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# *In Vitro* Bonding Performance of Self-etch Adhesives: II—Ultramorphological Evaluation

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### Clinical Relevance

All-in-one adhesives are being currently used in clinical dentistry. These simplified adhesives result in a deficient infiltration in dentin and deficient enamel etching, which may result in marginal leakage and compromised durability.


## SUMMARY

**Objective: To study the interfacial ultra-morphology formed by "all-in-one" self-etch adhesives. Methods: Forty-nine extracted molars were assigned to one of five all-in-one adhesives: Adper Prompt L-Pop (AP, 3M ESPE); Clearfil S³ Bond (S3, Kuraray); G-Bond (GB, GC America); iBond (iB, Heraeus Kulzer) and Xeno IV (XE, Dentsply Caulk). Adper Single Bond Plus (SB, 3M ESPE), a two-step etch&rinse adhesive, and Clearfil SE Bond (SE, Kuraray), a two-step self-etch adhesive, were used as controls. Dentin, unground enamel and ground enamel were used as bonding substrates. Dentin specimens were processed for FESEM and TEM analyses. Enamel specimens were processed for FESEM. Results: Dentin: GB, iB, S3, SE and XE resulted in a submicron-thick hybrid layer (0.2-0.7 µm), but only S3 and SE did not result in interfacial gaps. AP resulted in the thickest hybrid layer (1.7-2.9 µm) among the self-etch adhesives. SB resulted in a 3.4-5.2 µm thick hybrid layer. Unground enamel—GB, iB and SE resulted in a mostly featureless morphology resembling that of untreated enamel with areas in which the superficial enamel layer was removed without dissolving the subsurface enamel. XE resulted in areas of intra-prismatic etching and areas without any etching pattern. S3 resulted in frequent shallow intra-prismatic etching, while AP was able to unveil the enamel crystallites across the entire enamel surface. Phosphoric acid in SB resulted in the deepest intra- and inter-prismatic demineralization. Ground enamel—AP resulted in a well-defined inter-prismatic etching pattern. iB, GB, SE and S3 resulted in islands of superficially dissolved enamel within areas without evidence of enamel dissolution. XE resulted in etched enamel areas with mild intraprismatic exposure of crystallites. Phosphoric acid in SB resulted in deep enamel etching. Conclusions: Only AP, an aggressive self-etch adhesive, showed enamel morphological features that resemble those created by etch&rinse**



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**adhesives. S3 and SE were the only self-etch adhesives that did not result in dentin interfacial debonding.**

## INTRODUCTION

In 1955, Dr Buonocore reported the use of 85% phosphoric acid to improve the mechanical retention of an acrylic resin on enamel.[1] The micro-mechanical nature of the interaction of dental adhesives with enamel is a result of the infiltration of resin monomers into the microporosities left by the acid dissolution of enamel and subsequent envelopment of the exposed hydroxyapatite crystals with the polymerized monomers within the pores on the enamel surface.[2]

The latest dental adhesives are based on simplification and reduced application time, as they do not require etching and rinsing. These self-etch adhesives condition and prime enamel and dentin simultaneously, incorporating the smear layer and residual hydroxyapatite into the hybridized area.[2] The first self-etch non-rinsing adhesives were composed of two solutions, an acidic primer and a bonding resin. More recently, this trend has shifted to all-in-one adhesives, in which all components are combined into one solution. In spite of their user-friendliness and low technique sensitivity, all-in-one adhesives have resulted in low bonding effectiveness *in vitro*.[2-4] Additionally, they may behave as semi-permeable membranes that allow the movement of water across the bonded interface and potentially lead to hydrolytic degradation.[5-6]

Because of their higher pH, most of the first generation self-etch adhesives resulted in a shallow enamel demineralization compared to that of phosphoric acid.[7-8] Nevertheless, the instrumentation of enamel to remove prismless enamel and/or a separate phosphoric acid enamel etching step improved the enamel bonding ability of those self-etch adhesives.[9-10]

Several ultra-simplified all-in-one adhesives have been introduced to the dental market within the last few years without a comprehensive set of independent tests to validate the performance claimed by the respective manufacturers. Inadequate resin penetration into tooth substrates may result in accelerated degradation of the structure of the bonding interface.[4] As polymerization shrinkage stresses the bonding interface, dentin adhesives that are not well infiltrated into the substrate may not resist the stresses and result in bonding failure.[11] In light of the potential consequences of a deficient interfacial bonding, the objective of this *in vitro* study was to evaluate the ultrastructure of the interfaces produced by five all-in-one adhesives using two 2-step adhesives as controls.

## METHODS AND MATERIALS

Forty-nine extracted sound molars stored in 0.5% chloramine solution for up to one month were used in this study. The teeth were left in distilled water for 24 hours at 4°C prior to use, then cleaned with pumice and a prophy cup under slow speed for 15 seconds. The adhesives used are listed in Table 1: 1) Adper Prompt L-Pop (AP, 3M ESPE, St Paul, MN, USA); 2) Clearfil S$^3$ Bond (S3, Kuraray America, Inc, New York, NY, USA); 3) G-Bond (GB, GC America, Alsip, IL, USA); 4) iBond (iB, Heraeus Kulzer Inc, Armonk, NY, USA); 5) Xeno IV (XE, Dentsply Caulk, Milford, DE, USA); 6) Adper Single Bond Plus (SB, 3M ESPE) was used as a two-step etch&rinse control; 7) Clearfil SE Bond (SE, Kuraray America) was used as a two-step self-etch control. For each adhesive, the teeth were randomly subdivided into three bonding substrates: dentin, unground enamel and ground enamel.

