2.5) MPa in TU, and ranged from 37.6 (1.1) to 44.7 (2.9) MPa in CB, respectively. For the double-layer application, the mean SBS values ranged from 34.9 (2.2) to 47.5 (2.3) MPa in CU, ranged from 29.7 (2.2) to 34.5 (2.1) MPa in GP, ranged from 30.0 (3.8) to 46.4 (3.6) MPa in SU, ranged from 30.6 (3.0) to 50.3 (5.5) MPa in TU, and ranged from 37.2 (2.4) to 46.6 (1.6) MPa in CB, respectively. The SBS values of all adhesives increased with prolonged storage regardless of the application technique. However, three trends were observed when comparing the SBS values for single- and double-layer application for the same storage period. No significant differences in SBS were observed for GP and CB between single- and double-layer application for any storage period. Meanwhile, no differences in SBS between single- and double-layer application were noted for SU and TU after 5 min of storage, whereas the values were significantly higher for double-layer application in both groups for longer storage periods. Although significantly higher SBS values were observed for double-layer application for CU in the early phase storage groups, no significant differences were found between single- and double-layer application in the 12- and 24-h storage groups. Setting the SBS values of single-layer application in the 5-min storage group for each tested adhesive at 100%, the SBS values ranged from 98.9 to 189.1%. Comparing the SBS changes over time in the SE and ER modes, all adhesives displayed smaller proportional increases in the ER mode than in the SE mode (Fig. 2).

### Failure mode

Regarding the SE mode, the failure mode of the tested universal adhesives was adhesive failure regardless of the application technique or storage period. Conversely, although the predominant mode of failure for single-layer application of CB was adhesive failure for all storage groups, for double-



**Table 3** Influence of adhesive layer on early enamel bond strength (self-etch mode)

	5 min		1 h		6 h		12 h		24 h	
	Single layer	Double layer	Single layer	Double layer	Single layer	Double layer	Single layer	Double layer	Single layer	Double layer
CU	16.6 (1.1) <sup>cC</sup> [100%]	22.6 (2.4) <sup>bCB</sup> [136.1%]	16.5 (1.2) <sup>cC</sup> [99.4%]	24.0 (3.8) <sup>EB</sup> [144.6%]	16.9 (1.9) <sup>cC</sup> [101.8%]	31.2 (3.5) <sup>bA</sup> [188.0%]	22.9 (3.0) <sup>EB</sup> [138.0%]	32.8 (5.8) <sup>bA</sup> [197.6%]	26.9 (2.2) <sup>BB</sup> [162.0%]	34.1 (4.0) <sup>bA</sup> [205.4%]
GP	21.4 (2.5) <sup>BD</sup> [100%]	25.4 (1.1) <sup>bBC</sup> [118.7%]	21.7 (2.4) <sup>BD</sup> [101.4%]	26.0 (3.2) <sup>bBC</sup> [121.5%]	23.8 (2.4) <sup>bCD</sup> [111.2%]	25.4 (2.3) <sup>bBC</sup> [118.7%]	25.4 (3.7) <sup>bBC</sup> [118.7%]	33.2 (2.2) <sup>bA</sup> [155.1%]	28.9 (1.9) <sup>BB</sup> [135.0%]	34.4 (4.5) <sup>bA</sup> [160.7%]
SU	14.8 (1.4) <sup>BE</sup> [100%]	25.0 (2.7) <sup>bBC</sup> [168.9%]	18.1 (2.3) <sup>ED</sup> [122.3%]	24.8 (3.4) <sup>CC</sup> [167.6%]	19.9 (3.0) <sup>ED</sup> [134.5%]	28.7 (3.8) <sup>bCB</sup> [193.9%]	21.3 (2.0) <sup>ED</sup> [143.9%]	32.9 (3.1) <sup>bA</sup> [222.3%]	28.6 (1.7) <sup>BB</sup> [193.2%]	36.4 (4.5) <sup>bBA</sup> [245.9%]
TU	15.4 (1.7) <sup>BD</sup> [100%]	21.8 (2.4) <sup>CC</sup> [141.6%]	17.1 (2.5) <sup>CD</sup> [111.0%]	29.6 (2.5) <sup>BB</sup> [192.2%]	20.7 (4.7) <sup>bCC</sup> [134.4%]	30.3 (1.8) <sup>BB</sup> [196.8%]	28.1 (3.2) <sup>BB</sup> [182.5%]	35.7 (2.9) <sup>ABA</sup> [231.8%]	29.6 (4.1) <sup>BB</sup> [192.2%]	36.1 (1.1) <sup>ABA</sup> [234.4%]
CB	30.5 (1.4) <sup>CC</sup> [100%]	32.3 (1.5) <sup>CC</sup> [105.9%]	36.2 (2.3) <sup>AB</sup> [118.7%]	36.3 (2.3) <sup>BB</sup> [119.0%]	37.5 (1.8) <sup>uAB</sup> [122.9%]	39.7 (1.8) <sup>uAB</sup> [130.2%]	37.4 (2.9) <sup>uAB</sup> [122.6%]	37.5 (3.7) <sup>uAB</sup> [122.9%]	41.6 (3.4) <sup>uA</sup> [136.4%]	40.9 (3.9) <sup>uA</sup> [134.1%]

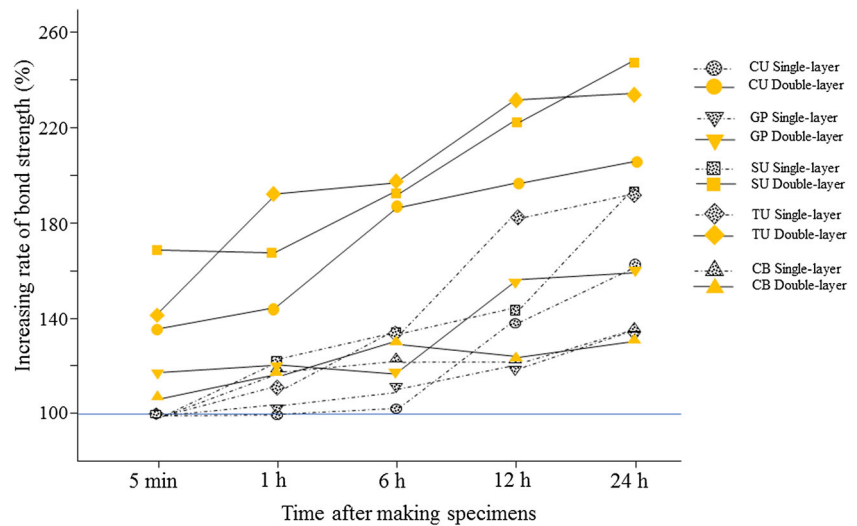
$n = 15$ , mean (SD) in MPa. Same lowercase letter in vertical columns indicates no difference at 5% significance level. Same capital letter in horizontal rows indicates no difference at 5% significance level. Values in parenthesis indicate standard deviation. Percentage values in brackets indicate strength relative to single layer strength at 5 min

