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博 士 論 文

Effect of an extra hydrophobic resin layer on the bond strength of universal adhesives

（ユニバーサルアドヒーシブの接着に対する疎水性ボンディング材の影響）

令和５年３月申請

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Effect of an extra hydrophobic resin layer on the bond strength of universal adhesives

ユニバーサルアドヒーシブの接着に対する疎水性ボンディング材の影響

WANG LINHONG

THIS THESIS SUBMITTED IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE DEPARTMENT OF RESTORATIVE DENTISTRY FACULTY OF DENTAL MEDICINE.

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This is to certify that the research work presented in this thesis, entitled 'Effect of an extra hydrophobic resin layer on the bond strength of universal adhesives.' was conducted by Wang Linhong under the supervision of Professor Hidehiko Sano. No part of this thesis has been submitted anywhere else for any other degree. This thesis is to submit to the Department of Restorative Dentistry, Graduate School of Dental Medicine, Hokkaido University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the field of Dental Medicine.

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Abstract

Purpose: To determine the effect of an extra hydrophobic resin layer (EHL) on the bond strength and durability of three different pH one-step universal adhesives (UAs).

Materials and methods: One hundred and nineteen extracted non-carious human molars were used in this study. The flat dentin surfaces were exposed and divided into 7 groups and 14 subgroups. Three one-step UAs with different pH, G-Premio Bond (GPB), Scotchbond Universal (SBU), All-Bond Universal (ABU) were used in this study, while the bonding agent of Clearfil SE Bond 2 (SE2) was selected as an EHL to GPB, SBU and ABU. The six experimental groups were: GPB, GPB+EHL, SBU, SBU+EHL, ABU, ABU+EHL, and SE2 was used as a control group. The adhesives were applied to flat dentin surfaces according to each manufacturer's instructions, while for the EHL groups, dentin surfaces were treated with one of these adhesives followed by the application of EHL prior to light cure. The microtensile bond strength (μTBS) was evaluated after 24 h water storage or after 15,000 thermal cycling (TC). The fracture modes, interfacial structures were analyzed using SEM, while nanoleakage (NL) was analyzed using SEM under backscattered mode. Elastic modulus (EM) and hardness (H) of the adhesive layer were evaluated by a nanoindenter. The data of μTBS were analyzed by three-way ANOVA to determine the effects of adhesive, EHL and TC and the interaction effect of these three factors, and the multiple comparisons were performed using one-way ANOVA, followed by Games-Howell test. As for the data of EM and H, two-way ANOVA was employed to

determine the effects of adhesive and EHL as well as the interaction of these two parameters, multiple comparisons between groups were performed using one-way ANOVA, followed by Tukey's test (α=0.05).

Results: Significantly higher μTBS was achieved in GPB+EHL group compared with GPB group both at 24 h and after 15,000 TC ($p<0.05$). The additional use of EHL did not significantly improve the μTBS of SBU and ABU groups both at 24 h and after 15,000 TC ($p>0.05$). After 15,000 TC, the bond strength of GPB+EHL, SBU, SBU+EHL, ABU+EHL and SE2 group significantly decreased compared with the 24 h groups, while no significant difference was found within GPB and ABU groups ($p>0.05$). Many bubbles were found in GPB group both at 24 h and after 15,000 TC, while the bubble formation was rare in other groups. GPB group showed higher NL than SE2 both at 24 h and after 15,000 TC, while GPB+EHL group demonstrated lower NL than GPB group. The mean EM and H of adhesive layer in GPB+EHL group was significantly decreased compared with GPB group ($p<0.05$).

Conclusions: The results indicated that the bond strength and durability of low pH one-step UA (GPB) were significantly improved by additional application of EHL both at 24 h and after 15,000 TC, while no significant improving effect for ultra-mild one-step UAs (SBU and ABU), GPB works well as a primer in a two-step bonding system while SBU, ABU does not work well.

Keywords: universal adhesive, primer, extra hydrophobic resin layer, microtensile bond strength, nanoleakage.

Background

Dental adhesives are wildly used in clinical practice. Contemporary dental adhesive systems can be classified in etch-and-rinse, self-etch and selective-etch approach based on their system mode. They can also be divided into three-step, two-step, and one-step according to the delivery mode. For self-etch bonding systems, the one-step self-etch adhesives are more easier to use in clinical practice, however the bond strength and durability of the one-step systems are considered to be inferior to the two-step systems[1,2]. The universal adhesives (UAs) have been used since 2011 in clinical practice, and are becoming increasingly popular because of their advantages including user-friendliness, acceptable bond strength, and less technique sensitivity[3]. UAs can be used in "multi-mode" including etch-and-rinse, self-etch, and selective-etch mode[4,5]. Compared with two-step self-etch adhesive systems, the simplified UAs are basically more hydrophilic[6], and the bonding effectiveness and durability of UAs are inferior to the two-step self-etch adhesive systems[7].

UAs can be further subdivided based on their acidity (ultra-mild, pH $\geqslant$ 2.5; mild, pH $\approx$ 2; or intermediately strong, pH $\approx$ 1.5)[8]. It is interesting to find that when a strong or intermediately strong pH self-etch adhesive was used, it was associated with a higher annual failure rate and inferior clinical effectiveness, this conclusion also has important implications in the UAs[9]. The pH of the adhesive seems to play an important role in its clinical effectiveness, when the bond strength was compared considering the type of self-etch adhesives, the adhesives with a milder pH presented much more beneficial, it could be concluded in previous studies that a mild pH self-etch adhesive was desired on dentin[10-12]. It was reported that for strong one-step self-etch adhesives, significantly higher failure rates have been demonstrated

(5.4%), compared with the mild one-step self-etch adhesives (3.6%)[13]. So far there are still few studies available on the comparison of UAs with different pH values *vs* bond strength.

According to a systematic review, the bond strength of UAs to dentin can be improved by using multiple layer applications[14]. The bond durability of UAs was also found to benefit from the application of an extra hydrophobic resin layer (EHL) in self-etch mode[15]. In recent years, a study proposed one-step UAs to go back to multi-step, to obtain higher bond strength and long-term stability[16]. Another study found that the latest two-step adhesive system (G2-Bond Universal) which using a universal adhesive-derived primer, offered a durable bonding performance[17]. The primer of G2-Bond Universal is based on the chemistry of a UA G-Premio Bond (GPB)[18]. The question was that the one-step UAs could go back to multi-step and work as a primer, and the effect of EHL on the bond performance of different UAs is still unknown.

Therefore, the objective of this study was to evaluate the short and long-term effect of EHL to UAs with different pH, including the bond strength, failure modes, interfacial structures, nanoleakage (NL) and elastic modulus (EM) and hardness (H) of the adhesive layer, and explore whether UAs could be used as a primer in two-step bonding system.

Chapter 1: Introduction

At present the latest generation of dental adhesive is so-called universal adhesives (UAs), which can be used with any adhesion strategies both in etch-and-rinse (ER), self-etch (SE), and selective-etch mode, and it can also offers a versatility of use with both direct and indirect restorative materials and procedures[19]. Due to their lots of advantages such as user-friendliness, acceptable bond strength, and less technique sensitivity, it becomes increasingly popular in clinical use. UAs can achieve substantial bonding to both dentin and enamel, regardless of the using modes[20]. Nevertheless, compared with two-step self-etch adhesive systems, simplified one-step self-etch adhesives are associated with lower bond strength in vitro and poorer longevity of restorations in vivo[1,2,7,21,22]. Simplified one-step adhesives always contain higher content of solvents, which might impair the complete volatilization of solvent and lead to poorer adhesive polymerization, lower adhesive conversion consequently[23].

On the other hand, bond strength of various UAs was compared considering the different pH and the result showed that a milder pH adhesives was more beneficial and the pH of adhesive seems to play an important role in their clinical effectiveness[11,12]. A strong pH adhesive was also related with a much higher failure rate for self-etch adhesives[9]. It has been reported that for strong one-step self-etch adhesives, the annual failure rate was around 5.4%, which was significantly higher than that of a mild one-step self-etch adhesives, which was about 3.6%[8,13]. On the other hand, the compatibility of adhesives with composites or resin cements are also

related with their pH. It has been indicated that a stronger acidity or lower pH adhesive is related with a less compatibility[24]. Moreover, irrespective of ER or SE mode, low pH adhesives showed a significant decrease of bond strength after aging, when mild UAs showed bond strength more stable in both strategies[11]. The current primarily commercial UAs in the market are listed in Table 1[11,19].

Table 1 List of current commercial UAs, their pH and functional monomer(s)

Universal Adhesives (UAs)	Classification by pH	Functional monomer(s)
All-Bond Universal (Bisco, Schaumburg, IL, USA)	Ultra-mild 3.2	10-MDP
Prelude One (Danville Materials, S. Ramon, CA, USA)	Ultra-mild 2.8	10-MDP
One Coat 7 Universal (Coltene-Whaledent, Allstetten, Switzerland)	Ultra-mild 2.8	10-MDP
Adhese Universal (Ivoclar-Vivaden, Schaan, Liechtenstein)	Ultra-mild 2.8	10-MDP, MCAP
Scotchbond Universal (3M Espe, St. Paul, MN, USA)	Ultra-mild 2.7	10-MDP, PAC
Prime&Bond Elect (Dentsply Caulk, Milford, DE, USA)	Mild 2.5	PENTA
Prime&Bond Universal (Dentsply Sirona, Konstanz, Germany)	Mild 2.5	10-MDP, PENTA
Optibond Universal (Kerr, Orange, CA, USA)	Mild 2.4	GPDM
Futurabond U (Voco GmbH, Cuxhaven, Germany)	Mild 2.3	10-MDP
Clearfil Universal Bond (Kuraray, Tokyo, Japan)	Mild 2.3	10-MDP
Clearfil Universal Bond Quick (Kuraray, Tokyo, Japan)	Mild 2.3	10-MDP
Peak Universal Adhesive (Ultradent, South Jordan, UT, USA)	Mild 1.2–2.0	4-META
iBond Universal (Heraeus Kulzer, GmbH, Hanau, Germany)	Intermediately strong	10-MDP, 4-META

	1.6–1.8	
G-Bond Plus (GC, Tokyo, Japan)	Intermediately strong 1.5	10-MDP, 4-META
G-Premio Bond (GC, Tokyo, Japan)	Intermediately strong 1.5	10-MDP, 4-META

Abbreviations: 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; MCAP, methacrylated carboxylic acid polymer; GPDM, glycero-phosphate dimethacrylate; PAC, polyalkenoic acid copolymer; PENTA, dipentaerythritol penta-acrylate monophosphate; 4-META, 4-methacryloxyethyl trimellitic acid.

