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SEM observation of novel characteristic of the dentin bond interfaces of universal adhesives

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ABSTRACT

Objective. The aim of this study was to observe the resin/dentin interfaces of universal adhesives by using scanning electron microscopy (SEM), and to compare their morphologies with conventional etch & rinse (ER) and self-etch (SE) adhesive systems.

Methods. Two three-step and one two-step ER adhesives and two two-step and two single-step SE adhesives were used for comparison with seven universal adhesives in ER mode and SE mode, respectively. Bonded surfaces with bovine teeth were longitudinally sectioned and mirror-polished. Half of the samples were treated with HCl and NaOCl solutions. The interfaces were subjected to argon ion beam etching and then observed by scanning electron microscopy.

Results. The thickness of the adhesive layer (AL) of most of the seven universal adhesives and single-step SE adhesives was similar. Universal adhesives in SE mode formed a hybrid smear layer as a high-density zone between the AL and dentin. The thickness of the hybrid layer (HL) of the universal adhesives in ER mode was ∼1–2 μm, with a high-density zone (reaction layer [RL]) below the HL.

Conclusion. The morphological features of most universal adhesives in SE mode and single-step SE adhesives are similar. Although resin–dentin interfaces of universal adhesives in ER mode resemble those of ER adhesives, universal adhesives have a distinctive feature, an RL.

Significance. The RL might be a sign of chemical bonding even when using universal adhesives in ER mode.

1. Introduction

Bonding mechanisms of resin composites to enamel and the dentin substrate look alike, but are quite different in nature. Enamel is primarily homogeneous and essentially comprises hydroxyapatite (HAp), while dentin is heterogeneous, comprising HAp, collagen fibrils, and a non-collagenous dentin extracellular matrix which includes biological molecules such as, phosphophoryn, osteocalcin, osteopontin, osteonectin, and so on. In addition, HAp's crystal structure and size differ between enamel and dentin: HAp crystals are hexagonal

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columns in enamel, but plate-like and comparatively smaller in dentin. Furthermore, the water content of dentin is considerably higher than that in enamel, which makes adhesion more complex.

There are two types of resin composite adhesives: etch-and-rinse (ER) and self-etch (SE) [1]. These two types are further divided into three-step or two-step procedures in ER adhesives and two-step or single-step procedures in SE adhesives [1]. In ER adhesives, the bonding mechanism is mainly mechanical interlocking between demineralized tissue and a cured resinous adhesive layer (AL) [2]. The strong acidity of phosphoric acid effectively demineralizes enamel and the dentin substrate and contributes to adhesion by micromechanical interlocking with the formation of a hybrid layer (HL) and the penetration of resin monomers into dentinal tubule branches. In contrast, in SE adhesives, chemical bonding occurs between HAp and functional monomers, and the mechanical interlocking might be weaker than that in ER adhesives [1,3]. The chemical bonding is believed to form self-assembled nanolayers of hydrolytically stable calcium salts for acidic challenges, which plays a key role in the prevention of secondary caries, sealing of restoration margins, and promotion of restoration durability [4–6].

To analyze the dentin bond effectiveness of adhesives, dentin bond strength tests are conducted [7]. Standardized dentin bond strength tests help quantify the dentin bond performance of adhesives, compare dentin bond strength values among different products and conditions, screen bonding ability, and help understand the bonding mechanism from the mechanical force point of view. However, it is unclear what kind of reactions occur between adhesives and the dentin substrate, for which qualitative analyses and morphological evaluations are required. Ultrastructure observations of resin–tooth interfaces have been made using scanning electron microscopy (SEM) and transmission electron microscopy in bonding mechanism research [8–10].

The universal adhesives have similarities to conventional single-step self-etch adhesives, although they can be used with either in the etch-&-rinse or self-etch mode to enamel and dentin [11]. This multimode usage enables the selection of the optimal etching mode, depending on cavity configurations and enamel and dentin proportions [12,13]. Although the pre-etching of dentin with phosphoric acid prior to SE mode adhesion is controversial, several studies on universal adhesives have shown that ER adhesives have dentin bond strength equal to or greater than SE adhesives [12,14,15]. However, dentin bond characteristics with universal adhesives used in different etching modes differ in more than dentin bond strength. Therefore, to understand the bonding mechanism of universal adhesives, the detailed ultrastructure of dentin bond interfaces in different etching modes should be analyzed and the interfaces compared to conventional adhesives.

In this study, we investigated dentin bond interfaces of universal adhesives in different etching modes using field-emission SEM (FE-SEM) and compared their morphologies to conventional ER and SE adhesives (three- and two-step ER and two- and single-step SE adhesives). We determined whether the ultrastructure of dentin bond interfaces of universal adhesives differs from that of interfaces of conventional adhesives.

2. Methods

2.1. Study materials

The materials used in this study are listed in Table 1. The seven universal adhesives used were: All Bond Universal [AB] (Bisco, Schaumburg, IL, USA), Adhese Universal [AU] (Ivoclar Vivadent Schaan, Lichtenstein), Clearfil Universal Bond Quick [CU] (Kuraray Noritake Dental, Tokyo, Japan), G-Premio Bond [GP] (GC, Tokyo, Japan), Prime&Bond Universal [PU] (Dentsply Sirona, Konstanz, Germany), OptiBond Universal [OU] (Kerr, Orange, CA, USA), and Scotchbond Universal [SU] (3M Oral Care, St. Paul, MN, USA). Two three-step ER adhesives, OptiBond FL [OF] (Kerr) and Scotchbond Multi-Purpose Plus [add "SM" and be consistent in document with abbreviation, Figure legend switches to "SP" different than Table 1 "SM"] (3M Oral Care), and a two-step ER adhesive, Single Bond Plus [SB] (3M Oral Care), were used as comparisons. In addition, two two-step SE adhesives, OptiBond XTR [OX] (Kerr), and Clearfil SE Bond [CS] (Kuraray Noritake Dental), and two single-step SE adhesives, G-ænial Bond [GB] (GC) and Clearfil Tri-S Bond ND Quick [CT] (Kuraray Noritake Dental) were also used as comparisons. A single resin composite, Clearfil AP-X (Kuraray Noritake Dental), was used for bonding to dentin. The same phosphoric acid pre-etching agent (Ultra-Etch, Ultradent, South Jordan, UT, USA) was used for ER adhesive systems and universal adhesives in etch-&-rinse mode. A tungsten halogen visible-light curing unit (Optilux 501, sds Kerr, Danbury, CT, USA), was used, and the power density (average 600 mW/cm^2) of the curing unit was monitored throughout the study using a dental radiometer (Model 100, Kerr).

2.2. Specimen preparation

In this study, we used bovine superficial dentin (extracted mandibular bovine incisors stored frozen for up to 2 weeks) as a substitute for human dentin, as described previously [16,17]. Approximately two-thirds of the apical root structure of each tooth was removed using a diamond-impregnated disk in an IsoMet 1000 slow-speed precision sectioning saw (Buehler, Lake Bluff, IL, USA). Labial surfaces were ground with wet Fuji Star Type DDC #240-grit silicon carbide (SiC) paper (Sankyo-Rikagaku, Saitama, Japan) to create a flat dentin surface.

2.3. SEM observations and evaluations

All bonding procedures were performed according to each manufacturer's instructions (Table 2). For each adhesive group, three teeth were used and the resin–dentin interface was observed in six bonded sectioned specimens. For bonding procedures of universal adhesives, we used different etching modes: without phosphoric acid etching and with phosphoric acid applied for 15 s prior to adhesive application. An adhesive was applied to the dentin surface, a resin composite was placed on the surface, and then the surface was

Table 1 – Materials used in this study.