**Table 4** Influence of adhesive layer on early enamel bond strength (etch-and-rinse mode)

	5 min		1 h		6 h		12 h		24 h	
	Single layer	Double layer	Single layer	Double layer	Single layer	Double layer	Single layer	Double layer	Single layer	Double layer
CU	28.2 (1.5) <sup>BD</sup> [100%]	34.9 (2.2) <sup>BC</sup> [123.8%]	28.0 (2.1) <sup>BD</sup> [99.3%]	37.0 (3.7) <sup>bBC</sup> [131.2%]	34.9 (3.1) <sup>BC</sup> [123.8%]	41.0 (3.4) <sup>BB</sup> [145.4%]	37.6 (3.2) <sup>bBC</sup> [133.3%]	40.1 (3.1) <sup>CB</sup> [142.2%]	46.8 (4.5) <sup>uA</sup> [166.0%]	47.5 (2.3) <sup>abA</sup> [168.4%]
GP	26.4 (2.0) <sup>BC</sup> [100%]	29.7 (2.2) <sup>bBC</sup> [112.5%]	29.4 (4.3) <sup>bBC</sup> [111.3%]	31.2 (1.7) <sup>cAB</sup> [118.2%]	31.0 (3.7) <sup>bAB</sup> [117.4%]	32.6 (2.2) <sup>cAB</sup> [123.5%]	35.0 (2.5) <sup>bCA</sup> [132.6%]	32.3 (2.2) <sup>dAB</sup> [122.3%]	34.8 (3.1) <sup>cA</sup> [131.8%]	34.5 (2.1) <sup>cA</sup> [130.7%]
SU	27.2 (2.7) <sup>BE</sup> [100%]	30.0 (3.8) <sup>cDE</sup> [110.3%]	29.4 (2.5) <sup>bDE</sup> [108.1%]	36.9 (2.8) <sup>BC</sup> [135.7%]	31.5 (3.6) <sup>bDE</sup> [115.8%]	41.8 (2.1) <sup>BB</sup> [153.7%]	32.2 (1.4) <sup>CD</sup> [117.5%]	42.9 (2.6) <sup>bCAB</sup> [157.7%]	39.1 (4.0) <sup>bCBC</sup> [143.8%]	46.4 (3.6) <sup>abA</sup> [170.6%]
TU	26.6 (2.1) <sup>BF</sup> [100%]	30.6 (3.0) <sup>eEF</sup> [115.0%]	28.1 (1.9) <sup>BE</sup> [105.6%]	38.7 (2.7) <sup>BC</sup> [145.5%]	34.3 (3.1) <sup>bDE</sup> [128.9%]	40.4 (1.8) <sup>bBC</sup> [151.9%]	38.6 (2.8) <sup>bCD</sup> [145.1%]	43.8 (2.9) <sup>abB</sup> [164.7%]	42.8 (2.5) <sup>abBC</sup> [160.9%]	50.3 (5.5) <sup>aA</sup> [189.1%]
CB	37.6 (1.1) <sup>CC</sup> [100%]	37.2 (2.4) <sup>CC</sup> [98.9%]	42.4 (2.0) <sup>AB</sup> [112.8%]	44.9 (2.2) <sup>uAB</sup> [119.4%]	44.8 (2.2) <sup>uAB</sup> [119.1%]	46.6 (1.6) <sup>uA</sup> [123.9%]	44.4 (4.4) <sup>uAB</sup> [118.1%]	46.2 (1.7) <sup>uA</sup> [122.9%]	44.7 (2.9) <sup>uAB</sup> [118.9%]	45.6 (2.8) <sup>bAB</sup> [121.3%]

$n = 15$ , mean (SD) in MPa. 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. Percentage values in brackets indicate strength relative to single layer strength at 5 min

**Fig. 1** Changes in shear bond strength (%) in self-etch mode. Abbreviations: CU, Clearfil Universal Bond Quick; GP, G-Premio Bond; SU, Scotchbond Universal; TU, Tokuyama Universal Bond; CB, Clearfil SE Bond



layer application, the proportions of mixed and cohesive failures in enamel increased with increasing storage time.

For the ER mode, the universal adhesives only exhibited adhesive failure for both single- and double-layer application in the 5-min and 1-h storage groups. However, excluding GP, mixed and cohesive failures in enamel were observed in the 6-, 12-, and 24-h storage groups regardless of the application technique. Contrarily, although only adhesive failure was observed for CB in the 5-min group for both application techniques, mixed and cohesive failures in enamel were found after 1 h of storage, and the frequencies of these failure modes increased with prolonged storage.

**Flexural properties**

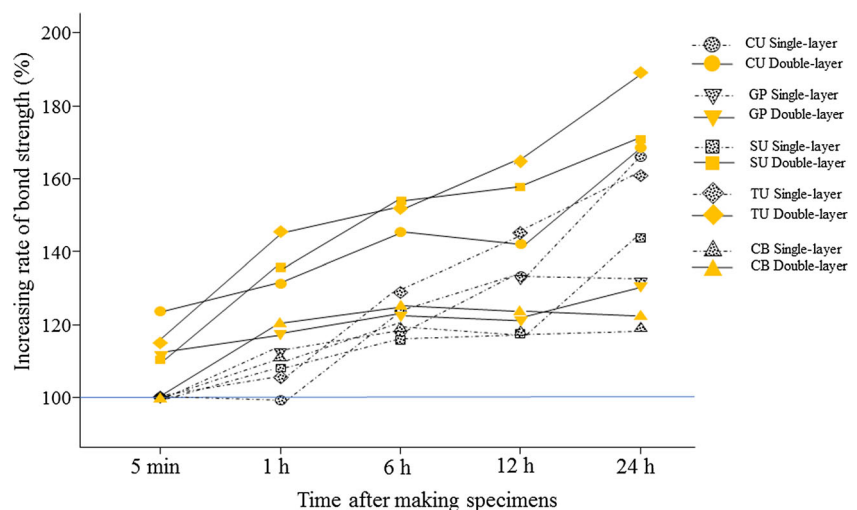
Changes in the flexural properties of the tested resin composite over time are shown in Table 5. The tested resin composite displayed increased  $\sigma_F$  and  $E$  with prolonged storage. Significant lower  $\sigma_F$  values were observed after 5 min and

1 h than after longer storage periods. Setting the  $\sigma_F$  values in the 5-min storage group for each for each adhesive at 100%, the  $\sigma_F$  values ranged from 118.0 to 138.9%. The tested resin composite exhibited significantly lower  $E$  values after 5 min and 1 h of storage than after longer storage periods. Setting the  $E$  values in the 5-min storage group for each adhesive at 100%, the  $\sigma_F$  values ranged from 134.1 to 175.8%.

**SEM observations**

Representative SEM images of restorative/enamel interfaces are shown in Figs. 3 and 4. For each adhesive, the thickness of the adhesive layer (AL) was similar between the SE and ER modes. However, the AL thickness differed between the application techniques. The AL thickness of universal adhesives was similar, ranging from 5 to 10  $\mu\text{m}$  for single-layer application and from 15 to 20  $\mu\text{m}$  for double-layer application. However, the AL thickness of CB was 30–40  $\mu\text{m}$  for single-layer application and 50–60  $\mu\text{m}$  for double-layer application.