A variety of alternative techniques or strategies have been used to improve the bonding performance of UAs to dentin such as by the use of multiple layers or the additional application of hydrophobic resin layer coating[14,15,21,25-32]. The bond durability of UAs applied in SE mode was found to benefit from the application of an extra hydrophobic adhesive layer when the UAs were first light cured[15]. It has been reported that the double application of UAs was beneficial and improve the bond strength to dentin affected by radiotherapy after ionizing radiation[31]. An extra hydrophobic resin coat could increase the bond strength and reduce the nanoleakage (NL) in eroded dentin after two-years of storage as reported[33]. The improvement of bonding performance and durability using an EHL are believed to be due to the fact that the thicker adhesive layers are associated with better stress distribution, increased the chemical interaction, improved the resin infiltration, better solvent volatilization, and leading to improved mechanical properties of the adhesive interface[26].

UAs basically work as traditional one-step SE adhesives when they are applied with the SE mode[34]. The stability of bond strength seemed to be material-dependent and was vulnerable to hydrolytic degradation. Taschner et al. showed that using the double application technique to improve the bond strength of one-step self-etch adhesives was adhesive dependent[32]. Ahmed et al. found that solely G-Premio Bond benefited from extra bonding layer when applied in SE mode. The overall effect of

extra adhesive layer depended on the specific UAs and their bonding modes both immediate and aged bonding efficacy[25]. However, the additional use of a hydrophobic resin bonding layer after the recommended application sequence of Scotchbond Universal Adhesive did not improve its clinical performance in non-carious cervical lesions after 5 years' clinical observation[35].

In recent years, a study proposed one-step UAs to go back to multi-step, to obtain higher bond strength and long-term stability[16]. Another study found that the latest two-step adhesive system (G2-Bond Universal) which using a universal adhesive-derived primer, offers a durable bonding performance[17]. The primer of G2-Bond Universal is based on the chemistry of a UA G-Premio Bond (GPB)[18]. The question was that could the one-step UAs go back to multi-step and works as a primer, and the effect of EHL on the bond performance of different UAs is still unknown.

Meanwhile, most of UAs contain the functional monomer 10-MDP. Adhesives containing 10-MDP showed higher bonding performance than those of materials formulated with other acidic ingredients[36]. 10-MDP also can produce an acid-base resistant zone at the adhesive interface, and form the stable calcium salts of MDP (MDP-Ca salts)[37]. It was reported that the efficacy of 10-MDP adhesives to demineralize the dentin was not directly related to the pH value[38]. There was a large heterogeneity on the bond strength and durability between UAs of different pH. So far, little data is available for the UAs regarding film forming properties and bonding mechanism[39,40]. The effect of the EHL on the bond strength and durability of different pH 10-MDP based UAs is still scant in literature, further investigation of their mechanisms and properties is deemed important.

Therefore, the aim of this study was to evaluate the laboratory performance of an extra hydrophobic resin layer on dentin bond strength and durability of 10-MDP

based UAs with different pH using SE mode. The null hypothesis was that the bonding performance of UAs would be improved by using an extra hydrophobic resin layer, and UAs could work as a primer in two-step bonding system.

Chapter 2: Materials and methods

2.1 Tooth selection and preparation

Extracted non-carious human molars (n=119) were used in this study. The teeth were collected under a protocol reviewed and approved by the Medical Ethics Committee requirements of Zhejiang Provincial People's Hospital (QT2022345) and the Ethics Committee of Hokkaido University (#2018-9). Extracted teeth were stored in an aqueous solution of 0.5% chloramine-T at 4 ºC and used within three months. The teeth were randomly assigned into 7 groups and 14 subgroups. 84 teeth were used for the microtensile bond strength (μTBS) test and were further randomly divided into 14 groups (6 teeth/group), including 24 h and 15,000 thermal cycling (TC) groups, and all beams from the central area of dentin were tested for μTBS. Additionally, 14 teeth (1 tooth/group) were used for resin-dentin interface observation using scanning electron microscopy (SEM) (24 h and 15,000 TC), and 14 teeth (1 tooth/group) were used for nanoleakage analysis (24 h and 15,000 TC), 7 teeth (1 tooth/group) were used for elastic modulus (EM) and hardness (H) test (24 h).

Flat dentin surfaces were obtained with a gypsum model trimmer (Jiantai JT-19C, Wuhan, Hubei, China) under a continuously cooling water, removing the coronal enamel of the each tooth, and a stereoscope was used to confirm that no enamel remained on the dentin surface. Then the exposed dentin surfaces were polished with 600-grit silicon carbide (SiC) abrasive papers for 1 min to produce a standardized smear layer prior to bonding under continuous water cooling.

2.2 Experimental design and bonding procedures

Three different pH 10-MDP based UAs were selected in this study, including G-Premio Bond (GPB, GC, Tokyo, Japan), Scotchbond Universal (SBU, 3M ESPE, St Paul, MN, USA), All-Bond Universal (ABU, Bisco, Schaumburg, IL, USA). The bond of Clearfil SE Bond 2 (SE2, Kuraray, Tokyo, Japan) was selected as an extra hydrophobic layer (EHL). The experimental groups were as follows: (1) GPB; (2) GPB+EHL; (3) SBU; (4) SBU+EHL; (5) ABU; (6) ABU+EHL, and SE2 was chosen as a control group. The pH, chemical compositions and application instructions of the materials were shown in the Table 2.

For the GPB, SBU, ABU groups, the adhesives were applied on the prepared dentin surface according to the manufactures' instructions, while for the GPB+EHL, SBU+EHL, ABU+EHL groups, EHL was applied after the use of primer and before light-curing. All bonded surfaces were built-up with resin composites (Clearfil AP-X, Kuraray, Tokyo, Japan), constructed in increments to a height of 4 mm, each incremental layer was light-cured for 20 s using a light source at 1200 mW/cm² (Bluephase G2, Ivoclar-Vivadent, Schaan, Liechtenstein). The prepared teeth were stored in distilled water at 37 ºC for 24 h.

Table 2 pH, chemical compositions of each group and instructions for application

Adhesives	pH	Chemical compositions	Manufacturers' instructions	Modified applications
Clearfil SE Bond 2 (SE2, Kuraray, Tokyo, Japan)	Mild 2.3	**Primer:** 10-MDP, HEMA, Hydrophilic aliphatic dimethacrylate, CQ, DEPT, Water. **Bond:** 10-MDP, Bis-GMA, HEMA, Hydrophobic aliphatic dimethacrylate, CQ, DEPT, Colloidals silica.	**Primer:** 1. Apply the primer and leave for 20 s. 2. Gentle air-blowing. **Bond (also used as extra hydrophobic resin layer, EHL):** 1. Apply the adhesive for 10 s. 2. Gentle air-blowing. 3. Light-cure for 10 s.	
G-Premio Bond (GPB, GC, Tokyo, Japan)	Interme-diately strong 1.5	10-MDP, 4-META, methacrylate acid ester, distilled water, acetone, photo initiators, silica.	1. Apply GPB using a microbrush. 2. Leave undisturbed for 10 s after application. 3. Dry thoroughly for 5 s with oil-free air under maximum air pressure. 4. Light-cure for 10 s.	

GPB+EHL		The component of GPB and the bond component of SE2		1. Apply GPB using a microbrush. 2. Leave undisturbed for 10 s after application. 3. Dry thoroughly for 5 s with oil-free air under maximum air pressure. 4. Apply the EHL for 10 s. 5. Gentle air-blowing. 6. Light-cure for 10 s.
Scotchbond Universal (SBU, 3M ESPE, MN, ST Paul, USA)	Ultra-mild 2.7	10-MDP, HEMA, ethanol, water, dimethacrylate resins, methacrylate-modified polyalkenoic acid copolymer, polyacrylic acid copolymer, silane, fillers, initiators.	1. Apply SBU on the surface and rub it for 20s. 2. Gently air-dry the adhesive for approximately 5 s for the solvent to evaporate. 3. Light cure for 10 s.	
SBU+EHL		The component of SBU and the bond component of SE2		1. Apply SBU on the surface and rub it for 20s. 2. Gently air-dry the adhesive for approximately 5 s for the solvent to evaporate. 3. Apply the EHL for 10 s. 4. Gentle air-blowing. 5. Light-cure for 10 s.
All-Bond Universal (ABU, Bisco, Schaumburg, IL, USA)	Ultra-mild 3.2	10-MDP, phosphoric acid ester monomer, Bis-GMA, HEMA, ethanol, water, initiators	1. Apply two separate coats of ABU, scrubbing the preparation with a microbrush for 10-15 s per coat. No light cure between coats. 2. Gently air-dry the adhesive for approximately 10 s for the solvent to evaporate. 3. Light cure for 10 s.	
ABU+EHL		The component of ABU and the bond component of SE2		1. Apply two separate coats of ABU, scrubbing the preparation with a microbrush for 10-15 s per coat. No light cure between coats. 2. Gently air-dry the adhesive for approximately 10 s for the solvent to evaporate. 3. Apply the EHL for 10 s. 4. Gentle air-blowing. 5. Light-cure for 10 s.

Abbreviations: 10-MDP, 10-methacryloxydecyl dihydrogen phosphate; 4-META, 4-methacryloyloxyethyl trimellitic anhydride; Bis-GMA, 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy) phenyl) propane; TEG-DMA, triethyleneglycol dimethacrylate; UDMA, urethane dimethacrylate; CQ, dl-camphorquinone; HEMA, 2-hydroxyethyl methacrylate; DPT: N,N-diethanol--toluldine; EHL: extra hydrophobic layer.