### Dentin-resin Interfaces with FESEM (Field Emission Scanning Electron Microscope)

The occlusal enamel of 21 molars (n=3) was removed with an Isomet 1000 diamond saw (Buehler Ltd, Lake Bluff, IL, USA) under water refrigeration, and dentin disks with a thickness of 800 ± 200 µm were obtained from middle dentin. A standard smear layer was created on the occlusal surface by wet sanding with 600-grit SiC sandpaper for 60 seconds.[12] After application of the respective adhesive, a 1-mm thick layer of a flowable resin composite (Filtek Flow A2, 3M ESPE) was applied to the treated dentin and light-cured for 80 seconds. The restored disks were cross-sectioned in two identical halves, using the water-cooled low-speed diamond saw. The specimens were immediately immersed in 2.5% glutaraldehyde/2% paraformaldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 for 12 hours at 4°C. After fixation, the specimens were rinsed with 20 mL of 0.1 M sodium cacodylate buffer at pH 7.4 for one hour with three changes, followed by distilled water for one minute. They were then dehydrated in ascending grades of ethanol (25%, 50% and 75% for 20 minutes, 95% for 30 minutes and 100% for 60 minutes). After the final ethanol step, the specimens were dried by immersion in hexamethyldisilazane (HMDS, Ted Pella Inc, Redding, CA, USA) for 10 minutes, placed on a filter paper inside a covered-glass vial and air-dried at room temperature.[13]

The dentin disks were embedded in self-curing Epo-Thin epoxy resin (Buehler Ltd) and stored at room temperature under a hood for 18 hours. After curing, the surface of each epoxy cast was polished with wet silicon carbide papers of decreasing abrasiveness (up to 1200-grit) and a soft polishing cloth, with increasingly fine diamond suspension to a particle size of 0.05 µm (Buehler Ltd). The top of the epoxy cast was protected with non-adhesive tape and sectioned with a diamond saw to separate the specimen from the epoxy block. The specimens were ultra-sonicated in 100% ethanol for five minutes to remove polishing debris, thoroughly dried, demineralized in 6 N HCl for 30 seconds and

Table 1: *Adhesives Tested with Corresponding pH[35,55-58] Composition, Instructions for Use and Type*

| Adhesives (pH) | Composition | Instructions for Use | Type |
|---|---|---|---|
| Adper Prompt L-Pop (AP) (0.9-1.0) | HEMA phosphates, HEMA, bis-GMA, modified poly-alkenoic acid, water, photo-initiator | 1. Activate the L-Pop Unit Dose Dispenser to mix the adhesive.<br>2. Apply mixed adhesive to entire surface, rubbing in with moderate finger pressure for 15 seconds.<br>3. Use a gentle stream of air to thoroughly dry the adhesive into a thin film.<br>4. Rewet the brush tip with adhesive and apply a second coat of adhesive to the tooth surface. The second coat does not require rubbing.<br>5. Use a gentle stream of air to thoroughly dry the adhesive into a thin film.<br>6. Light cure for 10 seconds. | All-in-one self-etch (mixing required) |
| Adper Single Bond Plus (SB) (0.4, silica thickened 35% $H_3PO_4$) | HEMA, bis-GMA, DMA's, methacrylate functional copolymer of polyacrylic and polyitaconic acids, water, ethanol, nanofiller, photo-initiator | 1. Apply Scotchbond Etchant to tooth surface for 15 seconds.<br>2. Rinse thoroughly for 10 seconds. Blot excess water using a cotton pellet or mini-sponge. Do not air dry.<br>3. Apply two-to-three consecutive coats of adhesive for 15 seconds with gentle agitation using a fully saturated applicator.<br>4. Gently air thin for five seconds to evaporate solvent.<br>5. Light cure for 10 seconds. | Two-step total-etch |
| Clearfil S$^3$ Bond (S3) (2.4) | 10-MDP, HEMA,bis-GMA, water, ethanol, silanated colloidal silica, camphorquinone | 1. Thoroughly wet brush tip with BOND. Apply BOND to the tooth surface and leave for 20 seconds.<br>2. Dry the entire surface sufficiently by blowing high-pressure air for more than five seconds while spreading the bond layer thinly.<br>3. Light cure for 10 seconds. | All-in-one self-etch |
| Clearfil SE Bond (SE) (1.8) | *Primer:* 10-MDP, HEMA, hydrophilic DMA, tertiary amine, water, photo-initiator<br>*Bond*: 10-MDP, HEMA, bis-GMA, hydrophilic DMA, tertiary amine, silanated colloidal silica, photo-initiator | 1. Thoroughly wet brush tip with Primer. Apply Primer to tooth surface and leave in place for 20 seconds.<br>2. Dry with a mild air stream to evaporate the volatile ingredients.<br>3. Dispense the necessary amount of BOND into second mix well.<br>4. Apply BOND to the tooth surface.<br>5. After applying BOND, create a uniform film using a gentle air stream.<br>6. Light cure for 10 seconds. | Two-step self-etch |
| G-Bond (GB) (1.8) | 4-MET, UDMA, phosphate monomer, DMA component, fumed silica filler, acetone, water, photo-initiator | 1. Prior to dispensing, shake the bottle thoroughly. Replace bottle cap immediately after use.<br>2. IMMEDIATELY apply to the prepared enamel and dentin using the micro-tip Applicator.<br>3. Leave undisturbed for 10 seconds.<br>4. After application, dry thoroughly using oil free air under MAXIMUM air pressure for five seconds, in the presence of vacuum suction to prevent splatter of the adhesive.<br>5. Light cure 10 seconds. | All-in-one self-etch |
| iBond (iB) (2.2) | UDMA, 4-MET, glutaraldehyde, acetone, water, stabilizer, photo-initiator | 1. iBond is applied in three consecutive layers and massaged into the prepared tooth structure for 30 seconds.<br>2. Following that, the solution is blown away with a gentle air stream.<br>3. Polymerize for 20 seconds. | All-in-one self-etch |
| Xeno IV (XE) (2.5) | UDMA, PENTA, acetone, polymerizeable trimethacrylate resin, and 2 polymerizeable DMA resins, photo-initiator | 1. Using the disposable microbrush applicator tip supplied, immediately apply and scrub surfaces with generous amounts of XENO IV Adhesive to thoroughly wet all the tooth surfaces for 15 seconds.<br>2. Apply a second application of XENO IV Adhesive with the microbrush as above, scrubbing for 15 seconds (20 seconds for larger restorations).<br>3. Remove excess solvent by gently drying with clean, dry air from a dental syringe for at least five seconds.<br>4. Cure for 10 seconds. | All-in-one self-etch |