Code	Universal adhesive	Main components	pH	Manufacturer
AB	All Bond Universal Lot No. 1300008503	MDP phosphate monomer, bis-GMA HEMA, ethanol, water, initiators	3.2	Bisco,Schaumburg, IL, USA
AU	Adhese Universal Lot No. U49302	MDP, bis-GMA, HEMA, MCAP, D3MA, ethanol, water, initiator, stabilizers, silicon dioxide	2.5–3.0	Ivoclar Vivadent Schaan, Lichtenstein
CU	Clearfil Universal Bond Quick Lot No. 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 Lot No. 4G0011	MDP, 4-MET, MEPS, BHT, acetone, dimethacrylate resins, initiators, filler, water	1.5	GC, Tokyo, Japan
PU	Prime&Bond Universal Lot No. 1706006938	Bi- and multifunctional acrylate, MDP, PENTA, initiator, stabilizer, isopropanol, water	2.5	Dentsply Sirona, Konstanz, Germany
OU	OptiBond Universal Lot No. 6689301	GPDM, GDMA, HEMA, acetone, ethanol, water	2.4	Kerr, Orange, CA, USA
SU	Scotchbond Universal Lot No. 666964	MDP, HEMA, dimethacrylate resins, Vitrebond copolymer, filler, ethanol, water, initiators, silane	2.7	3M Oral Care St. Paul, MN, USA
Code	Etch-&-rinse adhesive			
OF	OptiBond FL (three-step) Lot No. 6902900 (primer) Lot No. 6911571 (adhesive)	Primer: HEMA, GPDM, BHT, ethanol, water, CQ Adhesive: bis-GMA, UDMA, TEGDMA, GDMA, HEMA, filler, CQ, ODMAB, filler (fumed SiO2, barium aluminoborosilicate, Na2SiF6), coupling factor A174	Primer: 1.8	Kerr
SM	Scotchbond Multi-purpose plus (three-step) Lot No. N852287 (pimer) Lot No. N86909 (adhesive)	Primer: HEMA, polyalkenoic acid, water Adhesive: Bis-GMA, HEMA, amines	Primer: 3.3	3 M Oral Care
SB	Single Bond Plus (two-step) Lot No. N898889	Bis-GMA, HEMA, dimethacrylate, methacrylated polyalkenoic acid, Vitrebond copolymer, ethanol, water, initiator, nanofiller	Adhesive 3.4	3 M Oral Care
Code	Self-etch adhesive			
CS	Clearfil SE Bond 2 (two-step) Lot No. 6B0094 (primer) Lot No. 7R0147 (adhesive)	Primer: MDP, HEMA, water, initiators Adhesive: MDP, HEMA, bis-GMA, initiators, microfiller	Primer: 2.5	Kuraray Noritake Dental
OX	OptiBond XTR (two-step) Lot No. 5847004 (primer) Lot No. 5852494 (adhesive)	Primer: GPDM, HEMA, dimethacrylate monomers, acetone, ethanol, water, CQ Adhesive: MEHQ, ethanol, barium aluminoborosilicate glass, fumed silica, sodium hexafluorosilicate, CQ	Primer: 2.4	Kerr
CT	Clearfil TriS Bond ND Quick (single-step) Lot No. 5G0066	MDP phosphate monomer, bis-GMA, HEMA, ethanol, NaF, water, silica-based micro filler, CQ	2.3	Kuraray Noritake Dental
GB	G-Bond Plus (single-step) Lot No. 4G0011	4-MET, UDMA, TEGDMA, phosphoric acid monomer, acetone, water, silanated colloidal silica, initiator	1.5	GC
Pre-etching agent Ultra-Etch (G017)		35% phosphoric acid		Ultradent Products, South Jordan, UT, USA

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, 4-MET: 4-Methacryloyloxyethyl trimellitate, MEPS: methacryloyloxyalkyl thiophosphate methylmethacrylate, BHT: butylated hydroxytoluene, PENTA: dipentaerythritol pentacrylate phosphate, TEGDMA: triethyleneglycol dimethacrylate, CQ: *dl*-camphorquinone, GDMA: glycerol dimethacrylate, GPDM: glycerol dimethacrylate dihydrogen phosphate, ODMAB: 2-(ethylhexyl)-4-(dimethylamino)benzoate (co-initiator), MEHQ = 4-methoxyphenol mono(2-methacryloyloxy)ethyl phthalate,UDMA: urethane dimethacrylate.

Table 2 – Bonding procedures for the tested adhesives.

Universal adhesive	Etching method
ER mode	Dentin surface was phosphoric acid etched for 15 s. Etched surface was rinsed with water for 15 s (three-way dental syringe)
SE mode	Phosphoric acid pre-etching was not performed.
	Adhesive application protocol
AB	Adhesive was applied to dentin surface (do not desiccate) with rubbing action for 10–15 s per coat. No light cure between coats. Gentle stream of air applied over the liquid for at least 10 s. Light irradiated for 10 s.
AU	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 irradiated for 10 s.
CU	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 irradiated for 10 s.
GP	Adhesive was applied to air-dried dentin surface and immediately a strong stream of air 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.
PU	Adhesive was applied to dentin surface (do not desiccate) with rubbing action for 20 s. Gentle stream of air applied over the liquid for at least 5 s. Light irradiated for 10 s.
OU	Adhesive was applied to air-dried dentin surface with rubbing motion for 20 s, and then medium air pressure applied to surface for 5 s. Light irradiated for 10 s.
SU	Adhesive was applied to air-dried dentin surface with rubbing motion for 20 s, and then medium air pressure applied to surface for 5 s. Light irradiated for 10 s.
ER adhesives	Adhesive application protocol
OF (three-step)	Dentin surface was phosphoric acid etched for 15 s. Etched surface was rinsed with water for 15 s. Dried gently for 3 s (do not desiccate). Primer was applied to dentin surface with light brushing motion for 15 s. Air dried for 5 s. Using same applicator, adhesive was applied with light brushing motion for 5 s. Air thinned for 3 s. Light irradiated for 20 s.
SP (three-step)	Dentin surface was phosphoric acid etched for 15 s. Etched surface was rinsed with water for 15 s. Dried gently for 2 s. Left moist. Primer was applied to dentin. Dried gently for 5 s. Adhesive was applied to dentin. Light irradiated for 10 s.
SB (two-step)	Dentin surface was phosphoric acid etched for 15 s. Etched surface was rinsed and blotted dry. Priming adhesive was applied to dentin for 15 s. Dried gently for 5 s. Light irradiated for 10 s.
SE adhesives	Adhesive application protocol
CS (two-step)	Primer was applied to air-dried dentin surfaces for 20 s followed by medium air pressure for 5 s. Adhesive was then applied to primed surfaces and was air thinned gently. Adhesive was light irradiated for 10 s.
OX (two-step)	Primer applied to air-dried dentin surface with rubbing action for 20 s. Medium air pressure was applied to surface for 5 s. Adhesive was applied to primed surface with rubbing action for 15 s and then air thinned for 5 s. Adhesive light irradiated for 10 s.
CT (single-step)	Adhesive was applied to air-dried dentin surface for 10 s and then medium air pressure was applied to surface for 5 s. Adhesive light irradiated for 10 s.
GB (single-step)	Adhesive was applied to air-dried dentin surface for 10 s. Strong stream of air was applied over the liquid adhesive for 5 s or until adhesive no longer moved and the solvent had completely evaporated. Adhesive light irradiated for 10 s.