2.3 Thermal cycling (TC)

After 24 h water storage, 84 bonded teeth (n=14/group) were sectioned into beams (cross-sectional area: approximately 1 mm^2) with a low-speed diamond saw (Isomet Low Speed Saw 1000, Buehler, Lake Bluff, IL, USA) under copious water cooling. 28 bonded teeth (n=2/group) were sectioned into slabs perpendicularly to the interface using a low-speed diamond saw under copious water cooling. Half of the prepared specimens of each group were put into a thermal cycling device (PTC2c; PolyScience, Illinois, USA) being filled with distilled water. The conditions being set as follows: 15,000 thermal cycling at temperatures of 5 ºC, and 55 ºC, the dwell time was 30 seconds and the transfer time was 5 seconds[41].

2.4 Microtensile bond strength test (µTBS)

All beams of the central dentin area of each tooth were collected and fixed to a jig with cyanoacrylate glue (Zapit; Dental Ventures of America, Corona, CA, USA). The µTBS test was carried out at a crosshead speed of 0.5 mm/min with a universal testing machine (Bisco, Schaumburg, IL, USA) until failure occurred. The tensile load (N) at failure of each beam was divided by the cross-sectional bonding area (mm^2) to achieve the µTBS in MPa. The mean bond strength of central beams derived from each tooth represented the µTBS of that tooth. Specimens with premature failures (PF) were included in the tooth mean as zero MPa.

2.5 Fracture modes analysis

After the µTBS test, the fractured specimens were collected and mounted on aluminum stubs, coated with Pt-Pd using an ion sputter coater (JEC-3000FC, JEOL Ltd., Tokyo, Japan) for 150 s. The fractured dentin and resin surfaces were observed

using a scanning electron microscopy (SEM, JSM-IT200, JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 15 kV. Both dentin and resin surfaces were examined at low magnification (90×) to determine the mode of fracture, and specific features were further examined at 3000× magnification. The failure modes of the specimens were classified into four groups, including: adhesive failure (AF, failure at resin-dentin interface/within adhesive layer); cohesive failure (CC, > 95% failure within composite resin); cohesive failure (CD, > 95% failure within dentin); and mixed failure (MF, failure at resin-dentin interface, which included cohesive failure of composite resin or dentin)[42].

2.6 Interfacial structure observation

To investigate the typical morphology of the resin-dentin interface, one slab from the central part of the tooth was randomly selected and prepared for SEM observation. The sectioned slab surfaces were sequentially polished with SiC papers (600-, 800-, and 1000-grit) under running water. Subsequently, all the slabs were polished on a special soft cloth using diamond pastes (DP-Paste, Struers, Denmark) with a grit size of 6-, 3-, 1-μm sequentially and the specimens were cleaned using an ultrasonic device (SK10GT, Kudos ultrasonic instrument Co., LTD, Shanghai, China) for 3 min after each paste polishing. After polishing, the specimens were immersed in 1 M hydrochloric acid (HCl) for 30 s and 5% sodium hypochlorite (NaOCl) for 5 min, followed by rinsing with distilled water. The slabs were left to air dry for 24 h. Finally, the specimens were sputter coated with Pt-Pd by the ion sputter coater for 150 s and the resin-dentin interfaces were examined using the SEM at an accelerating voltage of 15 kV.

2.7 Nanoleakage (NL) evaluation

Fourteen additional human molars were assigned to the 14 groups with one tooth per group, they were prepared as previously described, and sectioned into slabs perpendicularly to the resin-dentin interface using the low-speed diamond saw. One slab from the central part of each tooth was randomly selected. The surface of the slabs was covered with two layers of nail varnish with the exception of the bonding interface area within about 1 mm, to prevent dye penetration into the other parts of the tooth. After the nail varnish completely dry, the specimens were immersed in 50% ammoniacal silver nitrate solution (pH 9.5) for 24 h at 37 ºC[41], washed with running water, and blow-dried. The specimens were then immersed in developing solution under fluorescent light for 8 h, washed with running water. Then the slabs were prepared for SEM observation by the following protocol. Slab surfaces were sequentially wet polished with SiC papers (600-, 800-, and 1000-grit) and 6-, 3-, and 1-µm diamond paste using a polishing cloth, and the specimens were cleaned using an ultrasonic device for 3 min after each paste polishing. The slabs were left to air dry for 24 h. Finally, they were sputter coated with Pt-Pd using the ion sputter coater for 150 s and examined by SEM under a backscattered mode.

2.8 Elastic modulus (EM) and hardness (H) test

The elastic modulus (EM) and hardness (H) test was proceeded by a nanoindenter. Seven additional teeth of each group (24 h) were prepared with the same procedure as mentioned above. After water storage (37°C for 24 h), the prepared teeth were cut perpendicularly to the bonded surface to obtain resin-dentin slices and one central slice from each tooth was selected, prepared, and tested following a protocol described by Chowdhury et al.[26] A Nano Indenter G200 system (G200, Agilent Tech.,

USA) equipped with a Berkovich indenter was employed to conduct nanoindentation tests at ambient temperatures around 23 ºC with a relative humidity around 56%. Three regions, i.e., the adhesive layer, adhesive-dentin interface, and sound dentin were targeted. An array that consists of 45 indents was carried out on the surface of the specimen. For indentations on the adhesive layer and adhesive-dentin interface, the indentation depth was exponentially increased to a maximum depth of 500 nm at a constant strain rate of 0.05 s^{-1}. To minimize the effect of dentin microstructures on the nanoindentation results, the maximum depth was set to be 2000 nm for indentations on dentin. With regard to the setting of oscillating load, the harmonic displacement target is 2 nm and the frequency target was 45 Hz. EM and H values were obtained from the default software of the testing device. The detection position in adhesive layer was mainly located in the middle of the adhesive layer depending on the thickness of adhesive layer, and at least a 3 μm distance between the interface indentations to avoid the influence of the residual stress from adjacent indentations (Figure 1). The Poisson's ratio takes a fixed value of 0.38.

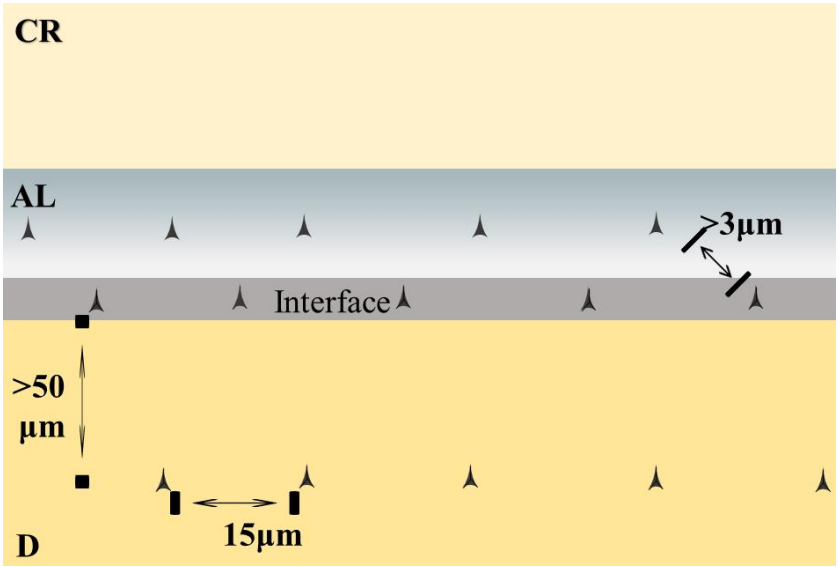

Figure 1 The protocol diagram of elastic modulus and hardness test by a nanoindenter, the triangle shapes refer to the indenter marks. (CR = composite resin; AL = adhesive layer; D = dentin).

2.9 Statistical analysis

The normality of the data distribution was assessed by Shapiro-Wilk test. As for the data of μTBS, three-way ANOVA was done to determine the effect of the adhesives, EHL, and TC as well as the interaction effect of these three factors. Comparison between groups were performed using one-way ANOVA, followed by Games-Howell test. As for the data of elastic modulus and hardness, two-way ANOVA was done to determine the interaction effect of the adhesives and EHL. Comparison between groups were performed using one-way ANOVA, followed by Tukey's test. All statistical analysis was done using IBM SPSS version 22.0 (SPSS Statistics 2.0, SPSS, Chicago, IL, USA), and the significance was set at 0.05.

Chapter 3: Results

3.1 µTBS test

The normality of all data were assessed using the Shapiro-Wilk test. Based on those test results, three-way ANOVA statistical analysis was employed to analyze the interaction effect of the adhesives, EHL, and TC on the µTBS. Comparison between groups were performed using one-way ANOVA, followed by Games-Howell test. The results indicated that a significant influence of the adhesives (F=12.713, p<0.001), EHL (F=12.573, p=0.001), and TC (F=51.645, p<0.001) on the µTBS. In addition, the interaction between these three variables was also statistically significant (F=10.675, p<0.001) (Table 3).

Table 3 Summary of three-way ANOVA for microtensile bond strength conducted at each level of interacting factor

Dependent variable: µTBS

Source	Type III Sum of Squares	*df*	Mean Square	*F*	*Sig.*
Adhesives	906.891	2	453.445	12.713	.000
EHL	448.454	1	448.454	12.573	.001
TC	1842.091	1	1842.091	51.645	.000
Adhesives*EHL	73.139	2	36.570	1.025	.404
Adhesives*TC	194.241	2	97.120	2.723	.095
EHL*TC	995.669	1	995.669	27.914	.000
Adhesives*EHL*TC	761.540	2	380.770	10.675	.000
Error	2140.116	60	39.807		
Total	125902.525	72			

(TC = thermal cycling, EHL= extra hydrophobic resin layer).