*10-MDP = 10-methacryloyloxy decyl dihydrogenphosphate*
*4-MET = 4-methacryloxyethyl trimellitic acid*
*bis-GMA = bisphenol glycidyl methacrylate*
*DMA = dimethacrylate*
*HEMA = 2- hydroxyethyl methacrylate*
*PENTA = Dipentaerythritol penta-acrylate phosphate*
*UDMA = Urethane dimethacrylate*

deproteinized in 1% NaOCl for 10 minutes. After drying at room temperature, the specimens were mounted on aluminum stubs with adhesive carbon disks (Ted Pella Inc) and colloidal quick drying silver paint (Ted Pella Inc). After sputter-coating with gold-palladium, the specimens were observed under an S-4700 Hitachi (Hitachi, Tokyo, Japan) FESEM at an accelerating voltage of 4.0-5.0 kV and a working distance of 12.0-13.0 mm.

Five measurements of the hybrid layer thickness in intertubular dentin were taken from each half-disk at equivalent areas across the interface, with a total of 30 measurements for each adhesive. All the measurements were carried out with microscope-linked imaging software (Quartz PCI 4.00, Quartz Imaging Co, Vancouver, BC, Canada).

**Dentin-resin Interfaces with TEM**

The dentin substrate of 14 molars (n=2) was obtained as described for the FESEM study. After application of the respective adhesive, a 1-mm thick layer of a flowable resin composite (Filtek Flow A2, 3M ESPE) was applied to the treated dentin and light-cured for 80 seconds. Two sticks with a cross-section of 1 x 1 $mm^2$ were obtained from the central area of each restored disk using the water-cooled low-speed diamond saw. The sticks were immersed in 2.5% glutaraldehyde/2% paraformaldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 for 12 hours at 4°C. After fixation, the bonded sticks were rinsed with 10 mL of 0.1 M sodium cacodylate buffer at pH 7.4 for two hours. The specimens were post-fixed with a solution of 2% osmium tetroxide in 0.1 M sodium cacodylate buffer for one hour and washed in 0.1 M sodium cacodylate for one hour. They were then rinsed with deionized water four times, with a dwell time of five minutes, and dehydrated in ascending grades of ethanol (50%, 70%, 95% for five minutes and 2x 100% for 10 minutes each). After the final ethanol step, the specimens were immersed in propylene oxide for two periods of 10 minutes each.

The specimens were then embedded in 50% propylene oxide/50% MedCast epoxy resin (Ted Pella Inc) in a Pelco Infiltron (Ted Pella Inc) rotator at 6 rpm. After six hours of rotation, the specimens were transferred to 100% epoxy resin at room temperature, then placed under vacuum for 12 hours to allow for resin infiltration into the specimens. The specimens were oriented in rubber molds so that the resin-dentin interface corresponding to the central area of the dentin disk was first exposed for sectioning. The molds were filled with fresh MedCast epoxy resin and left in an incubator for 12 hours at 65°C. The resulting resin-embedded specimen blocks were trimmed and sectioned in an ultramicrotome equipped with a material-sciences type III diamond knife (Micro Star Technologies Inc, Huntsville, TX, USA). After observing semi-thin specimens stained with toluidine blue under an optical microscope, the ultra-thin sections (85 ± 10 nm-thick) were sectioned and mounted on 150-mesh nickel grids (Ted Pella Inc). After drying at room temperature, the sections were analyzed under a JEOL 1200 TEM (JEOL Ltd, Tokyo, Japan) at an accelerating voltage of 80 kV. Selected sections were stained with 2% uranyl acetate for 20 minutes and 3% lead citrate for 15 minutes to highlight the contrast within the hybrid layer.