**Fig. 2** Changes in shear bond strength (%) in etch-and-rinse mode. Abbreviations: CU, Clearfil Universal Bond Quick; GP, G-Premio Bond; SU, Scotchbond Universal; TU, Tokuyama Universal Bond; CB, Clearfil SE Bond



**Table 5** Flexural properties of AP-X

Time	Flexural strength (MPa)	Elastic modulus (GPa)
5 min	121.8 (9.4) <sup>c</sup> [100%]	9.1 (0.7) <sup>c</sup> [100%]
1 h	143.7 (9.6) <sup>b</sup> [118.0%]	12.2 (0.9) <sup>b</sup> [134.1%]
6 h	158.1 (10.4) <sup>a</sup> [129.8%]	15.0 (1.2) <sup>a</sup> [164.8%]
12 h	163.2 (12.3) <sup>a</sup> [134.0%]	15.8 (0.8) <sup>a</sup> [173.6%]
24 h	169.2 (10.0) <sup>a</sup> [138.9%]	16.0 (0.9) <sup>a</sup> [175.8%]

Percentage values in brackets indicate flexural strength and elastic modulus values relative to values at 5 min. Same lowercase letter in vertical columns indicates no difference at 5% significance level

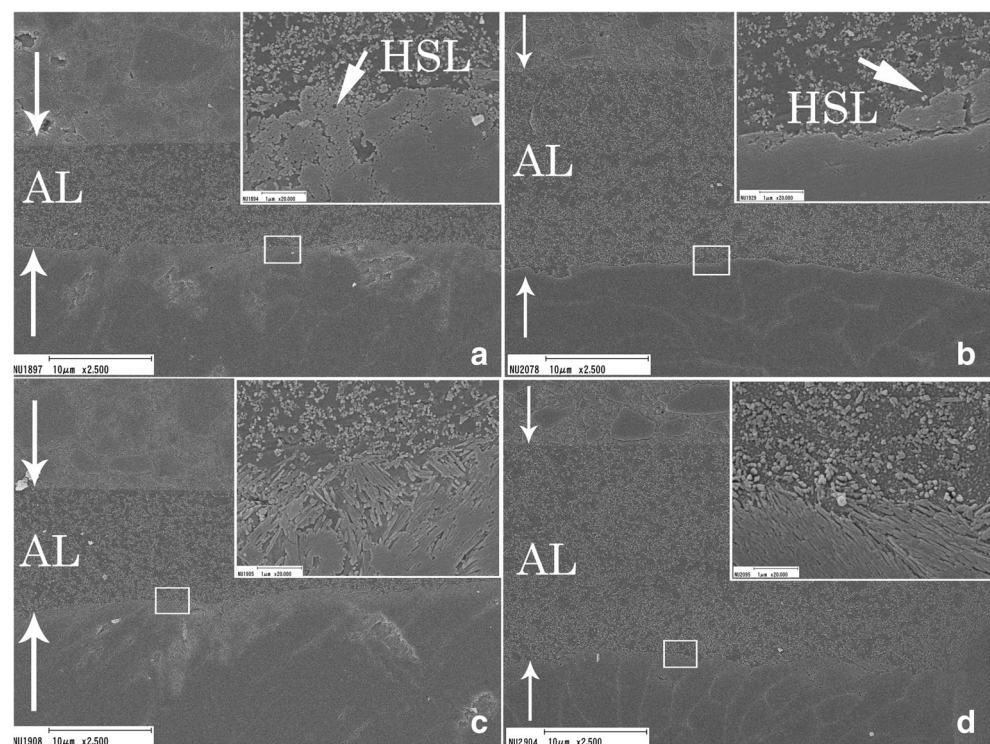
Regarding the AL/enamel interface, all of the tested adhesives displayed excellent adaptation between the enamel substrate and adhesive regardless of the application technique and etching mode. However, the morphological features near the AL-enamel interface differed between the etching modes. For all tested adhesives, the smear layer was completely dissolved in the ER mode regardless of the application technique. Additionally, adhesive interpenetration with enamel in the ER mode was indicated by the presence of the typical etching patterns. Regarding universal adhesives in the SE mode, the smear layer remained and formed a hybrid smear layer (indicated by small arrows) in which the resin monomers

penetrated beyond the smear layer into the intact enamel surface. Contrarily, although typical etching patterns were not observed for CB, the remaining smear layer appeared to be smaller than that of the universal adhesives, excluding GP.

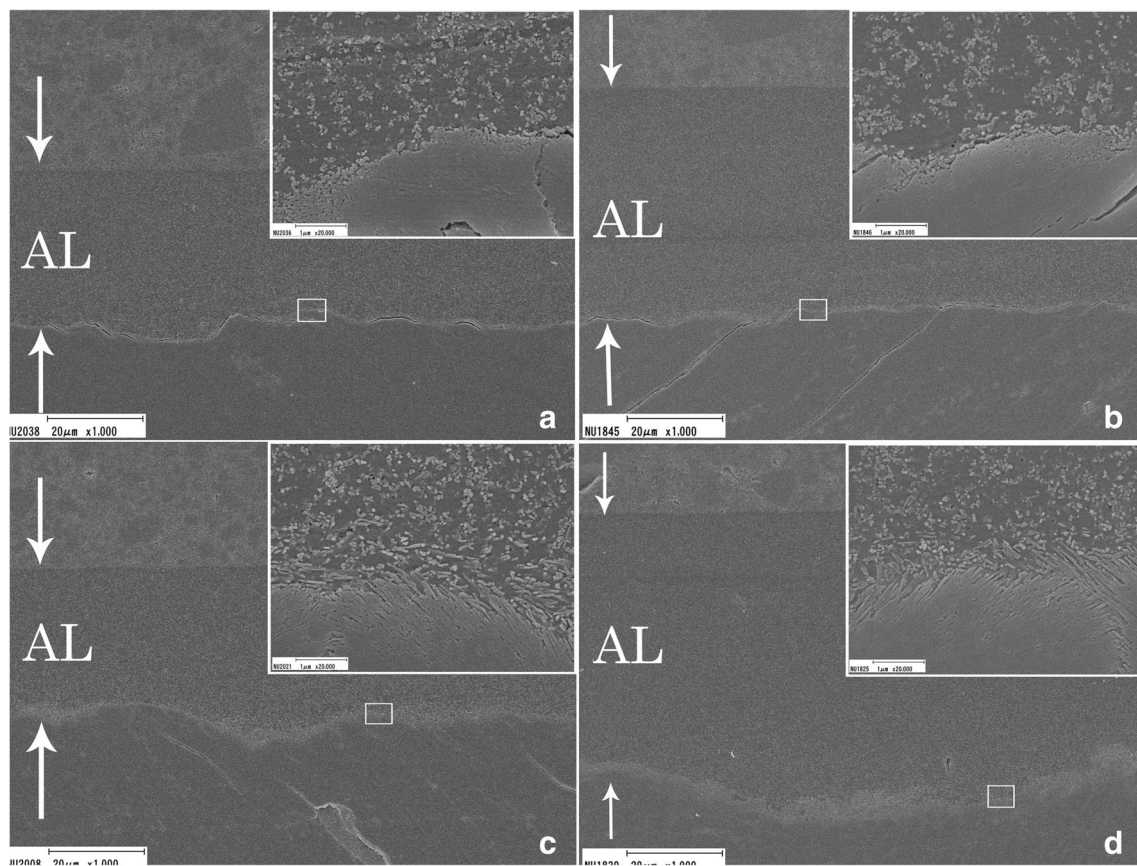
Representative SEM images of the debonded specimens are presented in Figs. 5 and 6. The appearance of the failure pattern was adhesive system-, application technique-, and etching mode-dependent. For the 5-min groups with single-layer application of the universal adhesives, although a similar morphological appearance typified by numerous bubbles was found at lower magnification in both the SE and ER modes (Fig. 5 a and e), attached enamel fragments were observed only in the ER mode at higher magnification. Contrarily, for the 5-min groups with double-layer application, failure patterns with cracks and cleavages were observed at lower magnification in both etching modes. In particular, the ER mode was associated with a higher number of attached enamel fragments (indicated by En) than the SE mode. The 24-h groups of both application techniques were typified by complicated failure patterns with cracks, cleavages with beach mark patterns (arrows), and clear evidence of enamel fragments (En), excluding the single-layer pattern for the SE mode.