irradiated with light for 30 s to provide 18 J/cm^2 radiant exposure. The bonded specimens were stored in distilled water at 37 °C for 24 h, embedded in epoxy resin, and then longitudinally sectioned using an IsoMet 1000 slow-speed saw. The sectioned surfaces were polished to a high gloss using Fuji Star Type DDC abrasive disks, followed by 0.25-μm particle size DP-Paste diamond paste (Struers, Ballerup, Denmark), and then ultrasonically cleaned for 3 min. Next, half of the bonded specimens were etched with 6 mol/L of HCl solution for 25 s and deproteinized by immersion in 6% NaOCl solution for 3 min to clearly visualize infiltrated resin tags (RTs). All SEM specimens were dehydrated in increasing 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 to a Model ID-3 critical-point dryer (Elionix, Tokyo, Japan) for 30 min. Then, the resin–dentin interface specimens were subjected to argon ion beam etching (EIS-200ER, Elionix) for 40 s, with the ion beam directed perpendicular to the polished surfaces; the accelerating voltage was 1.0 kV and the ion current density 0.4 mA/cm^2. Finally, all SEM specimens were coated with a thin gold film in an SC-701 Quick Coater vacuum evaporator (Sanyu Electric, Tokyo, Japan). dentin interfaces were observed by FE-SEM (ERA-8800FE, Elionix) at an operating voltage of 10 kV with different magnifications (×500, ×5000 for the group with HCl and NaOCl treatment, ×1000, 2500, or 10000 for the group with just argon-ion etching). The lower magnification (500×) for group 1 allows the visualization of long RTs, while the lower magnification (1000×) for three- and two-step SE adhesives allows the visualization of a thicker AL. We evaluated the following aspects of SEM images: AL thickness, HL thickness, filler type, infiltrated RT length, and alterations in the vicinity of the AL-dentin substrate interphase.

3. Results

Figs. 1–10 show representative SEM images of resin–dentin interfaces, and Table 3 summarizes the characteristics of each adhesive, as determined from the SEM images.

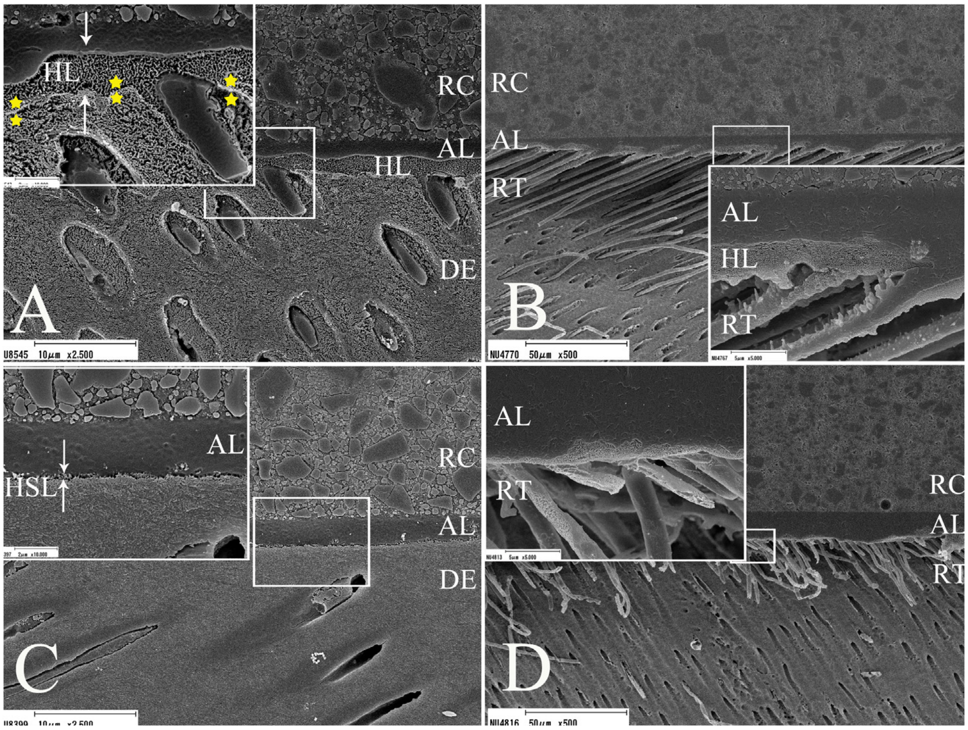

Fig. 1 – Representative SEM images of resin/dentin interfaces of the universal adhesives.
(A) AB in ER mode after argon-ion-beam etching (×2500 and ×10,000). (B) AB in ER mode after HCL and NaOCl treatment (×500 and ×5000). (C) AB in SE mode after argon-ion-beam etching (×2500 and ×10,000). (D) AB in SE mode after HCL and NaOCl treatment (×500 and ×5000).

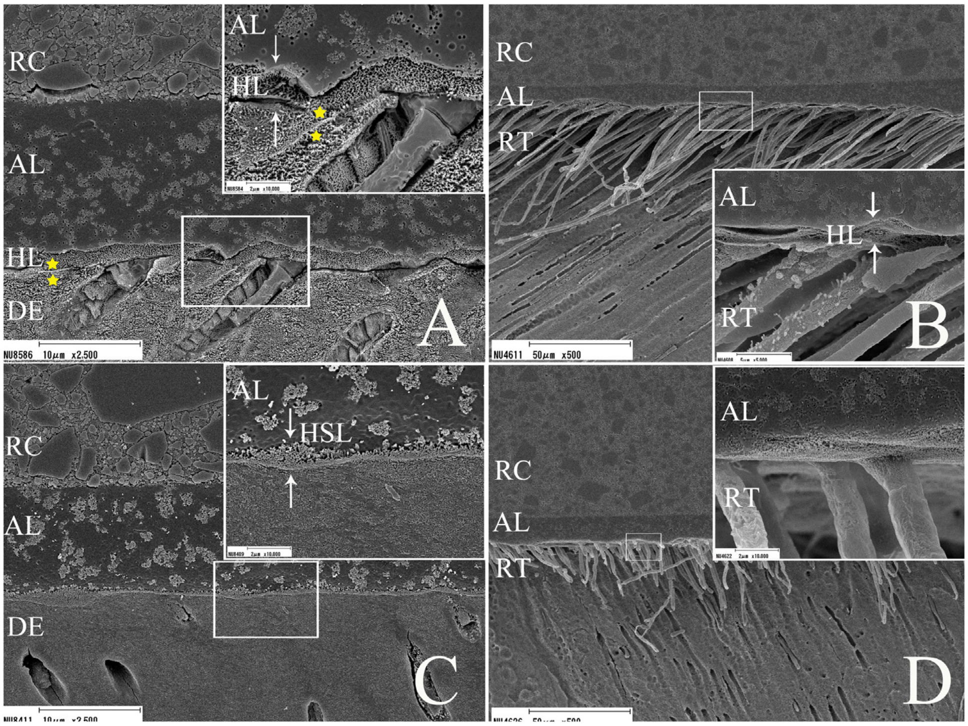

Fig. 2 – Representative SEM images of resin/dentin interfaces of the universal adhesives.
(A) AU in ER mode after argon-ion-beam etching (×2500 and ×10,000). (B) AU in ER mode after HCL and NaOCl treatment (×500 and ×5000). (C) AU in SE mode after argon-ion-beam etching (×2500 and ×10,000). (D) AU in SE mode after HCL and NaOCl treatment (×500 and ×5000).