Measurements of the hybrid layer thickness in intertubular dentin were taken from all viable sections. Means and standard deviations were not computed due to the disparity in the number of readings for each adhesive. All measurements were carried out with the Quartz PCI 4.00 software (Quartz Imaging Co, Vancouver, BC, Canada).

**Enamel Etching Pattern**

Fourteen lower molars were used for FESEM evaluation of the enamel etching pattern. An area approximately 8x4 $mm^2$ was marked on the enamel of each proximal surface and sectioned with a diamond saw under water-cooling. One half of each enamel rectangle was roughened with a coarse diamond bur (Two Striper, Premier Products Co, Plymouth Meeting, PA, USA) for five seconds under water, while the other half was left intact. The self-etch adhesives were applied to enamel per the manufacturers' instructions (Table 1) but not light-cured. For SE, only the SE Primer was applied on enamel. The specimens were then stirred continuously in acetone for 24 hours to dissolve the resin material from the surface of the enamel. A pilot study had indicated that dissolving the monomers for shorter periods might leave a residue on the monomers. For SB, the 35% phosphoric acid gel was applied to enamel for 15 seconds, rinsed for 10 seconds and air-dried. All specimens were allowed to dry for 24 hours under vacuum, mounted on Al stubs, sputter-coated with gold-palladium and observed under an S-4700 Hitachi (Hitachi) FESEM at an accelerating voltage of 4.0 kV-5.0 kV and a working distance of 12.0-13.0 mm.

## RESULTS

**Dentin-resin Interfaces with FESEM**

The resin-dentin interfaces obtained with FESEM are shown in Figure 1. AP (Figure 1A) was the only self-etch adhesive for which dentin demineralization/hybridization was deeper than 1 µm. The depth of the acid-resistant hybrid layer formed with AP ranged from 1.7 to 2.9 µm (average = 2.3 ± 0.4 µm). All other all-in-one adhesives formed submicron hybrid layers, which were only detectable starting at a 15,000x magnification.

For S3 (Figure 1B), the depth of the hybrid layer was 0.2-0.4 µm. The interface, however, was sealed with

Figure 1: *Dentin-resin interfaces with FESEM. (A = adhesive; H = hybrid layer; T = resin tag; D = dentin; C = composite; G = gap)*

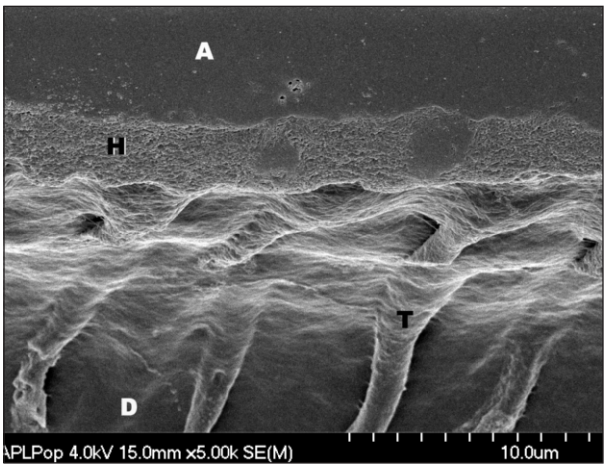


Figure 1A: *Representative interface formed with AP showing the reticular pattern of the hybrid layer (5000x).*

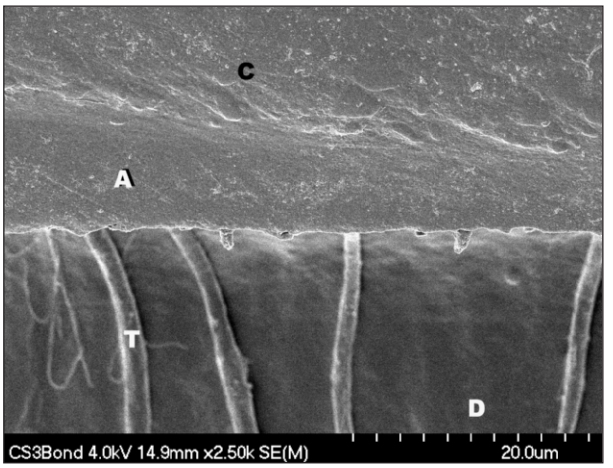


Figure 1B: *Representative interface formed with S3 at 2500x. The thin hybrid layer is only discernible starting at magnifications of 15,000x.*

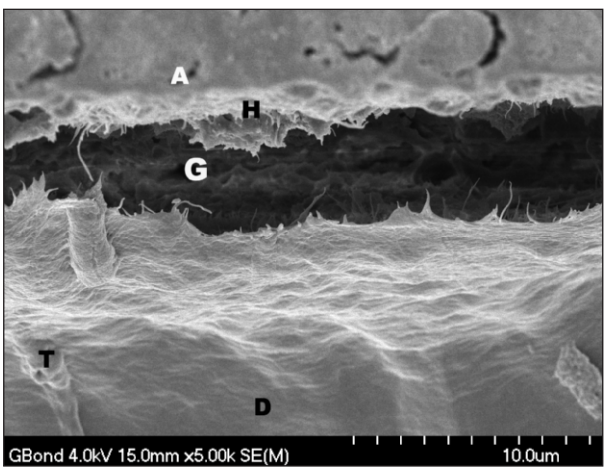


Figure 1C: *Representative interfaces formed with GB. Gaps formed frequently at the interface (5000x).*