The mean of µTBS values and standard deviations were shown in Table 4. In the short-term groups (24 h), the results showed that after the use of EHL in test groups,

the μTBS of GPB+EHL (53.79 ± 5.05 MPa) was significantly improved and higher than that of the GPB (33.39 ± 3.97 MPa) alone, however the additional use of EHL did not significantly improve the μTBS of SBU (50.90 ± 7.09 MPa) and ABU (33.88 ± 5.93 MPa) groups. Compared with the gold standard SE2 (59.31 ± 5.88 MPa), ABU and GPB alone was significantly lower than SE2, and there was no significant difference between other four groups and SE2.

In the long-term experiment (15,000 TC), GPB+EHL (42.35 ± 5.31 MPa) presented a relatively high μTBS and it was significantly higher than that in the GPB (30.91 ± 3.04 MPa) group alone. No significant difference was found between SBU (39.16 ± 6.39 MPa) and SBU+EHL (36.47 ± 4.05 MPa) group, ABU (32.81 ± 3.62 MPa) and ABU+EHL (31.40 ± 2.63 MPa) group.

Compared with the short-term 24 h groups, all long-term groups except for the GPB and ABU group, the bond strength significantly decreased.

Table 4 Mean ± SD (MPa) of μTBS of the tested groups (n=6/group)

Groups	μTBS (Mean ± SD)	
	24 h	15,000 TC
GPB	33.39 ± 3.97^{1Ba}	30.91 ± 3.04^{1Aa}
GPB+EHL	53.79 ± 5.05^{2Aa}	42.35 ± 5.31^{2Ab}
SBU	50.90 ± 7.09^{1Aa}	39.16 ± 6.39^{1Ab}
SBU+EHL	53.93 ± 8.67^{1Aa}	36.47 ± 4.05^{1Ab}
ABU	33.88 ± 5.93^{1Ba}	32.81 ± 3.62^{1Aa}
ABU+EHL	47.82 ± 10.56^{1Aa}	31.40 ± 2.63^{1Ab}
SE2	59.31 ± 5.88^{Aa}	42.77 ± 9.73^{Ab}

The different number in the individual column showed statistically significant differences ($p<0.05$) within the groups. The different upper case letters in the individual column showed statistically significant difference ($p<0.05$) between the experimental groups and control group. The different lower case letters in the individual row showed statistically significant difference ($p<0.05$) between 24 h and 15,000 TC.

3.2 Fracture modes analysis

The percentage of fracture modes was summarized in Figure 2. The GPB group showed 83.02% of AF, 3.77% of MF at 24 h and 56.52% AF, 19.57% MF after 15,000 TC. However, in the GPB+EHL group, there was only 55.93% of AF and 6.78% of MF at 24 h, 36.73% of AF and 18.37% of MF after 15,000 TC. In SBU group, the AF and MF of 24 h was 53.49% and 20.93%, after 15,000 TC was 39.22% and 19.61% respectively. The fracture mode of ABU was similar to GPB group both at 24 h and after 15000 TC. After the additional application of EHL in ABU group, the percentage of AF went down to 48.72% and cohesive failure in composite resin and dentin increased. Compared with the 24 h group, the incidence of AF of all the 15,000 TC groups decreased, and other failure modes increased. The incidence of AF was obviously higher in the group with low bond strength groups (GPB and ABU). After the additional application of EHL, the rate of AF decreased in all groups.

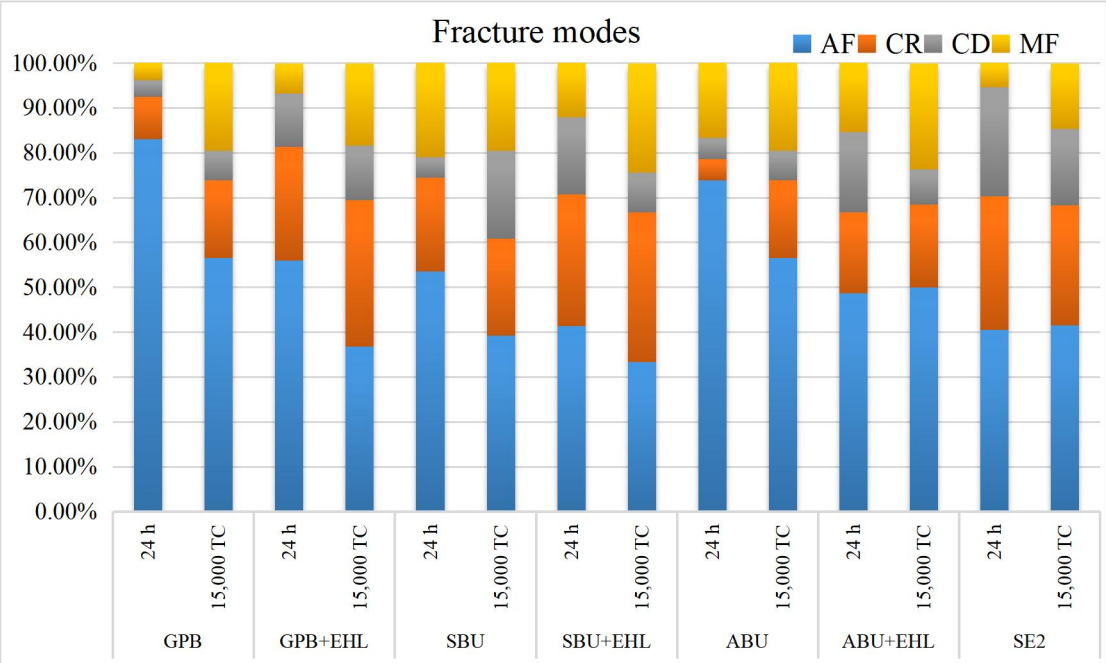

Figure 2 Fracture modes of each group. (AF: adhesive failure; CD: cohesive failure within dentin; CC: cohesive failure within composite resin; MF: mixed failure.)

SEM images taken at 90× and 3000× magnification after 24 h of each group were presented in Figure 3. Failure occurred mainly between adhesive and the composite resin in GPB group with a lot of bubbles (yellow zigzag mark) and round-shaped droplets (white block arrowhead) were presented within the adhesive layer, presumably resulting from the phase separation of its components, while in GPB+EHL group, bubbles were rare and failure mainly occurred within the adhesives. In SBU group, failure occurred within the adhesive layer without dentin tubules exposed, while most adhesive failure occurred on the top of smear layers in SBU+EHL group with a lot of scratches observed (white zigzag mark) and dentin tubules were plugged with resin tags, while little scratches were observed in SBU group. In ABU group, both open dentin tubules (yellow arrow) and occluded dentin tubules (white arrow) could be seen, as well as scratches were observed (white zigzag mark), while in ABU+EHL group, most dentin tubules were plugged with resin tags (white arrow). For the adhesive failure in SE2, most of the failure occurred within the adhesive layer or between the adhesive layer and composite resin; dentin tubules, smear layer or bubbles were not observed.

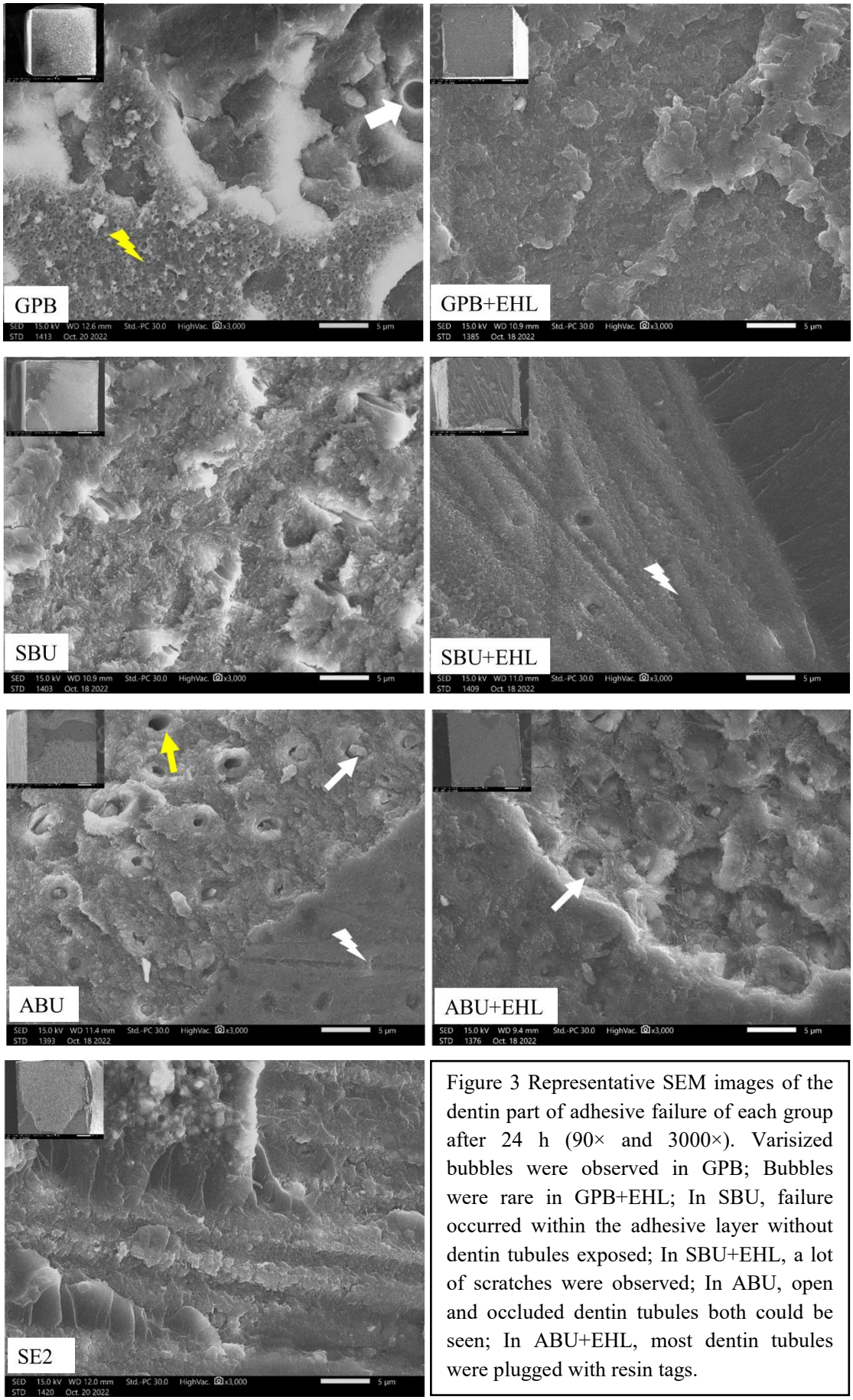

Figure 3 Representative SEM images of the dentin part of adhesive failure of each group after 24 h (90× and 3000×). Varisized bubbles were observed in GPB; Bubbles were rare in GPB+EHL; In SBU, failure occurred within the adhesive layer without dentin tubules exposed; In SBU+EHL, a lot of scratches were observed; In ABU, open and occluded dentin tubules both could be seen; In ABU+EHL, most dentin tubules were plugged with resin tags.