Although the 5-min group for the single-layer application of CB displayed a somewhat flat failure pattern in both etching modes at lower magnification, the ER mode was associated with cracks, cleavages (arrows) in the adhesive layer, and attached enamel fragments at higher magnification. However, for the 5-min group for double-layer application, clearer cracks and cleavages in the adhesive were observed

**Fig. 3** Representative SEM micrograms of the resin-enamel interface of the universal adhesive CU. **a** Single-layer application in SE mode at magnifications of  $\times 2500$  and  $\times 20,000$ . **b** Double-layer application in SE mode at magnifications of  $\times 2500$  and  $\times 20,000$ . **c** Single-layer application in ER mode at magnifications of  $\times 2500$  and  $\times 20,000$ . **d** Double-layer application in ER mode at magnifications of  $\times 2500$  and  $\times 20,000$ . Abbreviations: AL, adhesive layer; HSL, hybrid smear layer







**Fig. 4** Representative SEM micrograms of the resin-enamel interface of the two-step self-etch CB. **a** Single-layer application in SE mode at magnifications of  $\times 2500$  and  $\times 20,000$ . **b** Double-layer application in SE mode at magnifications of  $\times 2500$  and  $\times 20,000$ . **c** Single-layer

application in ER mode at magnifications of  $\times 2500$  and  $\times 20,000$ . **d** Double-layer application in ER mode at magnifications of  $\times 2500$  and  $\times 20,000$ . Abbreviation: AL, adhesive layer

at lower magnification. The 24-h groups of CB exhibited complicated failure patterns with cracks, cleavages, and clear evidence of enamel fragments in both etching modes.

## Discussion

Although conflicting opinions exist regarding whether bovine teeth can be used as an appropriate substitute for human teeth in bond strength tests, there have been many previous studies that found no significant differences in SBS test results between human teeth and bovine teeth [31]. The reasons for using bovine teeth are that it is easy to obtain large quantities of them in good condition, and they have a less variable composition than human teeth. In addition, bovine teeth have large flat areas and have not had prior acid challenges that might affect the test results. Therefore, bovine enamel was used as a substitute for human enamel in this study. However, the intact enamel of bovine teeth has more asperities and irregularities than human teeth, and therefore ground enamel must be used for bond strength testing.

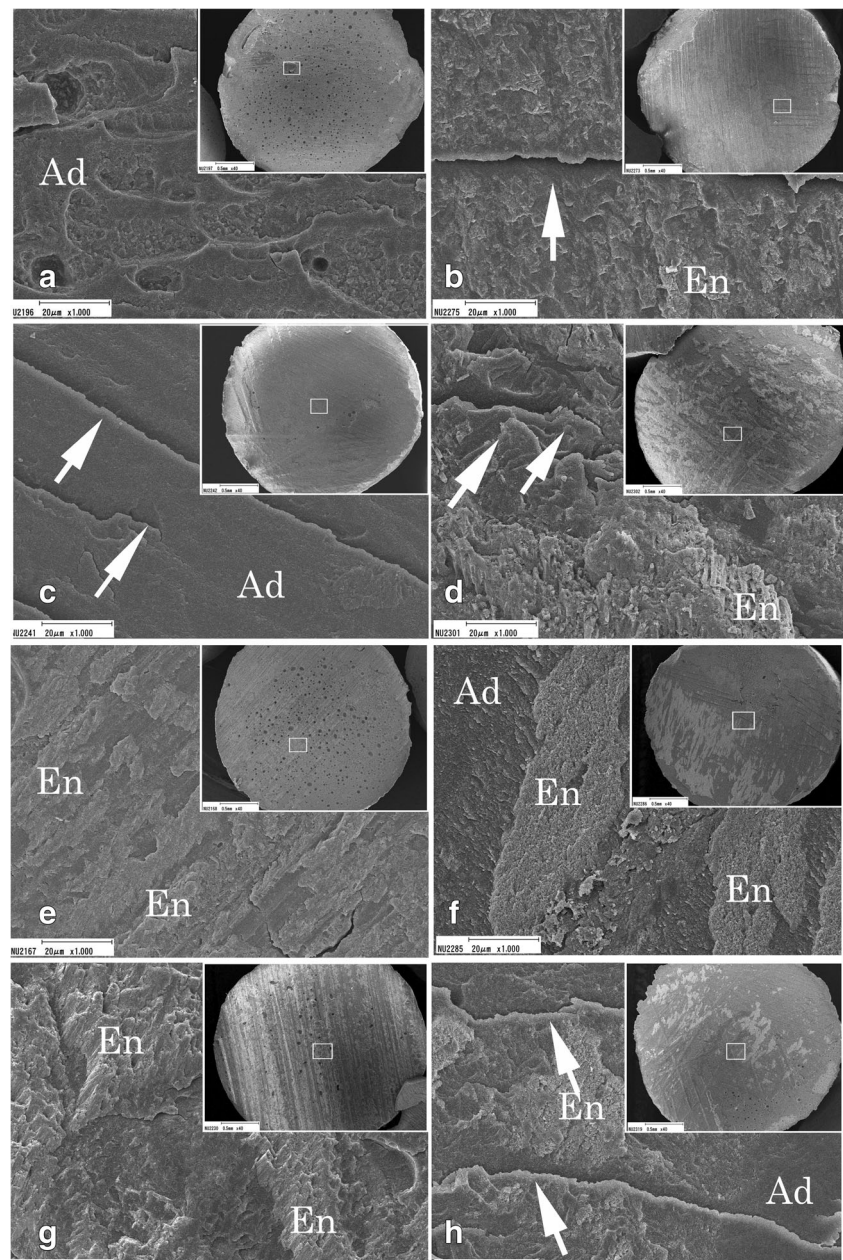
The bond strength test in this study was conducted in accordance with ISO 29022 [29]. The fabrication of bonded specimens for this test is relatively easy, and there is little risk of damage to bonded specimens before and during testing. The  $\mu$ -TBS (tensile bond strength) test is a well-established method and it is thought to be useful when human teeth are used [32]. However, there are concerns about technique sensitivity, inconsistent geometry, and labor intensity when fabricating specimens [33]. External forces applied to the bonded tooth during cutting or shaping may damage the interface of the specimen or cause pre-failure before testing, thus distorting the results. Further, when using universal adhesives in SE mode to enamel, it may be hard to fabricate bonded assemblies due to the weak enamel adhesion. In particular, specimens for the early phase of enamel bonding may be very hard to create.