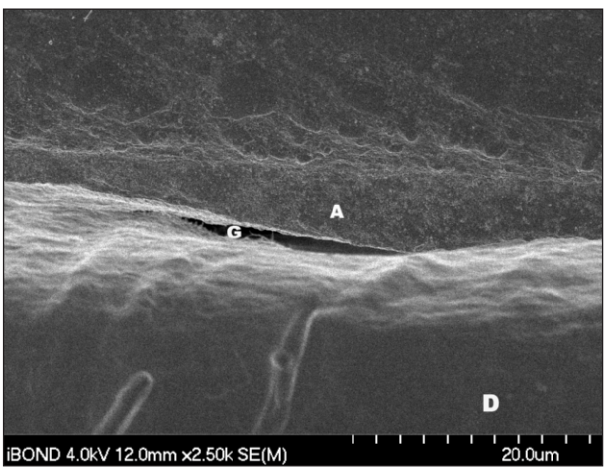


Figure 1D: *Representative interfaces formed with iB. Very poor tubular penetration with formation of interfacial gaps (2500x).*

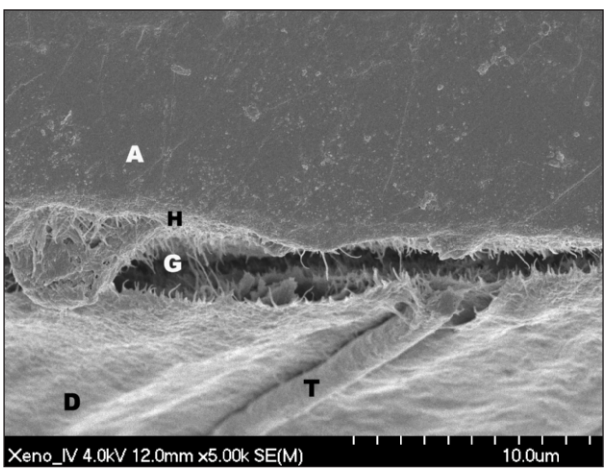


Figure 1E: *Representative interfaces formed with XE. Despite profuse tubular penetration, gaps formed at the interface (5000x).*

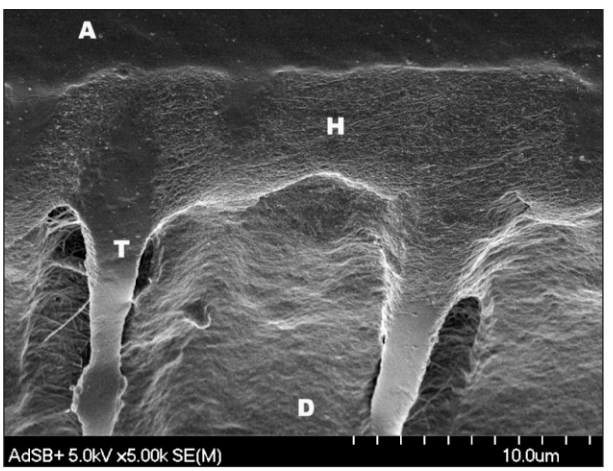


Figure 1F: *Representative interfaces formed with SB. Very thick (3.4-5.2 µm) and reticular hybrid layer without signs of separation throughout the interfaces (5000x).*

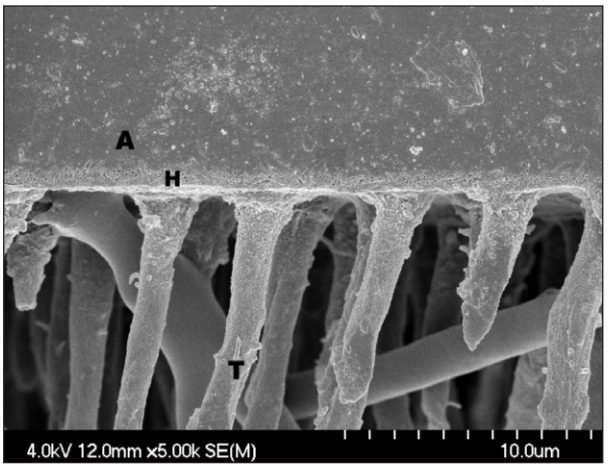


Figure 1G: *Representative interfaces formed with SE. Very thin (0.4-0.7 µm) and reticular hybrid layer without signs of separation throughout the interfaces (5000x).*

resin tags reaching 100 µm in length. GB (Figure 1C) resulted in a 0.3-0.4 µm thick hybridized area with wide debonded areas along the interface. Detachment commonly occurred between the hybridized area and the adhesive. iB (Figure 1D) formed a 0.4-0.6 µm thick hybrid layer and resulted in sporadic interfacial gaps with short and rare resin tags. XE (Figure 1E) hybridized dentin to a depth of 0.5-0.8 µm, resulting in interfacial gaps and formed more frequent resin tags than all other all-in-one adhesives with the exception of AP. SB (Figure 1F) resulted in a 3.4-5.2 µm thick hybrid layer, a high concentration of resin tags and characteristic funnel-shaped tags with peritubular triangular hybridization around the tag neck. SE (Figure 1G) resulted in a 0.4-0.7 µm thick hybrid layer, profuse infiltration of resin tags and a total absence of interfacial gaps.