The SEM images taken at 90× and 3000× magnification after 15,000 TC of each group were presented in Figure 4. Failure occurred in GPB group mostly occurred within the adhesive layer and lots of different sizes of bubbles were found within the adhesives (yellow zigzag mark), as well as some small circular droplets; while in GPB+EHL group, some deep dentin tubules were exposed and the collagen structures could be seen (white asterisk), cracks were found in adhesives (white arrow). In SBU group, failure mainly occurred within the adhesive layer without dentin tubules exposed and adhesives stratified could be seen (white block arrowhead), while in SBU+EHL group, failure mainly occurred on top of smear layer and many bubble-like cavities (yellow arrow) could be seen within the adhesives. In ABU group, dentin collagen network was exposed under the hybrid layer (yellow asterisk), and some scratches were found above the hybrid layer (white zigzag mark), while in ABU+EHL group, most dentin tubules were plugged with resin tags and scratches were also observed on the hybrid layer (white zigzag mark). In SE2 group, adhesive failures were demonstrated at a low magnification, cracks (yellow block arrowhead) were found and most of dentin tubules were plugged with resin tags.

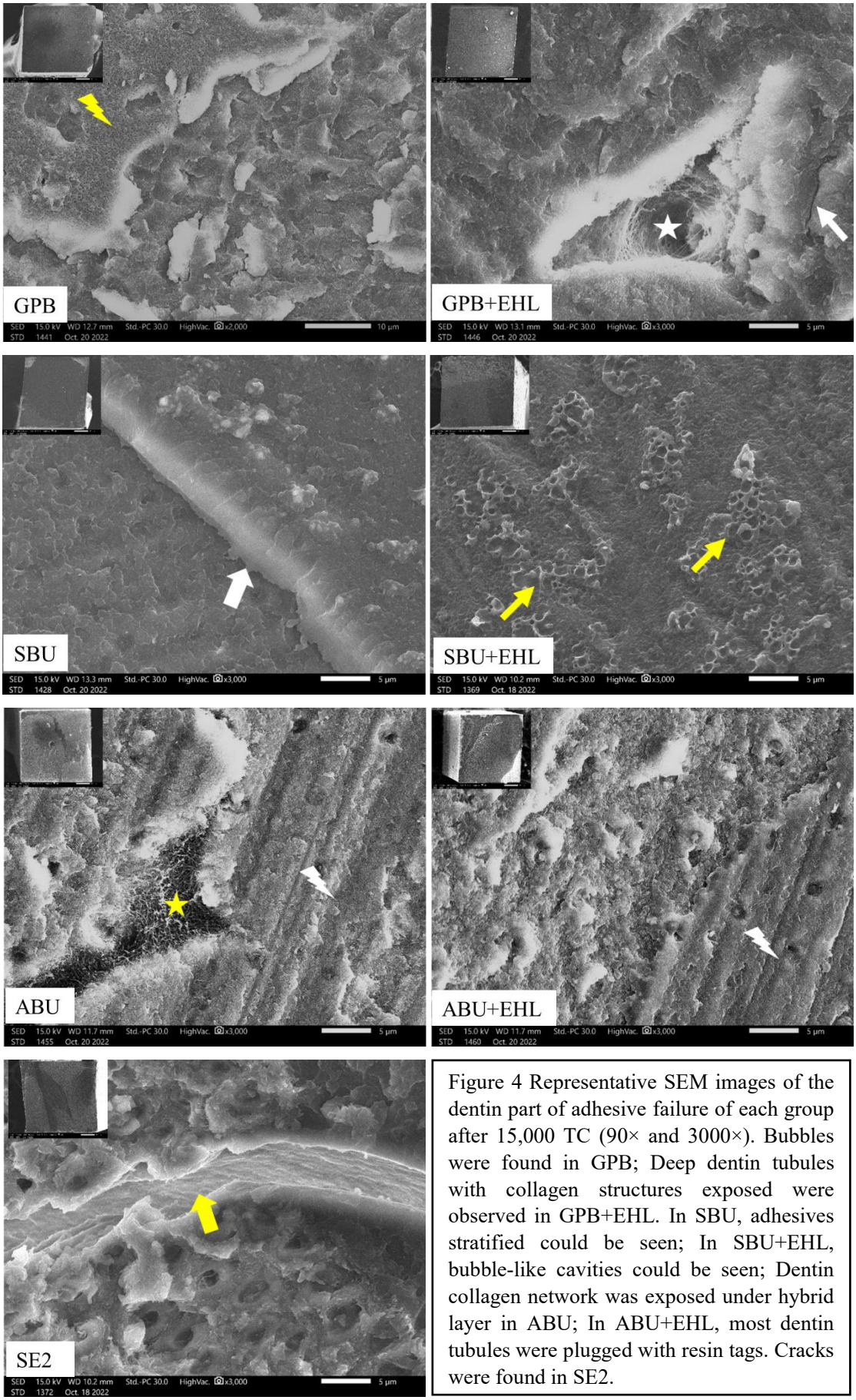

Figure 4 Representative SEM images of the dentin part of adhesive failure of each group after 15,000 TC (90× and 3000×). Bubbles were found in GPB; Deep dentin tubules with collagen structures exposed were observed in GPB+EHL. In SBU, adhesives stratified could be seen; In SBU+EHL, bubble-like cavities could be seen; Dentin collagen network was exposed under hybrid layer in ABU; In ABU+EHL, most dentin tubules were plugged with resin tags. Cracks were found in SE2.

3.3 Interfacial structure observation

Representative SEM images at 3000× magnification of resin-dentin interfaces of each group after 24 h were demonstrated in Figure 5. Additional application of EHL resulted in thicker adhesive layers in all groups. Thin adhesive layer with short and sparsely resin tags was found in GPB (approximately 3 μm) group. In GPB+EHL group, the thickness of adhesive layer was about 9 μm and longer and more resin tags were observed. In SBU group, the thickness of the adhesive layer was about 6 μm, and the resin tags were relatively few, while in SBU+EHL group, the thickness was about 10 μm, and the number of resin tags were much more. On the contrary, compared with ABU group, ABU+EHL group presented fewer and shorter resin tags. SE2 group presented a 10 μm thickness of adhesive layer with long and numerous resin tags.

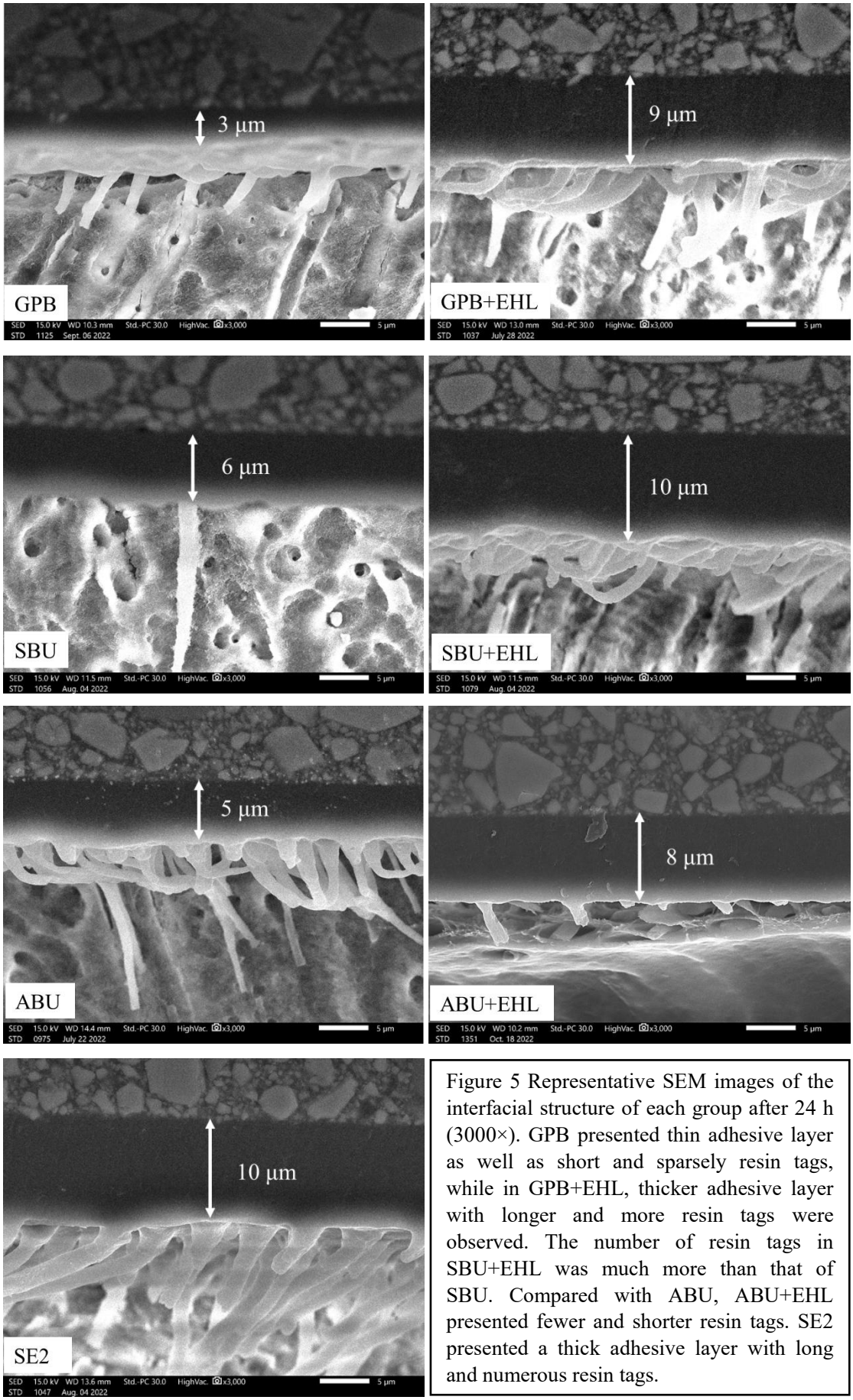

Figure 5 Representative SEM images of the interfacial structure of each group after 24 h (3000×). GPB presented thin adhesive layer as well as short and sparsely resin tags, while in GPB+EHL, thicker adhesive layer with longer and more resin tags were observed. The number of resin tags in SBU+EHL was much more than that of SBU. Compared with ABU, ABU+EHL presented fewer and shorter resin tags. SE2 presented a thick adhesive layer with long and numerous resin tags.