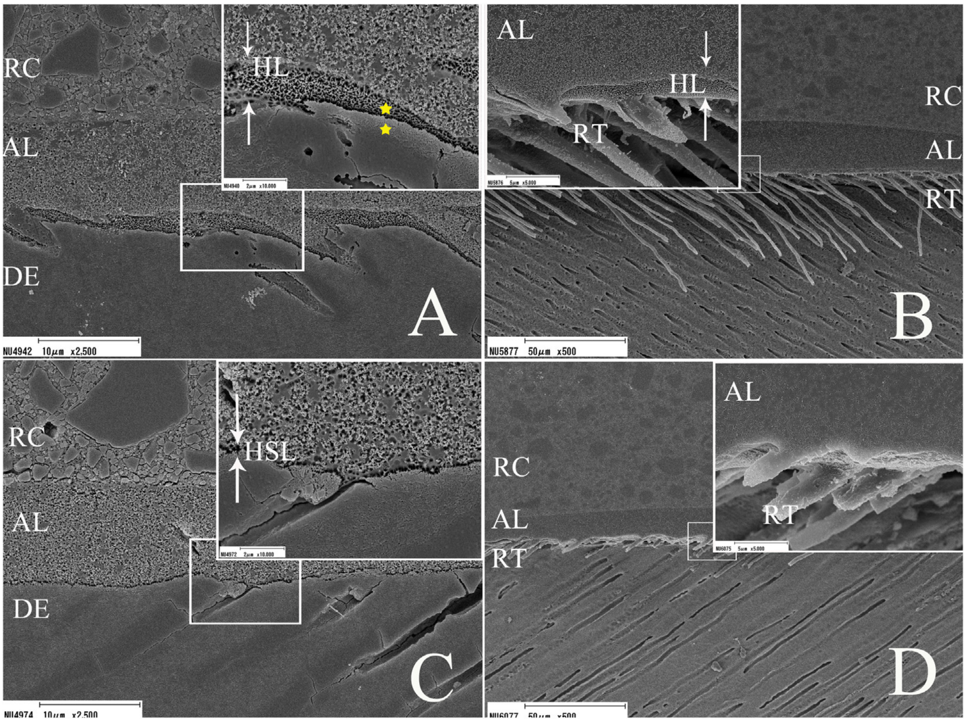

Fig. 3 – Representative SEM images of resin/dentin interfaces of the universal adhesives.
(A) CU in ER mode after argon-ion-beam etching (×2500 and ×10,000). (B) CU in ER mode after HCL and NaOCl treatment (×500 and ×5000). (C) CU in SE mode after argon-ion-beam etching (×2500 and ×10,000). (D) CU in SE mode after HCL and NaOCl treatment (×500 and ×5000).

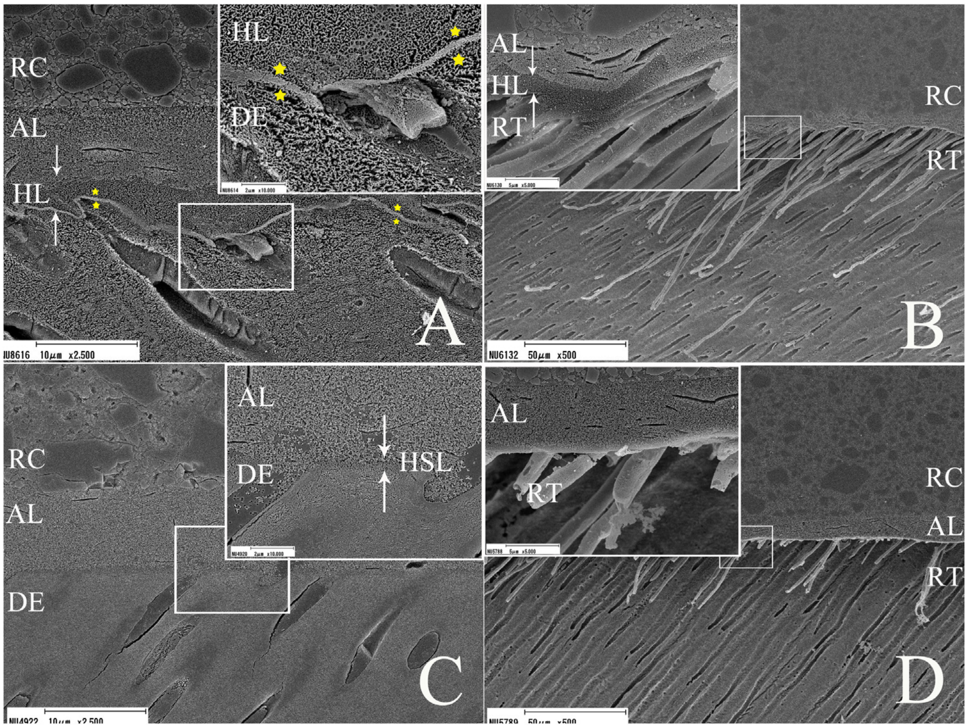

Fig. 4 – Representative SEM images of resin/dentin interfaces of the universal adhesives.
(A) GP in ER mode after argon-ion-beam etching (×2500 and ×10,000). (B) GP in ER mode after HCL and NaOCl treatment (×500 and ×5000). (C) GP in SE mode after argon-ion-beam etching (×2500 and ×10,000). (D) GP in SE mode after HCL and NaOCl treatment (×500 and ×5000).

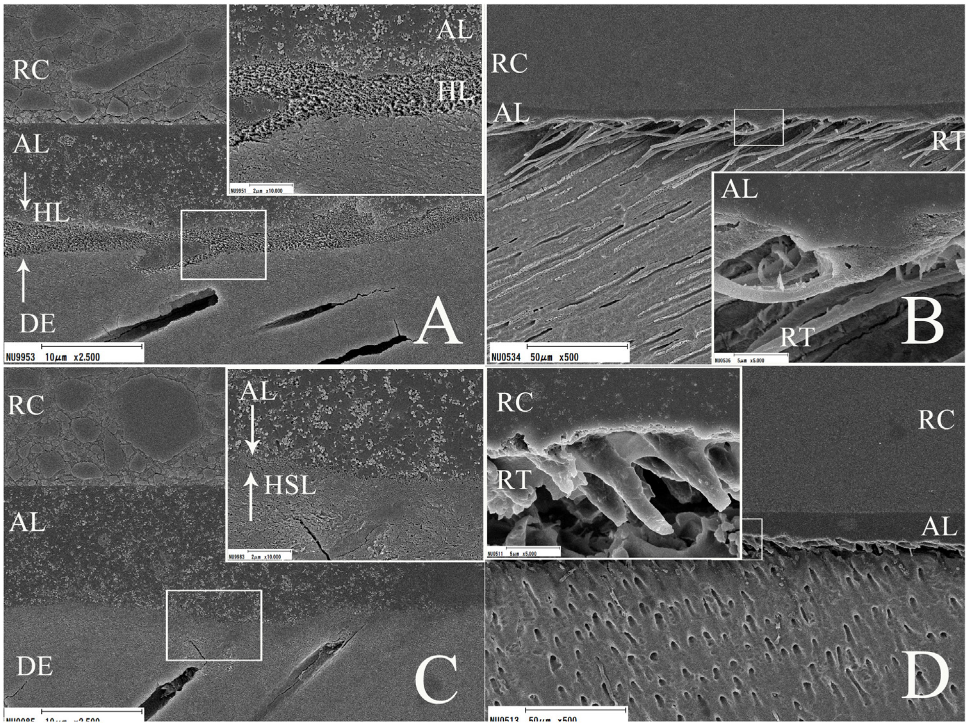

Fig. 5 – Representative SEM images of resin/dentin interfaces of the universal adhesives.
(A) OU in ER mode after argon-ion-beam etching (×2500 and ×10,000). (B) OU in ER mode after HCL and NaOCl treatment (×500 and ×5000). (C) OU in SE mode after argon-ion-beam etching (×2500 and ×10,000). (D) OU in SE mode after HCL and NaOCl treatment (×500 and ×5000).