### Dentin-resin Interfaces with TEM

For AP (Figure 2A), the absence of hydroxyapatite crystallites in the hybrid layer made the interfacial morphology resemble that of an etch&rinse adhesive. AP resulted in the thickest hybrid layer (1.9-2.3 µm) without the characteristic hybridized smear plugs observed for the other all-in-one adhesives. A dense mesh of individual collagen fibers was observed inside the hybrid layer, especially in the most superficial two-thirds. Although no hydroxyapatite crystals were depicted inside the hybrid layer, there was a gradation in density of crystallites in the transition from hybrid layer to the intact dentin.

Hydroxyapatite crystallites were observed in the hybridized area of all other self-etch adhesives. For S3 (Figure 2B), the hybrid layer was 0.2-0.5 µm thick, and the density of hydroxyapatite crystals inside the hybrid layer was very heavy, almost resembling that of intact dentin. The distinction between hydroxyapatite crystals and residual smear particles was not readily discernible even at higher magnifications. The thickness of the hybrid layer of GB ranged from 0.2 to 0.4 µm (Figure 2C). This morphology was very peculiar, as it included a very high concentration of hydroxyapatite crystals without the characteristic gradation in density. On the top of this hybridized area, a layer of electron-dense material, a residual resin-infiltrated smear layer, covered the hybrid layer, continuing inside the lumen of the tubules. The interfacial morphology of iB (Figure 2D) was quite different, as iB resulted in a gradation in density of hydroxyapatite crystallites from the resin to

Figure 2: *Dentin-resin interfaces with TEM. (C = composite; A = adhesive; H = hybrid layer; D = dentin)*

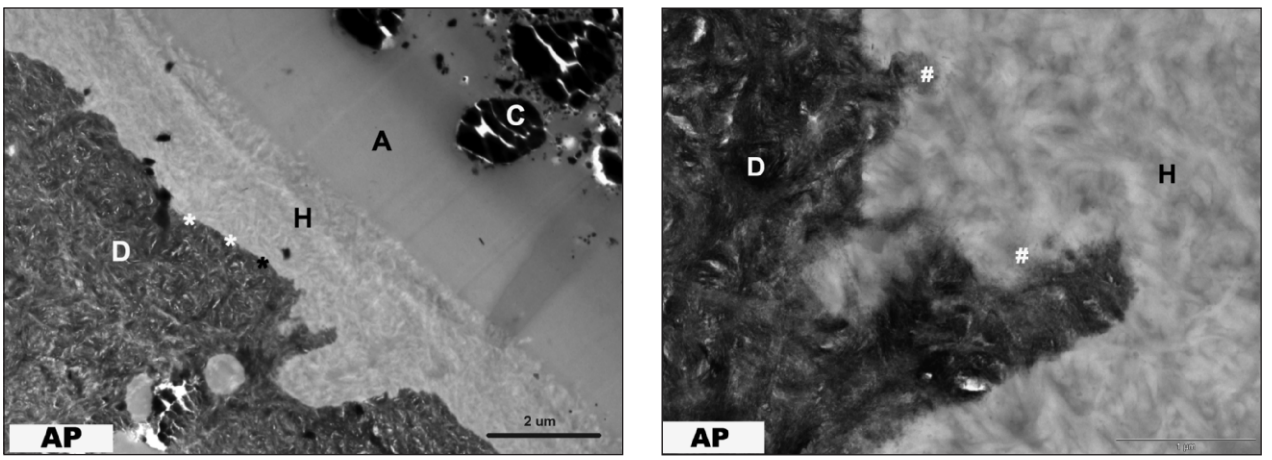


Figure 2A: *Representative interfaces formed with AP showing the reticular pattern of the hybrid layer resembling that of an etch&rinse adhesive. The image on the left shows a general view of the interface at 5,000x; the TEM micrograph on the right shows a high magnification (30,000x) of the transition from the hybrid layer (demineralized dentin) to unaffected dentin. Although the lower magnification micrograph shows an abrupt transition from the hybrid layer to the area of normal dentin (*), the image at higher magnification depicts a gradual transition (#) with an increase in the concentration of hydroxyapatite crystallites from the hybrid layer to the area of normal dentin.*