Representative SEM images at 3000× magnification of resin-dentin interfaces of each adhesive groups after 15,000 TC were demonstrated in Figure 6. The thickness of adhesive layer of each group was similar to 24 h groups. The resin tags in all groups became fewer and shorter and appears to be more disorganized. In GPB group, gaps at the bottom of the interface were noted (white arrow), while no gaps were found at the interface of GPB+EHL group. In SBU group, the resin tags were relatively few and short, while in the SBU+EHL group, the thickness of adhesive layer was about 12 μm, and the resin tags were densely observed. In the ABU group, the resin tags were few and gap was found at the interface (white arrow), while the ABU+EHL group presented a significantly thicker adhesive layer and more resin tags without gap formation. The SE2 group presented long and numerous bend resin tags.

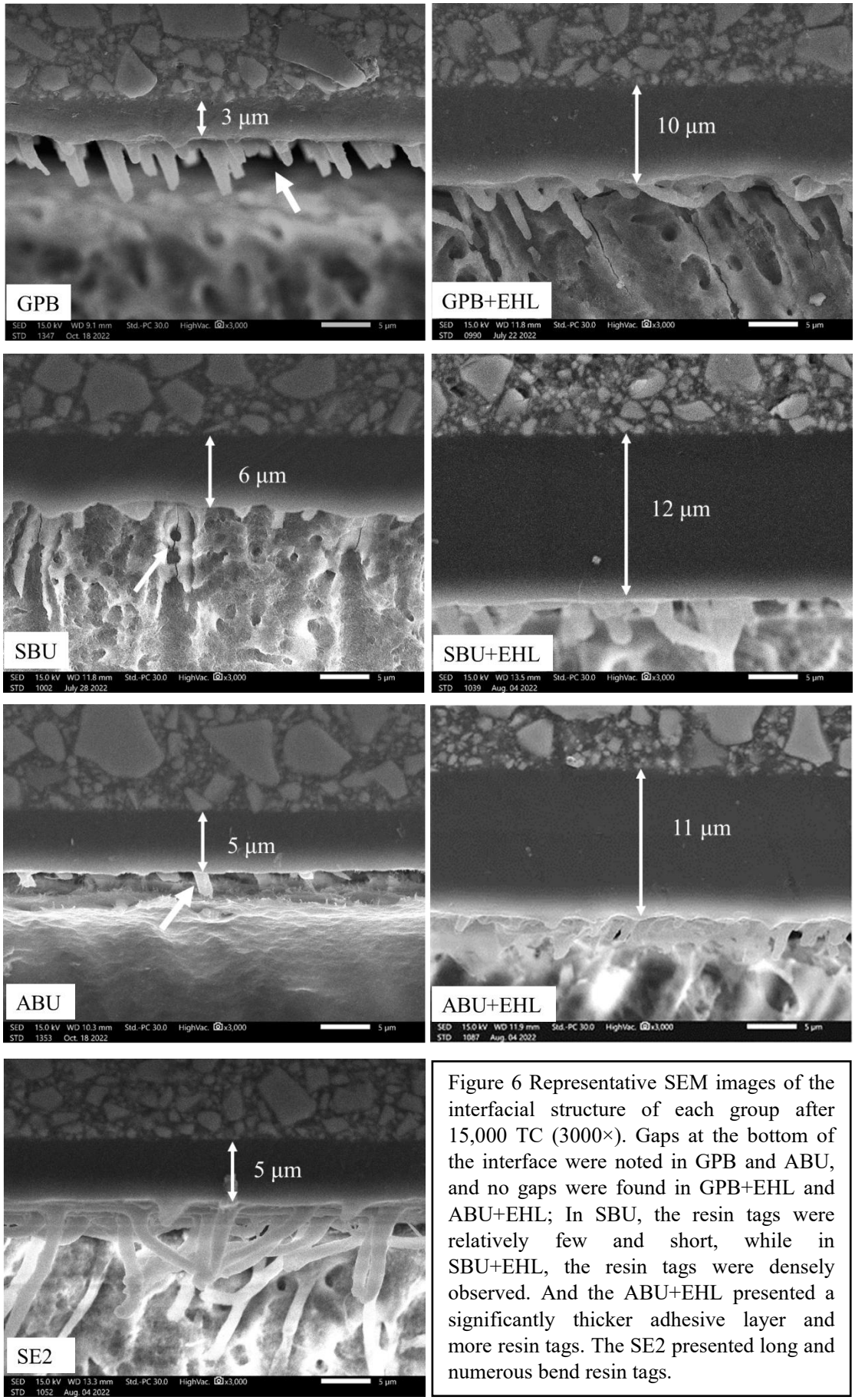

Figure 6 Representative SEM images of the interfacial structure of each group after 15,000 TC (3000×). Gaps at the bottom of the interface were noted in GPB and ABU, and no gaps were found in GPB+EHL and ABU+EHL; In SBU, the resin tags were relatively few and short, while in SBU+EHL, the resin tags were densely observed. And the ABU+EHL presented a significantly thicker adhesive layer and more resin tags. The SE2 presented long and numerous bend resin tags.

3.4 Nanoleakage (NL) evaluation

Representative backscattered SEM images of the nanoleakage at the interface of each group of 24 h and after 15,000 TC (1500× magnification) were presented in Figure 7 and Figure 8, respectively. All adhesives resulted in deposition of silver nitrate at the interface more or less.

At 24 h, a decrease in silver penetration was found after application of EHL in each group, specially in GPB group. In GPB group, it could be found that nanoleakage deposition at the interface presented as globular deposition and protruding into the dentin tubules (yellow arrow). Compared with GPB group, a fine linear deposition was seen in GPB+EHL group (white arrow). Thinner lines of silver nitrate infiltration was observed in SBU+EHL group (white arrow) compared with SBU group (white block arrowhead). Similarly the ABU+EHL group (white arrow) presented a less silver nitrate infiltration compared with ABU group (white block arrowhead). Compared with the SBU and SE2 group, GPB group resulted in the highest silver nitrate infiltration at the interface (Figure 7).