This study aimed to determine the changes in the bonding performance of universal adhesives in the early phase of creating bonded specimens and evaluate the effect of double-layer application on bonding effectiveness. From the results of the bond strength tests, the storage period of bonded specimens before testing significantly influenced the SBS values,

**Fig. 5** Representative de-bonded failure sites of CU. **a** Single-layer application in SE mode at 5 min at magnifications of  $\times 40$  and  $\times 1000$ . **b** Double-layer application in SE mode at 5 min at magnifications of  $\times 40$  and  $\times 1000$ . **c** Single-layer application in SE mode at 24 h at magnifications of  $\times 40$  and  $\times 1000$ . **d** Double-layer application in SE mode at 24 h at magnifications of  $\times 40$  and  $\times 1000$ . **e** Single-layer application in ER mode at 5 min at magnifications of  $\times 40$  and  $\times 1000$ . **f** Double-layer application in ER mode at 5 min at magnifications of  $\times 40$  and  $\times 1000$ . **g** Single-layer application in ER mode at 24 h at magnifications of  $\times 40$  and  $\times 1000$ . **h** Double-layer application in ER mode at 24 h at magnifications of  $\times 40$  and  $\times 1000$ .

Abbreviations: Ad, adhesive; En, enamel

Arrows indicate cracks and cleavages.



and the SBS values of all universal adhesives increased with increasing storage duration regardless of the etching mode or application technique. Therefore, the first null hypothesis that the early bond performance of universal adhesives would not change over time was rejected.

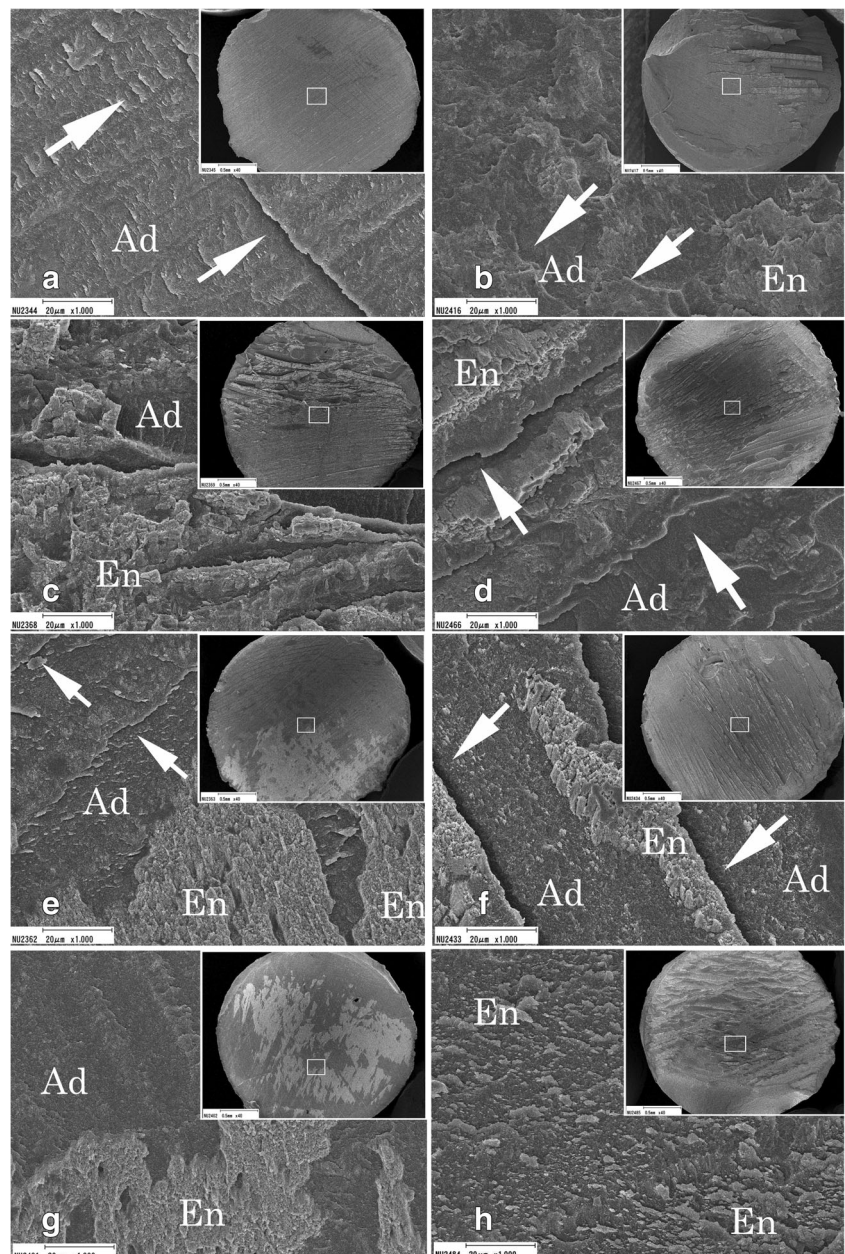
The main reasons for the increasing bond strength over time are believed to be increased mechanical properties of interface materials including adhesives and resin composites due to increased post-polymerization reaction over time [9–13, 34–36]. However, the details of the effects of polymerization of each resin-based material on the bonding effectiveness of universal adhesives have not yet been clarified. Therefore, to investigate the effects of resin composite

polymerization over time, only the single resin composite Clearfil AP-X was used as a restorative material for bonding to enamel. The results of the flexural strength test revealed that Clearfil AP-X was associated with increased  $\sigma_F$  and  $E$  values with prolonged storage, in line with the findings for SBS. Regarding the changes in the flexural properties of the resin composite,  $\sigma_F$  and  $E$  were not significantly different among measurements conducted after 6 h or more of storage. Theoretically, strong and stiff resin composite is believed to lead relatively higher bond strength [37]. A previous study investigated the relationship between the mechanical properties and tensile bond strength of eight resin composites with an experimental adhesive system, and a strong positive



**Fig. 6** Representative de-bonded failure sites of CB. **a** Single-layer application in SE mode at 5 min at magnifications of  $\times 40$  and  $\times 1000$ . **b** Double-layer application in SE mode at 5 min at magnifications of  $\times 40$  and  $\times 1000$ . **c** Single-layer application in SE mode at 24 h at magnifications of  $\times 40$  and  $\times 1000$ . **d** Double-layer application in SE mode at 24 h at magnifications of  $\times 40$  and  $\times 1000$ . **e** Single-layer application in ER mode at 5 min at magnifications of  $\times 40$  and  $\times 1000$ . **f** Double-layer application in ER mode at 5 min at magnifications of  $\times 40$  and  $\times 1000$ . **g** Single-layer application in ER mode at 24 h at magnifications of  $\times 40$  and  $\times 1000$ . **h** Double-layer application in ER mode at 24 h at magnifications of  $\times 40$  and  $\times 1000$ .

Abbreviations: Ad, adhesive; En, enamel. Arrows indicate cracks and cleavages



correlation was observed between bond strength and mechanical properties [38]. In addition, Irie et al. [6] also measured and compared the immediate flexural properties of nine resin composites with specimens fabricated after 24 h. Their findings were in line with the present data, in which significantly higher  $\sigma_F$  and  $E$  were observed after 24 h of storage than for shorter storage periods. One possible explanation is that the increasing  $E$  values of resin composites may result in more uniform stress distribution at the bonding interface and avoid a concentration of stress at the point of load application [39]. Therefore, the increased strength and stiffness of the resin composite over time were confirmed as important factors for the increased bond strength with increasing storage time.