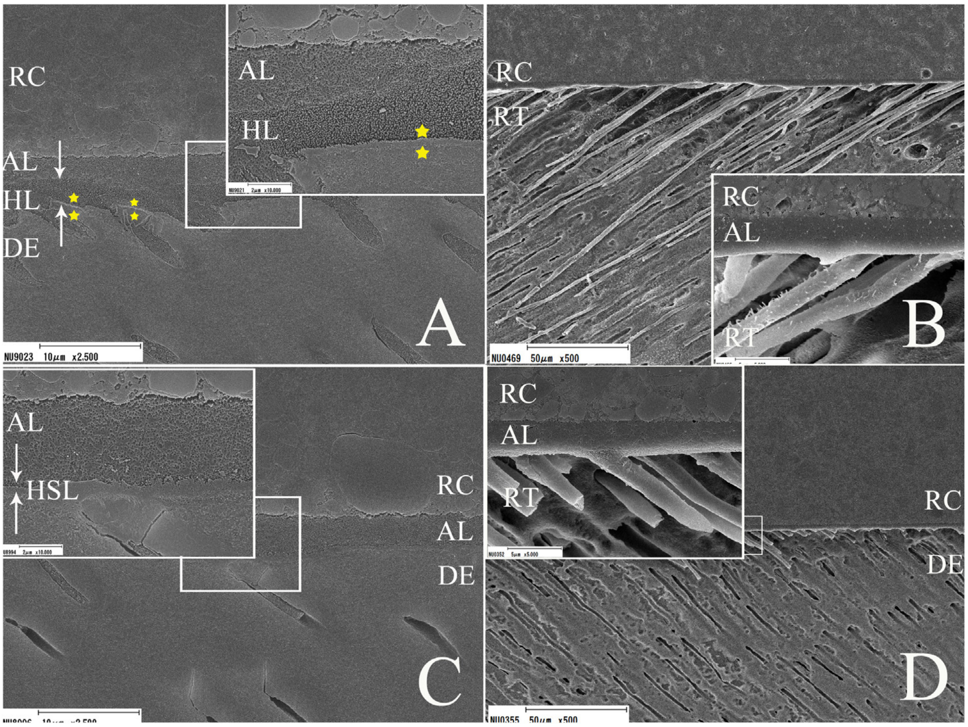

Fig. 6 – Representative SEM images of resin/dentin interfaces of the universal adhesives.
(A) PU in ER mode after argon-ion-beam etching (×2500 and ×10,000). (B) PU in ER mode after HCL and NaOCl treatment (×500 and ×5000). (C) PU in SE mode after argon-ion-beam etching (×2500 and ×10,000). (D) PU in SE mode after HCL and NaOCl treatment (×500 and ×5000).

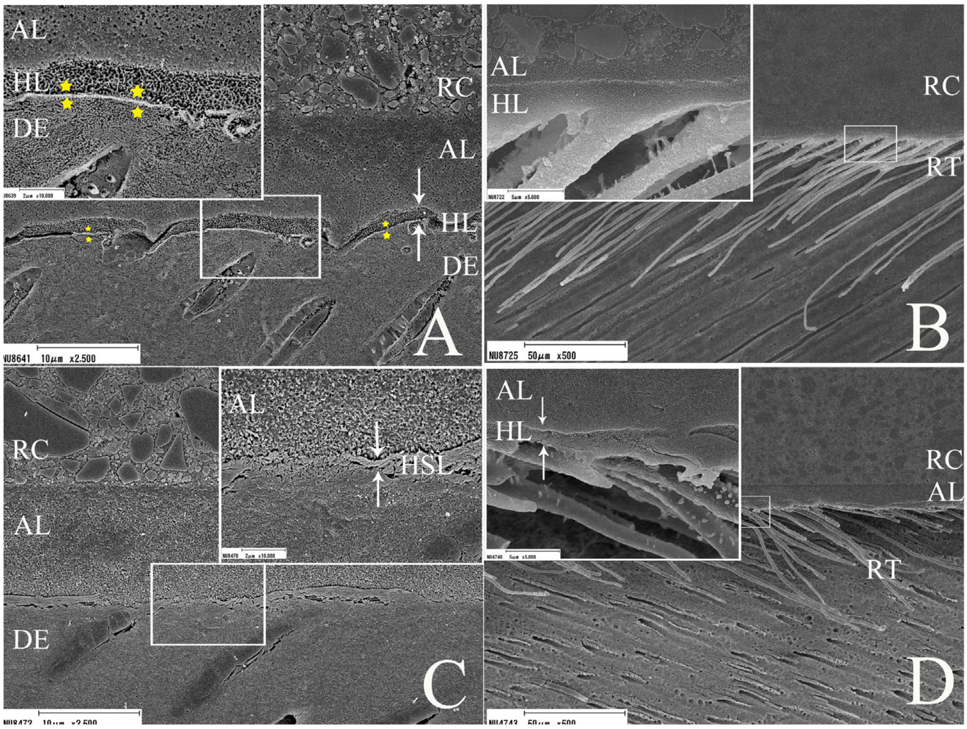

Fig. 7 – Representative SEM images of resin/dentin interfaces of the universal adhesives.
(A) SU in ER mode after argon-ion-beam etching (×2500 and ×10,000). (B) SU in ER mode after HCL and NaOCl treatment (×500 and ×5000). (C) SU in SE mode after argon-ion-beam etching (×2500 and ×10,000). (D) SU in SE mode after HCL and NaOCl treatment (×500 and ×5000).

Table 3 – Characteristics of resin/dentin interfaces.