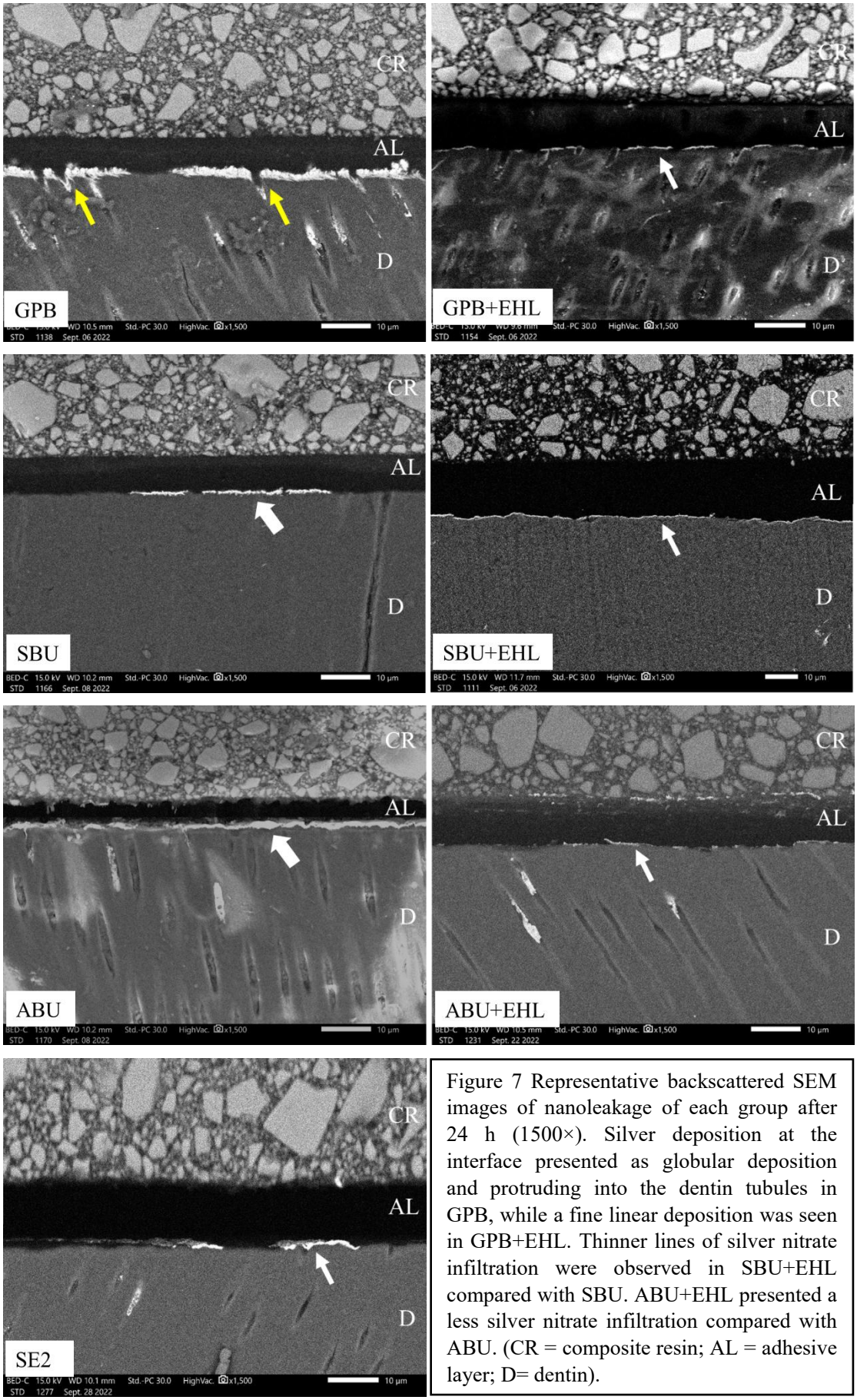

Figure 7 Representative backscattered SEM images of nanoleakage of each group after 24 h (1500×). Silver deposition at the interface presented as globular deposition and protruding into the dentin tubules in GPB, while a fine linear deposition was seen in GPB+EHL. Thinner lines of silver nitrate infiltration were observed in SBU+EHL compared with SBU. ABU+EHL presented a less silver nitrate infiltration compared with ABU. (CR = composite resin; AL = adhesive layer; D= dentin).

After 15,000 TC, each experimental group resulted in a higher silver nitrate deposition than that of 24 h groups regardless of the use of EHL. Abundant deposition of silver nitrate within the adhesive layer, resin-dentin interface and resin tags was found in GPB group. Dendritic depositions as well as glomerular-like spots (yellow block arrowhead) within the adhesive layer were observed in GPB group. However, GPB+EHL group showed a less nanoleakage with a relatively finer line (white block arrowhead) compared with GPB group. SBU+EHL group (white arrow) presented more silver nitrate deposition compared with SBU group (yellow arrow). On the contrary, ABU+EHL group showed less nanoleakage compared with the ABU group and a dendritic pattern was depicted within the resin-dentin interface of ABU group (yellow zigzag mark). SE2 group showed the lowest nanoleakage compared with other groups (Figure 8).

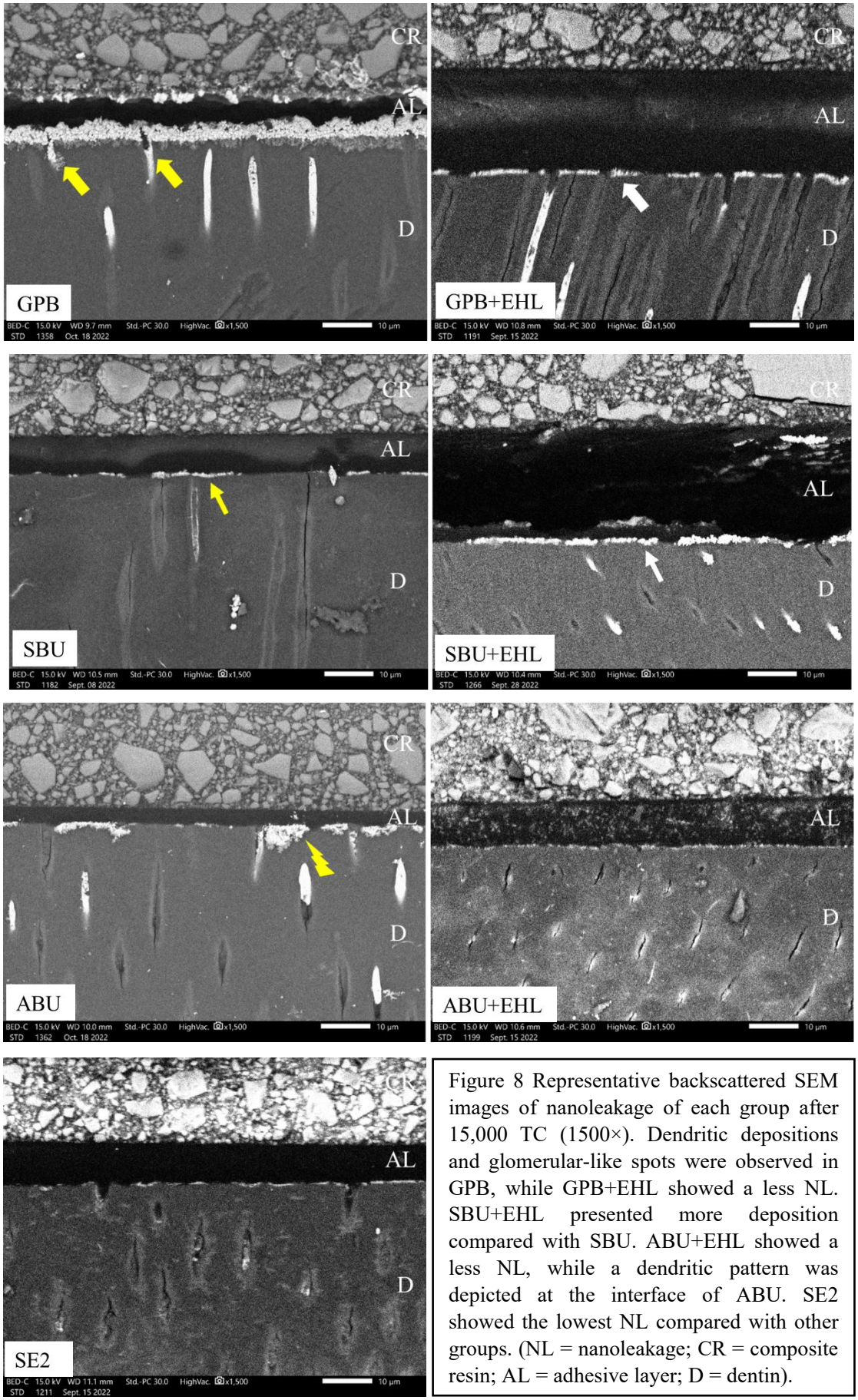

Figure 8 Representative backscattered SEM images of nanoleakage of each group after 15,000 TC (1500×). Dendritic depositions and glomerular-like spots were observed in GPB, while GPB+EHL showed a less NL. SBU+EHL presented more deposition compared with SBU. ABU+EHL showed a less NL, while a dendritic pattern was depicted at the interface of ABU. SE2 showed the lowest NL compared with other groups. (NL = nanoleakage; CR = composite resin; AL = adhesive layer; D = dentin).

3.5 Elastic modulus (EM) and hardness (H) test

Two-way ANOVA revealed that the EM of the adhesive layer was statistically significantly influenced by the type of adhesives (F = 15.990, $p < 0.001$) and with or without EHL (F = 61.373, $p < 0.001$). The interaction between these two factors was also statistically significant (F =10.511, $p < 0.001$) (Table 5). Similar results were found in the H of adhesive layer, two-way ANOVA revealed that the H of the adhesive layer was statistically significantly influenced by the type of adhesives (F = 12.108, $p < 0.001$) and with or without EHL (F = 21.163, $p < 0.001$). The interaction between these two factors was also statistically significant (F =19.382, $p < 0.001$) (Table 6).

Comparison between groups were performed using one-way ANOVA, followed by Tukey's test. The means and standard deviations of EM and H of the tested groups were shown in Table 7. Compared with GPB group, the EM and H values of adhesive layer in GPB+EHL group significantly decreased ($p < 0.05$), while no significant difference was found between SBU and SBU+EHL group ($p > 0.05$). The EM of adhesive layer in ABU was significantly higher than ABU+EHL ($p < 0.05$), while the H of adhesive layer in ABU was significantly lower than ABU+EHL ($p < 0.05$).

Table 5 Summary of two-way ANOVA for elastic modulus conducted at each level of interacting factor
(Dependent variable: EM)

Source	Type III Sum of Squares	*df*	Mean Square	*F*	*Sig.*
Adhesives	20.222	2	10.111	15.990	.000
EHL	38.809	1	38.809	61.373	.000
Adhesives*EHL	13.293	2	6.646	10.511	.000
Error	53.117	84	0.632		
Total	2957.930	90			

(EHL= extra hydrophobic resin layer).

Table 6 Summary of two-way ANOVA for hardness conducted at each level of interacting factor

(Dependent variable: H)

Source	Type III Sum of Squares	*df*	Mean Square	*F*	*Sig.*
Adhesives	0.313	2	0.157	12.108	.000
EHL	0.274	1	0.274	21.163	.000
Adhesives*EHL	0.502	2	0.251	19.382	.000
Error	1.035	80	0.013		
Total	18.880	86			

(EHL= extra hydrophobic resin layer).

Table 7 Mean ± SD (GPa) of elastic modulus (EM) and hardness (H) of adhesive layer

Group	Adhesive layer	
	EM	H
GPB	7.4600 ± 1.15623^{1A}	0.4133 ± 0.05038^{1A}
GPB+EHL	5.0667 ± 0.34572^{2C}	0.2913 ± 0.01959^{2B}
SBU	5.7467 ± 1.33195^{1B}	0.2560 ± 0.06620^{1C}
SBU+EHL	5.0800 ± 0.42292^{1C}	0.2660 ± 0.03542^{1C}
ABU	5.5933 ± 0.59338^{1B}	0.2780 ± 0.02305^{1C}
ABU+EHL	4.7133 ± 0.18074^{2C}	0.3107 ± 0.02052^{2B}
SE2	4.5200 ± 0.81082^{C}	0.2560 ± 0.06843^{C}

The different number in the individual column showed statistically significant differences ($p<0.05$) within the groups with or without EHL. The different upper case letters in the individual column showed statistically significant difference ($p<0.05$) between the experimental groups and control group.