Although the same resin composite was used for each adhesive system, the changes of the SBS values differed by adhesive type. Compared with the findings in the 5-min group for single-layer application as a baseline, CB exhibited a smaller rate of increase of the SBS value than the universal adhesives after 24 h of storage. In addition, no differences in SBS were observed for CB among the 6-, 12-, and 24-h storage groups in the SE mode or for any storage period other than 5 min in the ER mode. On the contrary, the SBS values for most of the universal adhesives increased with prolonged storage. This is probably primarily attributable to the higher hydrophilicity and water content of the universal adhesives, leading to lower C=C double-bond conversion in the early storage

of bonding. For universal adhesives, decalcification of mineralized tissue, penetration of resin monomers, and polymerization of adhesive occur simultaneously, and thus, the composition of universal adhesives should be complex [20].

After adhesive application, some ingredients such as remaining water and solvents and the low pH of functional monomers might inhibit the polymerization reaction [1, 14–16]. Meanwhile, although CB contains low-pH 10-methacryloyloxydecyl dihydrogen phosphate (MDP), the adhesive is separately applied onto the primed surface, leading to a hydrophobic nature compared with the features of the universal adhesives. Therefore, polymerization of this adhesive might proceed more quickly, leading to better bonding performance in the early phase of creating specimens and thus smaller increases of SBS values with prolonged storage.

The effect of chemical interactions between the functional monomer and hydroxyapatite (HAp) over time should be considered. Chemical interactions between the functional monomers and HAp might be time-dependent, leading to increasing SBS values with prolonged storage regardless of the etching mode or application technique. Fukegawa et al. [40] reported that MDP ionically bonds to HAp. This reaction was detectable after 5 min, and it became more widespread after 1 and 24 h. Although most of the adhesives used in this study contain MDP, the quantity and purity of MDP might be different. Previous studies found that differences in purity and quantity affected adhesive performance [41]. Therefore, such differences might explain the different rates of increases in SBS values among the different adhesive systems.

The effect of double-layer application on universal adhesives differed from the findings for CB. Compared with the findings for the SE mode, double-layer application was associated with significantly higher SBS values than single-layer application groups for the same storage period for all universal adhesives, excluding GP after 6 h of storage. Although the effect of double-layer application in the ER mode was adhesive-dependent, a similar trend was also observed for the SE mode. Therefore, the second null hypothesis that double-layer application would not increase the enamel bond strength was also rejected.

Double-layer application of universal adhesives may be performed in two ways, with or without light irradiation of the first layer. When light irradiation is not performed for the first layer, unpolymerized acidic monomers from the second layer may improve the etching ability of the adhesive and increase of adhesive layer thickness to a certain degree [42, 43]. However, this study protocol was designed to test the hypothesis the double layer application of universal adhesives increases their enamel bond effectiveness due to increased adhesive layer thickness. Because universal adhesives have a thin adhesive layer, mechanical destruction may occur in the vicinity of the enamel/adhesive interface [44]. Therefore, in order to more easily obtain a thicker adhesive layer, we

investigated the influence of double-layer application with light irradiation of the first layer.

The present findings are in line with previous data illustrating that the double-layer application of universal adhesives in the SE mode effectively enhances enamel SBS [28]. In addition, this technique was also effective even when repeated subcritical loads were applied [28]. Load stress from bond strength testing generates cracks at the bonded interface, and plastic deformation zones are formed near the ends of cracks during testing [44]. The crack propagation and subsequent fracture might be influenced by the size of the plastic deformation zone. The plastic deformation zone is defined as a zone in the crack tip region where permanent deformation or change in shape of the solid body occurs without fracture under the action of a sustained force [44]. When the thickness of the adhesive layer is much greater than the size of the plastic deformation zone, the stress distribution at the interface during testing might be more dispersed. According to SEM observations of the bonding interface, the AL thickness was higher for double-layer application. An increased AL thickness for universal adhesives is believed to improve tolerance to load stress. Ikeda et al. [45] reported a correlation between the microtensile fracture strength of cured adhesive and the microtensile bond strength. From the perspective of the mechanical properties of the adhesive layer, the properties of the first layer of universal adhesives might be improved due to the repeated light irradiation and additional opportunities for the evaporation of remaining water and solvent. Meanwhile, the thin adhesive layers might exhibit incomplete resin polymerization due to oxygen-mediated inhibition, thereby explaining the lower mechanical properties of the adhesive layer [46, 47].

Conversely, CB exhibited showed no significant differences in enamel strength between single- and double-layer application for the same storage period regardless of the etching mode. Meanwhile, SEM revealed that the AL thickness was higher for the double-layer application of CB. Thus, an increased AL thickness did not have positive effects on its early enamel bond strength. Fujiwara et al. [28] investigated the effect of double-layer application on enamel and dentin bond durability for several types of adhesive systems under fatigue stress. Although the single-step SE adhesives and universal adhesives displayed higher shear fatigue bond strength (SFS) after double-layer application than after single-layer application in both enamel and dentin, the opposite result was observed for the two-step CB adhesive. This suggests that there is an optimal AL thickness for each adhesive, and double-layer application may be counterproductive for two-step SE adhesives in terms of bond durability [28].

When comparing the early enamel bond effectiveness of the tested universal adhesives over time in different etching modes, most showed similar trends. However, for the SE mode, GP in the 5-min and 1-h storage groups with single-layer application showed significantly higher SBS values



when compared to the same storage period of the other universal adhesives. This might be due to its lower pH value and distinct composition. The pH value of GP has been reported to be 1.5, indicating higher acidity than the other universal adhesives [48, 49]. The higher acidity of GP induces demineralization of the enamel surface, creating micro mechanical retention. In addition, a strong stream air-blow and the acetone content might induce volatility and hygroscopicity of the adhesive layer of GP [50]. It is probable that these factors contribute to a higher bond strength of GP in SE mode in the early phase of enamel adhesion. On the other hand, both single- and double-layer application groups of GP in ER mode showed significantly lower SBS values at 24-h storage when compared to the same storage period of the other universal adhesives. Although it is difficult to determine the reasons for this phenomenon, one possible explanation is that the higher acidity of GP may cause excessive decalcification of a phosphoric acid etched enamel surface, and might create vulnerable regions leading to lower SBS values.

One of the concerns about double-layer application is prolonged application time. However, previous studies have reported acceptable performance when using abbreviated bonding procedures (quick bonding), such as reduction of application time before light irradiation of the adhesive [51–53]. Therefore, when selecting optimal universal adhesives that allow quick bonding, it is possible to use this double-layer application technique with acceptable bonding procedure time in clinical situations. The clinical implication from this study is that care should be taken when applying universal adhesives to enamel in the SE mode because of the lower enamel performance in the early phase of bonding procedures. Therefore, it is important to handle resin composite restorations with caution, taking care to not apply extra loads after filling the resin composite during the finishing and polishing procedures. To increase the enamel bonding of universal adhesives in the early phase, both phosphoric acid pre-etching and double-layer application might be useful.

## Conclusion

The results of this study indicated that the adhesive application technique, storage period, and adhesive system significantly influence the SBS values of adhesives regardless of the etching mode. The SBS values of all adhesives increased with prolonged storage regardless of the application technique or etching mode. However, these findings differed from those for a two-step SE adhesive. Double-layer application might be effective for increasing enamel bond strengths of universal adhesives in the early phase of bonding procedures, similarly as observed for phosphoric acid pre-etching.