Adhesive	Etching mode	Thickness of AL	Thickness of HL	Observation of RL	Filler type	Length of RT
Universal adhesive						
AB	ER	2–3 μm	1–1.5 μm	Yes	—	50–100 μm
AU	ER	10–12 μm	1–1.5 μm	Yes	Aggregate nanofillers	50–100 μm
CU	ER	10–12 μm	1–2 μm	Yes	Nanofillers	50–100 μm
GP	ER	8–10 μm	1–2 μm	Yes	Nanofillers	50–100 μm
PU	ER	2–3 μm	1–1.5 μm	Yes	—	50–100 μm
OU	ER	8–10 μm	1–2 μm	No	Nanofillers	30–50 μm
SU	ER	8–10 μm	1–2 μm	Yes	Nanofillers	50–100 μm
AB	SE	2–3 μm	—	No	—	20–40 μm
AU	SE	8–10 μm	—	No	Aggregate nanofillers	20–40 μm
CU	SE	8–10 μm	—	No	Nanofillers	5–15 μm
GP	SE	8–10 μm	—	No	Nanofillers	30–50 μm
PU	SE	2–3 μm	—	No	—	15–20 μm
OU	SE	8–10 μm	—	No	Nanofillers	5–15 μm
SU	SE	8–10 μm	—	No	Nanofillers	30–50 μm
Three-step ER adhesive						
OF	ER	40–50 μm	2–3 μm	No	> 0.5 μm irregular filler sized	50–100 μm
SP	ER	40–50 μm	3-4 μm	No	—	50–100 μm
Two-step ER adhesive						
SB	ER	10–15 μm	2–3 μm	No	—	50–100 μm
Two-step SE adhesive						
CS	SE	40–50 μm	—	No	Nanofillers	15–20 μm
OX	SE	10–15 μm	0.1–0.5 μm	No	>0.5 μm irregular filler sized	20–30 μm
Single-step SE adhesive						
CT	SE	8–10 μm	—	No	Nanofillers	10–30 μm
GB	SE	8–10 μm	—	No	Nanofillers	5–15 μm

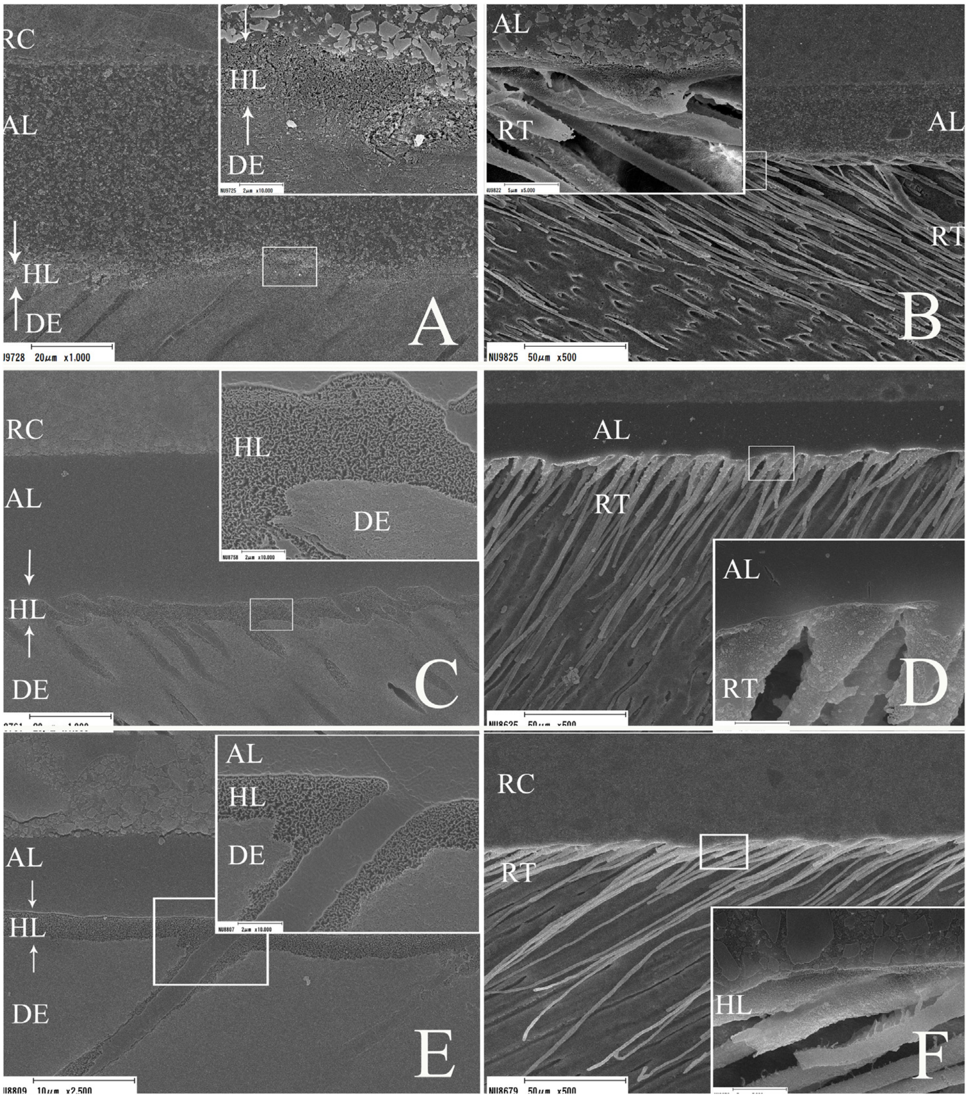

Fig. 8 – Representative SEM images of resin/dentin interfaces of the ER systems.
(A) OF (three-step) after argon-ion-beam etching (×1000 and ×10,000). (B) OF (three-step) after HCL and NaOCl treatment (×500 and ×5000). (C) SP (three-step) after argon-ion-beam etching (×1000 and ×10,000). (D) SP (three-step) after HCL and NaOCl treatment (×500 and ×5000). (E) SB (two-step) after argon-ion-beam etching (×2500 and ×10,000). (F) SB (two-step) after HCL and NaOCl treatment (×2500 and ×10,000).

3.1. *Thickness of the adhesive layer*

Most universal adhesives and single-step SE adhesives formed ALs of similar thickness (Figs. 2–5, 7, and 10) regardless of the etching mode (~10 µm). However, AB and PU formed a thin 2–3-µm AL (Figs. 1 and 6). The AL thickness for Scotchbond Multi-Purpose Plus [use abbreviation "SM"] and OF (40–50 µm) and CS (40–50 µm) was four to six times more than that for other adhesives (Figs. 8A,C and 9 A). SB (Fig. 8E) and OX (Fig. 9C) showed similar AL thickness (10–15 µm).

3.2. *Thickness of the hybrid layer*

The HL thickness was dependent on both the etching mode and the adhesive. Universal adhesives in SE mode or single-step SE adhesives did not form an HL (Figs. 1C, 2 C, 3 C, 4 C, 5 C, 6 C, 7 C, 10 A, and C). However, universal adhesives in ER mode formed an HL ~1–2 µm thick (Figs. 1A, 2 A, 3 A, 4 A, 5 A, 6 A, and 7 A). OF, SM, and SB formed a thicker HL than universal adhesives in ER mode (Fig. 8A, B, and C, respectively). OX formed a thin ~0.1–0.5 µm HL (Fig. 9C).

3.3. *Filler types in the adhesive layer*

In this study, the tested adhesives were classified into three groups: without fillers, with nanofillers, and with irregular fillers. AB, PU, SP, and SB did not contain any fillers (Figs. 1, 6, 8C, and E). OF and OX used irregular fillers <0.5 µm