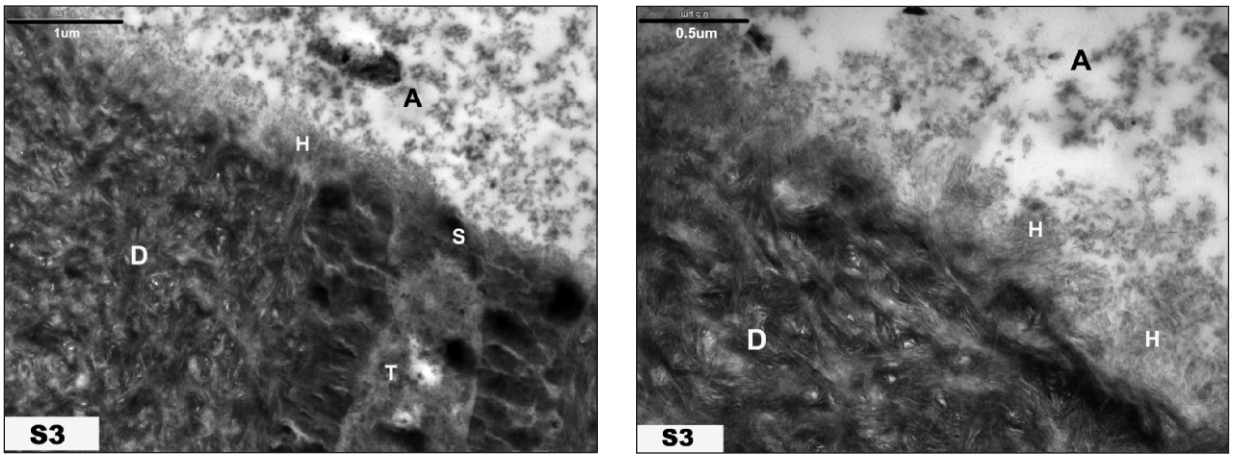


Figure 2B: *Representative interfaces formed with S3. The left micrograph (25,000x) shows the entrance of a dentin tubule still obliterated with a smear plug (S). The hybrid layer contains a high concentration of hydroxyapatite crystallites and residual smear layer particles. The micrograph on the right (50,000x) shows the hybrid layer with the needle-like hydroxyapatite crystallites and globular smear layer particles. (A = adhesive; H = hybrid layer; D = dentin; T = resin tag)*

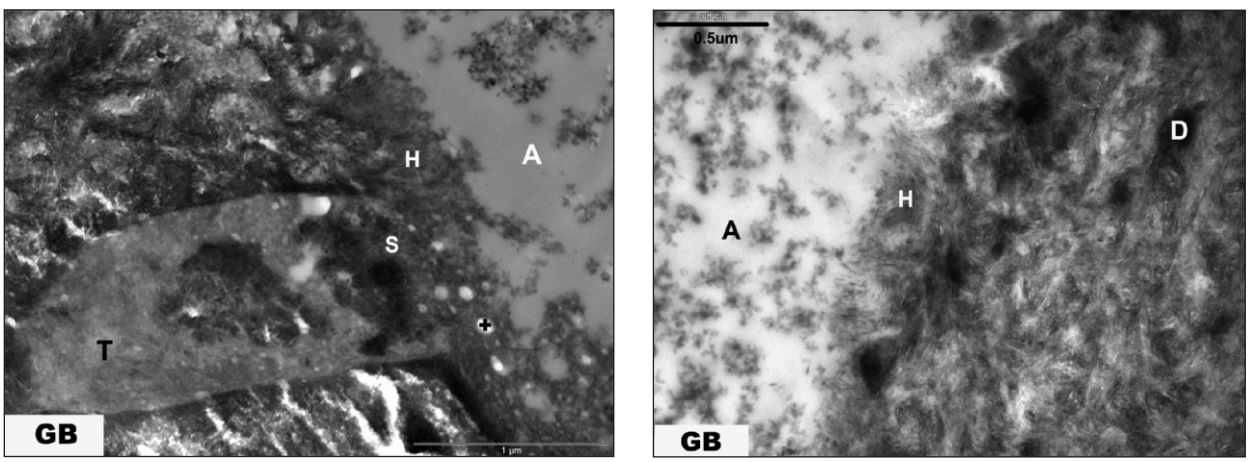


Figure 2C: *Representative interfaces formed with GB. On the left micrograph (40,000x), note the high electron-density of the hybrid layer with hydroxyapatite crystallites mixed with smear layer particles. The smear plug (S) obliterates the tubule. Some blisters (+) were likely caused by insufficient drying time of the self-etch adhesive. The right micrograph (50,000x) depicts a close-up of a dense hybrid layer with the characteristic needle-like hydroxyapatite crystallites. (T = resin tag; H = hybrid layer; A = adhesive; D = dentin)*

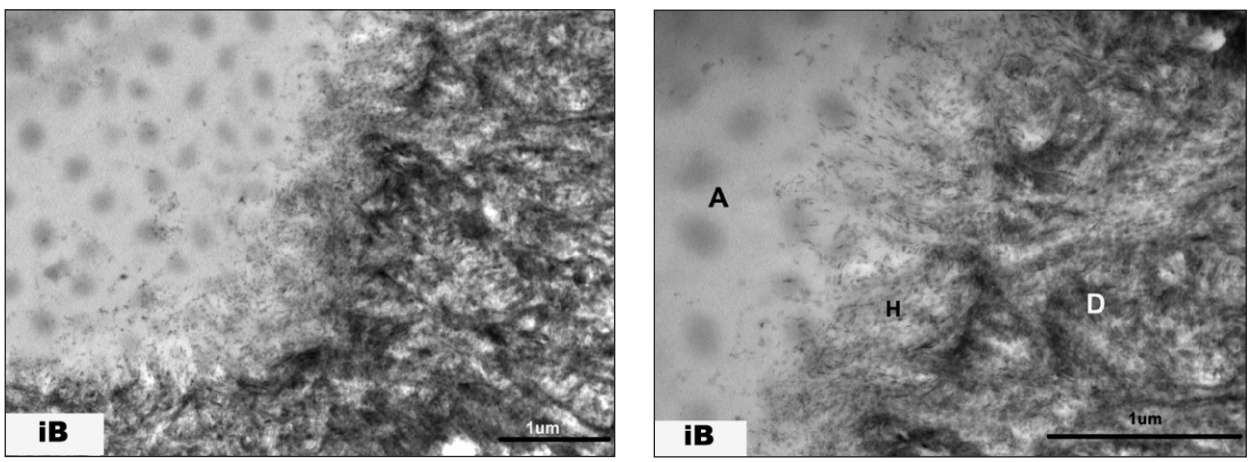


Figure 2D: *Representative interfaces formed with iB. The hybrid layer contains a low concentration of hydroxyapatite crystallites (needle-like) without evidence of residual smear layer. The electron density of the hybrid layer is therefore lower than that of S3 and GB. Magnifications—25,000x (left); 40,000x (right). (A = adhesive; H = hybrid layer; D = dentin)*