**Funding information** This work was supported in part by Grants-in-Aid for Scientific Research, Nos. 19K10158 and 17K11716, from the Japan Society for the Promotion of Science. This project was also supported in part by the Sato Fund, Uemura Yasuo · Haruko fund, and by a grant from the Dental Research Center of the Nihon University School of Dentistry, Japan.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This study does not contain any studies with human participants and subjects or animals performed by any of the authors.

**Informed consent** For this type of study, formal consent is not required.

## References

1. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL (2011) State of the art of self-etch adhesives. *Dent Mater* 27:17–28. <https://doi.org/10.1016/j.dental.2010.10.023>
2. Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, Tezvergil-Mutluay A (2011) State of the art etch-and-rinse adhesives. *Dent Mater* 27:1–16. <https://doi.org/10.1016/j.dental.2010.10.016>
3. Miyazaki M, Tsujimoto A, Tsubota K, Takamizawa T, Kurokawa H, Platt JA (2014) Important compositional characteristics in the clinical use of adhesive systems. *J Oral Sci* 56:1–9. <https://doi.org/10.2334/josnusd.56.1>
4. Ferracane JL (2008) Buonocore lecture. Placing dental composites—a stressful experience. *Oper Dent* 33:247–257. <https://doi.org/10.2341/07-BL2>
5. Petrovic LM, Atanackovic TM (2008) A model for shrinkage strain in photo polymerization of dental composites. *Dent Mater* 24:556–560. <https://doi.org/10.1016/j.dental.2007.11.015>
6. Irie M, Maruo Y, Nishigawa G (2017) Performance of class I composite restorations when polished immediately or after one-day water storage. *PLoS One* 12:e0183381. <https://doi.org/10.1371/journal.pone.0183381>
7. Irie M, Maruo Y, Nishigawa G, Suzuki K, Watts DC (2010) Physical properties of dual-cured luting-agents correlated to early no interfacial-gap incidence with composite inlay restorations. *Dent Mater* 26:608–615. <https://doi.org/10.1016/j.dental.2010.02.012>
8. Shinagawa J, Inoue G, Nikaido T, Ikeda M, Burrow MF, Tagami J (2019) Early bond strengths of 4-META/MMA-TBB resin cements to CAD/CAM resin composite. *Dent Mater J* 8:28–32. <https://doi.org/10.4012/dmj.2017-438>
9. Mohamad D, Young RJ, Mann AB, Watts DC (2007) Post-polymerization of dental resin composite evaluated with nanoindentation and micro-Raman spectroscopy. *Arch Orolfac Sci* 2:26–31
10. Alshali RZ, Silikas N, Satterthwaite JD (2013) Degree of conversion of bulk-fill compared to conventional resin composites at two time intervals. *Dent Mater* 29:e213–e217. <https://doi.org/10.1016/j.dental.2013.05.011>
11. Par M, Gamulin O, Marovic D, Klaric E, Tarle Z (2014) Effect of temperature on post-cure polymerization of bulk-fill composites. *J Dent* 42:1255–1260. <https://doi.org/10.1016/j.jdent.2014.08.004>
12. Par M, Gamulin O, Marovic D, Klaric E, Tarle Z (2015) Raman spectroscopic assessment of degree of conversion of bulk-fill resin