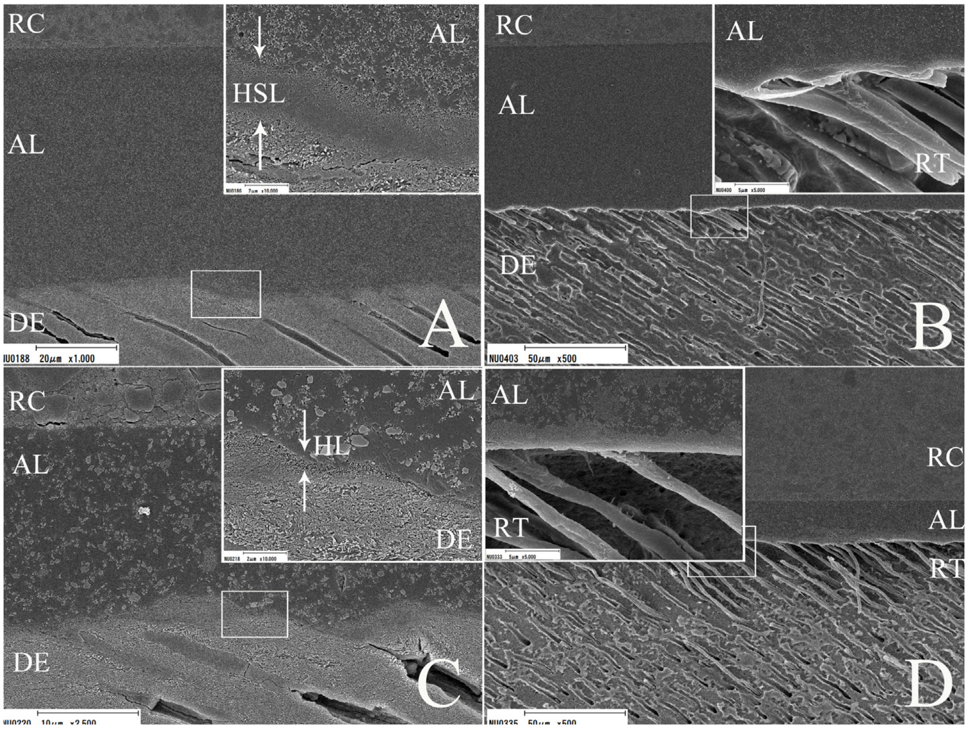

Fig. 9 – Representative SEM images of resin/dentin interfaces of the two-step SE systems.
(A) CS after argon-ion-beam etching (×1000 and ×10,000). (B) CS after HCL and NaOCl treatment (×500 and ×5000). (C) OX after argon-ion-beam etching (×2500 and ×10,000). (D) OX after HCL and NaOCl treatment (×500 and ×5000).

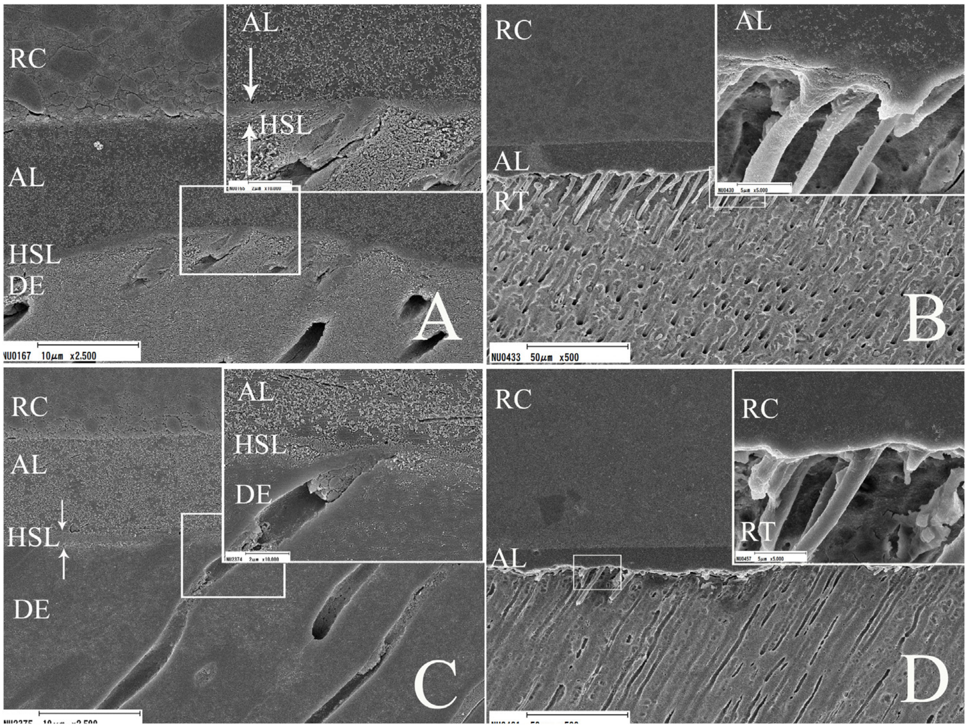

Fig. 10 – Representative SEM images of resin/dentin interfaces of the single-step SE systems.
(A) CT after argon-ion-beam etching (×2500 and ×10,000). (B) CT after HCL and NaOCl treatment (×500 and ×5000). (C) GB after argon-ion-beam etching (×2500 and ×10,000). (D) GB after HCL and NaOCl treatment (×500 and ×5000).
The visible material is indicated by abbreviations: AL: adhesive layer, DE: dentin, RC: resin composite, RT: resin tag, HL: hybrid layer, HSL: hybrid smear layer, RL: reaction layer (between star marks).

in diameter (Figs. 8A and 9 C). The remaining adhesives contained nanofillers, which in AU were aggregated (Fig. 2A).

3.4. *Length of infiltrated resin tags*

It was difficult to precisely measure a representative RT length due to variation across dentin locations, a wide-range of RT length, and artifacts in specimens prepared for observation. Therefore, the aim of this observation was to grasp the penetration ability of resin monomers into dentinal tubules rather than to measure exact RT lengths. The RTs of universal adhesives in ER mode and ER adhesives (Figs. 1B, 2 B, 3 B, 4 B, 5 B, 6 B, 7 B, 8 B, D, and F) showed longer RTs with a higher density than universal adhesives in SE mode and SE adhesives (Figs. 1D, 2 D, 3 D, 4 D, 5 D, 6 D, 7 D, 9 B, 9 D, 10 B, and D). Universal adhesives in ER mode and ER adhesives showed more resin monomer penetration into dentinal tubule branches. For universal adhesives in SE mode and SE adhesives, RT length was dependent on adhesives and locations. Specifically, AB, AU, GP, and SU in SE mode showed longer RTs than OX, CS, GB, and CT (Figs. 1D, 2 D, 4 D, and 7 D).

3.5. *Alterations in the vicinity of the adhesive layer–dentin substrate interface*

For OF, SM, and SB and the universal adhesives in ER mode, we observed alterations in the vicinity of the AL–dentin substrate interface (Figs. 1A, 2 A, 3 A, 4 A, 5 A, 6 A, 7 A, 8 A, 8 C, and 8E). For all universal adhesives except OU, we observed a high-density zone (star) below the AL (Figs. 1A, 2 A, 3 A, 4 A, 6 A, and 7 A). However, we could not clearly observe this high-density zone in OF, SM, and SB.

In contrast, universal adhesives in SE mode, CS, GB, and CT formed a hybrid smear layer (HSL) as a high-density zone between the AL and the dentin substrate (Figs. 1C, 2 C, 3 C, 4 C, 5 C, 6 C, 7 C, 9 A, 10 A, and C).

4. Discussion

Ion etching of dental materials and/or mineralized tissue makes morphological features stand out clearly [18,19]. Since 1962, argon ion beam etching and sputter-etching have been applied in dental research when conducting SEM observations [18]. In 1990, Inokoshi et al. introduced the technique of argon ion beam etching to observe resin–dentin interfaces [20]. The substances remaining in the vicinity of the AL–dentin substrate interface after argon ion beam etching depend on the composition and structure of the material [8]. Argon ion beam etching is a helpful technique to bring out complex structures in sharp contrast by selectively removing the softer resin matrix surface. For instance, after argon ion beam etching, HLs (where resin monomers penetrate dentin after phosphoric acid etching) are clearly observed as collagen mesh structures. Therefore, argon ion beam etching has contributed to our understanding of the bonding mechanisms of adhesives from the point of view of morphological features. However, it is difficult to observe the condition of the inside of bonded mineralized tissue infiltrated by resin monomers, namely, the RTs. Another technique used in this study—treating the specimens with HCl and NaOCl solutions before argon ion beam etching—helps to clearly visualize not only RTs but also the vicinity of the AL–dentin substrate interface, such as HLs or HSLs.