(EM = elastic modulus; H =hardness; EHL= extra hydrophobic resin layer)

Chapter 4: Discussion

The bonding effectiveness and durability of one-step bonding systems have been reported to inferior to two-step bonding systems due to their higher hydrophilicity[1,2]. UAs basically work as traditional one-step adhesives when they are applied with the SE mode[34]. A latest two-step UA system (G2-Bond Universal) which utilizes universal adhesives' benefits in its primer, offers a durable clinical bonding performance[17]. Converting one-step UAs into a two-step system might be a promising approach to improve their bonding performance. In this study, three different pH UAs were selected as a primer, and the bond agent of SE2 was used as a bond, the results showed that the EHL significantly improved the bond strength and reduced the nanoleakage at the interface when they were used to GPB both at 24 h and after 15,000 TC, but no significant improvement was found in other ultra-mild UAs (SBU and ABU). Therefore, the null hypothesis that the bonding performance of UAs would be improved by using an extra hydrophobic resin layer, and UAs could work as a primer in two-step bonding system was partially rejected.

In the present study, with the application of an EHL, both immediate and aged bond strength were significantly improved in GPB group. The most important thing was that after 15,000 TC, the μTBS of GPB+EHL was significantly higher than that of GPB. After 15,000 TC, the bond strength of GPB+EHL was similar to the control group of SE2, the nanoleakage of the GPB+EHL group was also lower than GPB alone both at 24 h and after 15,000 TC. This promoting effect was more significant in

the GPB group with higher hydrophilicity and more water content. Several studies have advocated the fact that the additional placement of EHL improved the short and long-term bonding performance of one-step self-etch adhesives[43,44]. The extra hydrophobic coat has been used recently to improve the bonding performance of UAs to dentin[45], by means of a higher hydrophobicity, and superior polymerization[15,25], enhancing monomer penetration into the hybrid layers and increasing the chemical interactions[26,46]. Hydrophobic extra layer supplements insoluble monomers, subsequently reduces the concentration of unreacted monomers and retains solvents in the adhesive layer[47], providing a thicker and uniform adhesive layer with better polymerization, and helps to protect the adhesive layer against water absorption, increasing the bond strength of the adhesive interface[48].

On the contrary, the additional application of EHL did not improve the μTBS in SBU and ABU group. This result was similar to a recent study showed that the application of extra hydrophobic resin or double layer did not improve the μTBS of SBU[28]. The reason why the μTBS was not improved by the EHL might probably relate to their high pH and hydrophobic properties.

Firstly, unlike hydrophilic GPB, EHL may couple more hydrophobic monomers to the adhesive interface and enhance the degree of conversion. Compared with ultra-mild SBU and ABU, the EHL reduced the inhibition of polymerization caused by a highly acidic pH UAs such as GPB[49,50]. The acidity of intermediately strong UAs will probably not be completely buffered from the demineralized hydroxyapatite, resulting in continued etching[51]. The placement of an EHL in GPB group resulted in

higher hydrophobicity, thicker film thickness, increased chemical interaction, and improved mechanical properties of the resin-dentin interface[26].

Secondly, most of UAs were based on the functional monomer 10-MDP, which can chemically interact to form stable 10-MDP-Ca salts in the hybrid layer[52], and it may take an appropriate time of 20 s for its chemical interaction to take place. Applying a second coat of such monomers without curing the first layer permits the primer to sufficiently interact with Ca^{2+} and promotes supplementary bonding[53]. Although the manufacturer's manual states that it is possible to do without waiting time of GPB (no-waiting self-etch; Japanese brochure), a precious study has confirmed the importance of waiting time in GPB[54,55]. According to the manufacturers' instructions, when using the SBU, it was required to rub for 20 s. When utilizing the ABU, it was applied two separate coats and scrubbed the preparation with a microbrush for 10-15 s per coat. However, it was just left GPB undisturbed for 10 s after application. This might be one of the reasons refer to waiting time and scrubbing technique might lead to different improve effect on bond strength between each group.

Moreover, a large amount of various bubbles were observed on the fracture surface of GPB group. The blister-like areas were depicted as phase separation in HEMA-free adhesive system or under insufficient solvent evaporation conditions. It may imply that the weak point of GPB adhesion was between the adhesives and resin[54,56]. On the other hand, in GPB+EHL group, no bubbles were found, suggesting that weak areas of the adhesive layer were eliminated, which might be related with

higher μTBS and better durability. The intermediately strong and ultra-mild UAs with thick adhesive layer might present no significant change after the additional use of EHL.

For one-step UAs, back to the multi-step with a hydrophobic bonding agent to improve their bonding effectiveness and durability, to be a new next-generation two-step universal system, might be a promising approach to expand its clinical application[16]. In the present study, the improving effect was achieved when we use GPB as a primer and EHL as the bond agent. It can be concluded that only GPB could easily go to 2-step system, while SBU and ABU need major modifications of their components if they want to go to 2-step self-etch universal system.

More interestingly, GPB and ABU presented lower bond strength compared with SBU and SE2 at 24 h. However, these two lower μTBS UAs seemed to be more stable than other higher μTBS UAs. The long-term bond strength of majority groups decreased after 15,000 TC except for the GPB and ABU group. In this study, this result contradicted with a systematic review which suggested a significant decrease in the bond strength after any type aging processes was observed with the use of intermediately strong adhesives, irrespective of the substrate or adhesion strategy used[11]. It is really hard to explain this result, but this might be related to the lower bond strength of GPB and ABU. Multiple adhesive coats significantly affected bond strength to dentin. The thicker adhesive layer might adversely affect the mechanical properties, negatively influencing the strength and the quality of adhesion[57]. Previous studies have stated that the effect of EHL application depended on the adhesive composition[21,25].

In the present study, it was found that GPB group presented highest nanoleakage compared with other groups, and the additional use of EHL obviously decreased the

nanoleakage compared with GPB used alone. Nanoleakage was originally used to describe microporous zones beneath or within hybrid layers[58], and nowadays it is used as an indirect method to evaluate the quality of resin-dentin bonds and represents the degradation pathway. Aging stability of adhesive was reported to be material dependent[25]. A thicker adhesive layer by EHL was reported to be related with better sealing of the adhesive interface, which indicated better bond degradation prevention[44]. The fluid flow across the adhesive interface would decrease due to the increase in the adhesive layer thickness[21,59]. Different nanoleakage expressions depended on the adhesives used as previously reported[60]. The higher nanoleakage in GPB group might be related with the existence of phase separation which could impair the bonding effectiveness to dentin[61], while the extra use of EHL significantly decreased the nanoleakage in GPB. Our result was also similar to a previous study revealing that the application of EHL resulted in a significantly reduced nanoleakage at 24 h only for G-Bond Plus and no significant change in nanoleakage for SBU and ABU using the SE strategy[30]. Since a meta-analysis found that nanoleakage expression depending on the UAs being used, the lower nanoleakage with these adhesives may be due to their lower aggressiveness of high pH and incorporation of 10-MDP[62]. As an extra bonding layer compensates UAs thin film thickness, the overall benefit of EHL on the short-term and aged bonding efficacy differed for the different UAs tested[63].

As for the relationship between the bond strength and nanoleakage of UAs, in the present study, the long-term of nanoleakage in GPB+EHL group was obviously lower than that of GPB group, while the bond strength of GPB+EHL group was still maintained in high level. The bond strength of ABU+EHL group was significantly

decreased after 15,000 TC, while the nanoleakage was still maintained in a low level. It can be found from this result that the decrease of μTBS was not consistent with less nanoleakage, and an increase in silver penetration over time might not be related to the decrease in μTBS. This result seemed contradictory as a previous study indicated that an increase in silver penetration is correlated with a decrease in bond strength[64]. However, this issue was controversial, other researchers stated that no correlation was found between the bond strength and nanoleakage among different adhesives[65]. Another possible reason should be that in this study, the number of TC might not be enough. According to the SEM observation of interfacial structure of each group, there was no particular degradation sign. Moreover, the stability of the adhesive layer is also strongly influenced by the of conversion of the bonding system[66]. Due to their high level of acidity of intermediately strong UAs, the presence of unpolymerized monomers remains and continues to demineralize the dentin, promoting dentin-adhesive interfaces with low hydrolytic stability[12].

With regard to the EM and H of the adhesive layer, the additional application of EHL obviously decreased the EM and H in GPB group and resulted in a remarkable increase in bond strength. The EM and H of GPB+EHL group was close to SE2 group. A gradual increase of EM and H values was found started from adhesive layer and rising through the harder adhesive-dentin interface to end with dentin in all groups, and this result was consisting with a previous study[67]. The result of this study indicated that a lower EM and H of adhesive layer might be related with a higher bond strength, this inverse correlation suggests adequate resistance of the adhesive to

elastic deformation under stress and a lower EM and H of adhesives yielded higher bond strength. These results were also in agreement with a previous study reported that within one-step and two-step self-etch adhesives, lower E yielded higher bond strength[68]. The superior bonding performance of GPB+EHL could be attributed to their better mechanical property and increased adhesive thickness imparting better stress relief at the interface[69].

In terms of the relationship between the pH and functional monomer 10-MDP in adhesives, it was reported that the pH value was strongly affected by the composition and concentration of the 10-MDP based all-in-one adhesives[70], a strong negative correlation between 10-MDP concentration and pH was observed[71]. However, the efficacy of 10-MDP-based all-in-one adhesives to demineralize the dentin was not directly related to their pH value[38]. An appropriate water content, pH, and 10-MDP concentration in adhesives is essential to obtain a promising bond strength and durability. Further studies are needed to explore new universal adhesives with strong and durable bond strength.

Conclusion

Within the limitations of this study, it can be concluded that the additional application of extra hydrophobic resin layer significantly improved the μTBS and reduce the nanoleakage only for low pH UAs GPB both at 24 h and after 15,000 TC, but not for ultra-mild (SBU and ABU) UAs, GPB works as a primer in a two-step bonding system while SBU, ABU does not work well.

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