- composites—changes at 24 hours post cure. *Oper Dent* 40:E92–E101. <https://doi.org/10.2341/14-091-L>
13. Schneider LF, Consani S, Ogliari F, Correr AB, Sorbrinho LC, Sinhoretto MA (2006) Effect of time and polymerization cycle on the degree of conversion of a resin composite. *Oper Dent* 31:489–495. <https://doi.org/10.2341/05-81>
  14. Salz U, Zimmermann J, Salzer T (2005) Self-curing, self-adhesive cements systems. *J Adhes Dent* 7:7–17
  15. Vrochari AD, Eliades G, Hellwig E, Wrbas KT (2009) Curing efficiency of four self-etching, self-adhesive resin cements. *Dent Mater* 25:1104–1108. <https://doi.org/10.1016/j.dental.2009.02.015>
  16. Zorzini J, Petschelt A, Ebert J, Lohbauer U (2012) pH neutralization and influence on mechanical strength in self-adhesive resin luting agents. *Dent Mater* 28:672–679. <https://doi.org/10.1016/j.dental.2012.03.005>
  17. 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. *Oper Dent* 46:520–530. <https://doi.org/10.2341/15-347-L>
  18. 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. *Dent Mater* 32:e9–e21. <https://doi.org/10.1016/j.dental.2015.11.005>
  19. Cuevas-Suárez CE, da Rosa WLO, Lund RG, da Silva AF, Piva E (2019) Bonding performance of universal adhesives: an updated systematic review and meta-analysis. *J Adhes Dent* 21:7–26. <https://doi.org/10.3290/j.jad.a41975>
  20. Nagarkar S, Theis-Mahon N, Perdigão J (2019) Universal dental adhesives: current status, laboratory testing, and clinical performance. *J Biomed Mater Res B Appl Biomater* 107:2121–2131
  21. Kadowaki Y, Kakuda S, Kawano S, Katsumata A, Ting S, Hoshika S, Ikeda T, Tanaka T, Carvalho RM, Sano H (2016) Bond performance of “touch and cure” adhesives on resin core systems. *Dent Mater J* 35:386–391. <https://doi.org/10.4012/dmj.2015-330>
  22. Takeda M, Takamizawa T, Imai A, Suzuki T, Tsujimoto A, Barkmeier WW, Latta MA, Miyazaki M (2019) Immediate enamel bond strength of universal adhesives to unground and ground surfaces in different etching modes. *Eur J Oral Sci* 127:351–360. <https://doi.org/10.1111/eos.12626>
  23. Ogura Y, Shimizu Y, Shiratsuchi K, Tsujimoto A, Takamizawa T, Ando S, Miyazaki M (2012) Effect of warm air-drying on dentin bond strength of single-step self-etch adhesives. *Dent Mater J* 31:507–513. <https://doi.org/10.4012/dmj.2011-258>
  24. Shiratsuchi K, Tsujimoto A, Takamizawa T, Furuichi T, Tsubota K, Kurokawa H, Miyazaki M (2013) Influence of warm air-drying on enamel bond strength and surface free-energy of self-etch adhesives. *Eur J Oral Sci* 121:370–376. <https://doi.org/10.1111/eos.12061>
  25. Perdigão J, Muñoz MA, Sezinando A, Luque-Martinez IV, Staichak R, Reis A, Loguercio AD (2014) Immediate adhesive properties to dentin and enamel of a universal adhesive associated with a hydrophobic resin coat. *Oper Dent* 39:489–499. <https://doi.org/10.2341/13-203-LR>
  26. Taschner M, Kümmerling M, Lohbauer U, Breschi L, Petschelt A, Frankenberger R (2014) Effect of double-layer application on dentin bond durability of one-step self-etch adhesives. *Oper Dent* 39:416–426. <https://doi.org/10.2341/13-168-L>
  27. Sezinando A, Luque-Martinez IV, Muñoz MA, Reis A, Loguercio AD, Perdigão J (2015) Influence of a hydrophobic resin coating on the immediate and 6-month dentin bonding of three universal adhesives. *Dent Mater* 31:e236–e246. <https://doi.org/10.1016/j.dental.2015.07.002>
  28. Fujiwara S, Takamizawa T, Barkmeier WW, Tsujimoto A, Imai A, Watanabe H, Erickson RL, Latta MA, Nakatsuka T, Miyazaki M (2018) Effect of double-layer application on bond quality of adhesive systems. *J Mech Behav Biomed Mater* 77:501–509. <https://doi.org/10.1016/j.jmbbm.2017.10.008>
  29. ISO 29022: 2013 Dentistry-Adhesion-Notched-edge shear bond strength test. 1<sup>st</sup> edn. Geneva, Switzerland: International Organization for Standardization, ISO (2013) 1–12
  30. ISO 4049: Dentistry-Polymer-based restorative materials. Geneva, Switzerland, International Organization for Standardization
  31. Yassen GH, Platt JA, Hara AT (2011) Bovine teeth as substitute for human teeth in dental research: a review of literature. *J Oral Sci* 53:273–282. <https://doi.org/10.2334/josnusd.53.273>
  32. Pashley DH, Carvalho RM, Sano H, Nakajima M, Yoshiyama M, Shono Y, Fernandes CA, Tay FR (1999) The microtensile bond test: a review. *J Adhes Dent* 1:299–309
  33. Armstrong S, Geraldini S, Maia R, Raposo LH, Soares CJ, Yamagawa J (2010) Adhesion to tooth structure: a critical review of “micro” bond strength test methods. *Dent Mater* 26:e50–e62. <https://doi.org/10.1016/j.dental.2009.11.155>
  34. Neves Ade A, Countinho E, Poitevin A, Van der Sloten J, Van Meerbeek B, Van Oosterwyck H (2009) Influence of joint component mechanical properties and adhesive layer thickness on stress distribution in micro-tensile bond strength specimens. *Dent Mater* 25:4–12. <https://doi.org/10.1016/j.dental.2008.04.009>
  35. Marchesi G, Navarra CO, Cadenaro M, Carrilho MR, Codan B, Sergio V, Di Lenarda R, Breschi L (2010) The effect of aging on the elastic modulus and degree of conversion of two multistep adhesive systems. *Eur J Oral Sci* 118:304–310. <https://doi.org/10.1111/j.1600-0722.2010.00736.x>
  36. Aromaa MK, Vallittu PK (2018) Delayed post-curing stage and oxygen inhibition of free-radical polymerization of dimethacrylate resin. *Dent Mater* 34:1247–1252. <https://doi.org/10.1016/j.dental.2018.06.019>
  37. Thomsen KB, Peutzfeldt A (2007) Resin composites: strength of the bond to dentin versus mechanical properties. *Clin Ora Investig* 11:45–49. <https://doi.org/10.1007/s00784-006-0077-9>
  38. Hasegawa T, Itoh K, Koike T, Yukiitani W, Hisamitsu H, Wakumoto S, Fujishima A (1999) Effect of mechanical properties of resin composites on the efficacy of the dentin bonding system. *Oper Dent* 24:323–330
  39. Miyazaki M, Tsubota K, Takamizawa T, Kurokawa H, Rikuta A, Ando S (2012) Factors affecting the in vitro performance of dentin-bonding systems. *Jpn Dent Sc Rev* 48:53–60. <https://doi.org/10.1016/j.jdsr.2011.11.002>
  40. Fukegawa D, Hayakawa S, Yoshida Y, Suzuki K, Osaka A, Van Meerbeek B (2006) Chemical interaction of phosphoric acid ester with hydroxyapatite. *J Dent Res* 85:941–949. <https://doi.org/10.1177/154405910608501014>
  41. Yoshihara K, Nagaoka N, Okihara T, Kuroboshi M, Hayakawa S, Maruo Y, Nishigawa G, De Munck J, Yoshida Y, Van Meerbeek B (2015) Functional monomer impurity affects adhesive performance. *Dent Mater* 31:1493–1501. <https://doi.org/10.1016/j.dental.2015.09.019>
  42. Cardenas AM, Siqueira F, Rocha J, Szesz AL, Anwar M, El-Askary RA, Loguercio AD (2016) Influence of conditioning time of universal adhesives on adhesive properties and enamel-etching pattern. *Oper Dent* 41:481–490. <https://doi.org/10.2341/15-213-L>
  43. Siqueira FSF, Armas-Vega A, Izquierdo-Bucheli A, Pinto TF, Hanzen TA, Bauer J, Cardenas AFM, Loguercio AD (2019) Does the conditioning mode and duration of universal adhesives affect the bonding effectiveness to fluorotic enamel? *J Adhes Dent* 21:525–536. <https://doi.org/10.3290/j.jad.a43695>
  44. Wakasa K, Yamaki M, Matsui A (1995) Calculation models for average stress and plastic deformation zone size of bonding area in dentine bonding systems. *Dent Mater J* 14:152–165. <https://doi.org/10.4012/dmj.14.152>
  45. Ikeda T, De Munck J, Shirai K, Hikita K, Inoue S, Sano H, Lambrechts P, Van Meerbeek B Effect of fracture strength of

- primer-adhesive mixture on bonding effectiveness. *Dent Mater* 21: 413–420
46. Pashley EL, Agee KA, Pashley DH, Tay FR (2002) Effects of one versus two applications of an unfilled, all-in-one adhesive on dentin bonding. *J Dent* 30:83–90. [https://doi.org/10.1016/s0300-5712\(02\)00002-7](https://doi.org/10.1016/s0300-5712(02)00002-7)
  47. Tsujimoto A, Barkmeier WW, Takamizawa T, Latta MA, Miyazaki M (2016) Influence of the oxygen-inhibited layer on bonding performance of dental adhesive systems: surface free energy perspective. *J Adhes Dent* 18:51–58. <https://doi.org/10.3290/j.jad.a35515>
  48. da Rosa WL, Piva E, da Silva AF (2015) Bond strength of universal adhesives: a systematic review and meta-analysis. *J Dent* 43:765–776. <https://doi.org/10.1016/j.jdent.2015.04.003>
  49. Saikaew P, Chowdhury AF, Fukuyama M, Kakuda S, Carvalho RM, Sano H (2016) The effect of dentine surface preparation and reduced application time of adhesive on bonding strength. *J Dent* 47:63–70. <https://doi.org/10.1016/j.jdent.2016.02.001>
  50. Pashley EL, Zhang Y, Lockwood PE, Rueggeberg FA, Pashley DH (1998) Effects of HEMA on water evaporation from water-HEMA mixtures. *Dent Mater* 14:6–10. [https://doi.org/10.1016/s0109-5641\(98\)00003-7](https://doi.org/10.1016/s0109-5641(98)00003-7)
  51. 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. *J Adhes Dent* 20:65–77. <https://doi.org/10.3290/j.jad.a39913>
  52. Saito T, Takamizawa T, Ishii R, Tsujimoto A, Hirokane E, Barkmeier WW, Latta MA, Miyazaki M (2020) Influence of application time on dentin bond performance in different etching modes of universal adhesives. *Oper Dent* 45:183–195. <https://doi.org/10.2341/19-028-L>
  53. Ahmed MH, Yoshihara K, Mercelis B, Van Landuyt KL, Peumans M, Van Meerbeek B (2019) Quick bonding using a universal adhesive. *Clin Oral Investig* (in press). <https://doi.org/10.1007/s00784-019-03149-8>

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.