Conventional adhesives tend to have thicker ALs, and ALs in multistep adhesives are much thicker (~4–6 times) than single-step SE adhesives and universal adhesives, which can be attributed to a hydrophobic bonding agent that does not include water. A thicker AL might be beneficial for durability [21,22]. Load stress from mastication creates cracks at the bonded interface, and plastic zones are formed near the ends of the cracks. The AL plastic zones might be related to crack propagation and fracture. We believe that stress distribution at the bonded interface during mastication can be more dispersed when the AL thickness is much greater than the plastic zone size [23]. Therefore, because of superior mechanical properties compared to hydrophilic ALs, somewhat thicker, hydrophobic ALs have benefits such as enduring fatigue stress from mechanical load and resisting degradation from hydrolysis [24,25]. In contrast, because of a discrepancy in thermal expansion between the AL and the other materials, an excessively thick AL might cause problems related to bonding restorations in aesthetic areas and long-term marginal integrity [26]. However, currently, there is no consensus on the optimal AL thickness for long-term dentin bond durability.

One of the characteristics of ER adhesives is HL formation [1,2]. An HL is defined as a layer of dentin that is conditioned to remove the adherent smear layer and where resin monomers have penetrated into the decalcified region to form a collagen–resin phase [27]. The HL thickness is similar in both ER adhesives and universal adhesives in ER mode, but is not exactly the same: ER adhesives exhibit a slightly thicker HL than universal adhesives. In addition, all universal adhesives in ER mode, except OU, which does not contain MDP, exhibit a high-density zone below the HL, which is not observed clearly in conventional ER adhesives. Also, the HL in three-step ER adhesives is much thicker than other adhesives. The reasons for different HL thicknesses in different adhesives might be related to the pH of the primer or adhesive, application time, and bonding procedure. Although the HL plays a key role in micromechanical interlocking, there are concerns about the degradation of the scaffold by hydrolysis and enzymes because of the presence of collagen fibrils not protected by resin monomers [28,29]. An important study suggested that 10-methacryloyloxydecyl dihydrogen phosphate (MDP) has a relatively stable interaction with collagen because of hydrophobic interactions between MDP moieties and the collagen surface, as measured by the saturation transfer difference using nuclear magnetic resonance (NMR) [30]. Most universal adhesives used in this study contain MDP as a functional monomer, although the purity and quantity of each ingredient in each adhesive might differ [31]. Therefore, although the HL appears similar in both universal adhesives and conventional ER adhesives, the interaction between naked collagen fibrils and resin monomers might differ. In addition, there is a possibility that the reaction between resin monomers and the non-collagenous dentin extracellular matrix might also differ.

Many laboratory studies have shown little or no difference between dentin bond strengths of universal adhesives in different etching modes [12,14,15]. The high penetration ability of universal adhesives and the inclusion of functional monomers might modify the intact dentin substrate below the decalcified dentin. This interaction zone might be evidence of chemical bonding, even when phosphoric acid etching is applied. A similar but much thicker, high-density layer is observed with universal adhesives in SE mode, which is believed to be the site of chemical bonding between the adhesive and the superficial dentin and to be central to dentin bond strength [1,3]. Adhesives in ER mode rely more on mechanical interlocking, which might explain the consistency in performance observed between the two etching modes. We propose calling this thicker, high-density layer the reaction layer (RL). However, we did not clearly observe RLs for all the tested universal adhesives, which might be because of different adhesive compositions and application methods.

The results of this SEM observation study tell us little about the composition or mode of creation of the RL. The RL was only clearly observed in universal adhesives containing MDP, which suggests that MDP may be important to its formation. One possibility is that the RL is a layer of concentrated CA salt formed by reactions between the functional monomer and HAp. MDP is known to form a more stable Ca salt than the other functional monomers used in these adhesives (GPDM, 4-MET, phenyl-P) [32,33], which could explain the observed differences in the RL. Further, as mentioned earlier, Hiraishi et al. [30] have shown that MDP has a relatively stable interaction with collagen, which may also contribute to the RL. However, the non-collagenous extracellular matrix has a complex composition, and may also be involved.

In order to understand the RL, it is important to determine its composition. However, given the thinness of the RL and its location within the bonding interface, such studies would be technically challenging. Methods such as Raman spectroscopy, X-ray diffraction (XRD) spectroscopy, or NMR may be informative. Similarly, alternative approaches to visualizing the morphology, such as scanning tunneling microscopy (STM), atomic force microscopy (AFM), may be informative. However, this is clearly a matter for future work.

There is some controversy over whether fillers in adhesives are beneficial for dentin bond durability. Fillers in adhesives are believed to reinforce the mechanical properties of the cured AL and to exert a pinning effect to inhibit crack propagation by load stress [34,35]. In addition, a specific AL thickness and the viscosity achieved might help the application process. However, hydrolysis induces filler debonding from the cured AL of SE adhesives, deteriorating restorations over time [36], and including fillers might impair the penetration ability of resin monomers into demineralized dentin. Although most universal adhesives contain nanofillers, AB and PU do not contain any fillers. The AL of these two adhesives is much thinner than that of other universal adhesives. In terms of the penetration of resin monomers into decalcified tissue, although omitting fillers is believed to be advantageous for universal adhesives in both ER and SE modes, AB and PU do not clearly show superior penetration ability compared to other universal adhesives used in this study.

ER adhesives and universal adhesives in ER mode show much longer RTs than SE adhesives and universal adhesives in SE mode. In addition, the phosphoric acid pre-etching of dentin prior to the application of the adhesive induces the formation of dense RTs that penetrate dentinal tubule branches. However, the role of RT formation in influencing dentin bond performance is not yet clear. Studies have reported that there is no correlation between RT length and dentin bond strength of two-step ER adhesives [37]. In addition, RT formation in a conventional single-step SE adhesive, produced under vacuum treatment of dentin, does not contribute to dentin bond durability [38]. However, one study that investigated the influence of surface wetness on the bonding effectiveness of universal adhesives in ER mode reported some relationship between RT length and dentin bond fatigue durability and suggested that RT length affects dentin bond fatigue durability in some universal adhesives [39]. The penetration ability of resin monomers might closely depend on adhesive composition (solvent type, water content, presence or absence of inorganic fillers, and hydrophilicity or hydrophobicity of resin monomers) and application methods. In any case, when using universal adhesives in ER mode, the formation of longer RTs might induce chemical bonding between the universal adhesives' functional monomers and peritubular dentin. However, it is difficult to determine the chemical interaction between decalcified dentin and functional monomers only from morphological observations; therefore, further studies of the dentin bond interface are required.

5. Conclusion

Within the limitations of this *in vitro* study, the morphological features of most universal adhesives in SE mode were similar to the single-step SE adhesives GB and TS, suggesting that the bonding mechanism of universal and single-step SE adhesives is similar. The appearance of a high-density RL below the HL might be a sign of chemical bonding when using universal adhesives in ER mode and requires continued investigation.

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