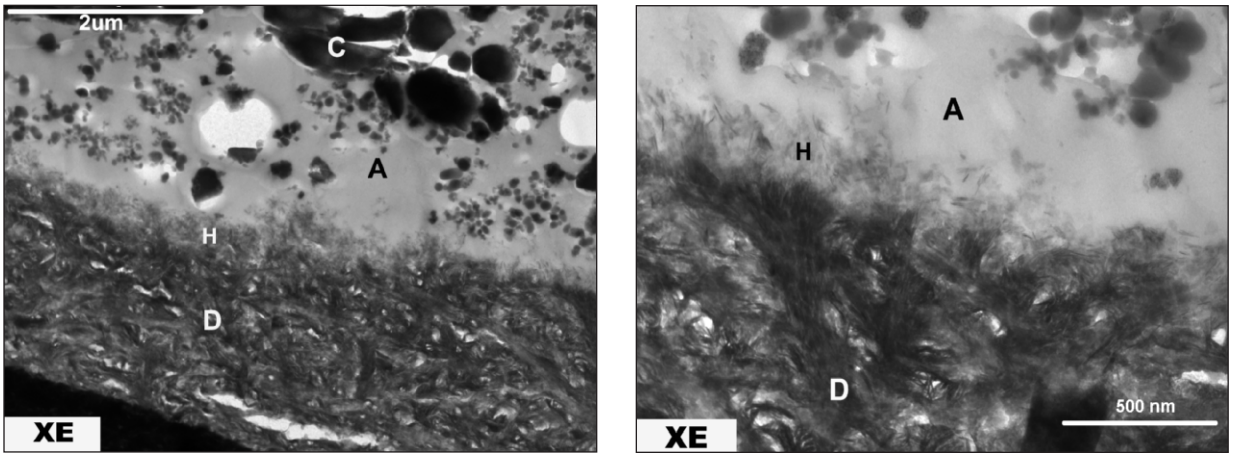


Figure 2E: *Representative interfaces formed with XE. The thickness of the adhesive layer (A) was lower for XE than for the previous groups. On the left micrograph (20,000x), composite particles almost contact the area of the hybrid layer, which shows a gradation in concentration of hydroxyapatite crystallites. The presence of these needle-like crystallites are observed at higher magnification (60,000x) in the micrograph on the right. (C = composite; H = hybrid layer; D = dentin)*

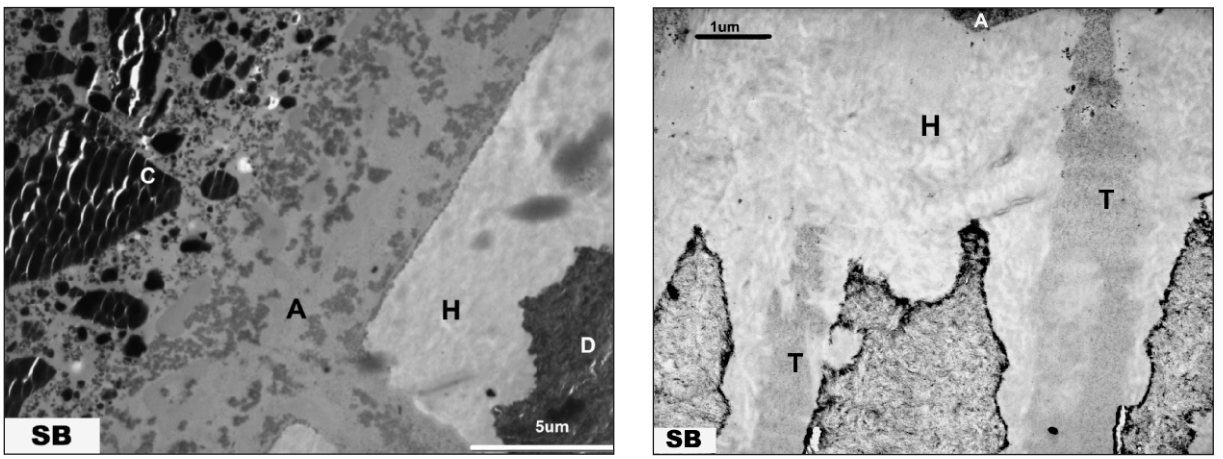


Figure 2F: *Representative interfaces formed with SB. The left micrograph shows a very thick and reticular hybrid layer (6,000x) with electron-dense precipitates in the adhesive layer. The micrograph on the right (10,000x) depicts the small dots in the resin tags (T) that represent the nanofiller particles present in the composition of SB. Note that the hybrid layer is completely demineralized as it does not show evidence of the needle-like crystallites observed in the TEM micrographs of S3, GB, and iB. (C = composite; A = adhesive; H = hybrid layer; D = dentin)*

the intact dentin, forming a hybrid structure of 0.4-0.5 µm without the residual smear layer on the top of the hybridized structure. For XE (Figure 2E), the hybrid layer was in the range of 0.3-0.7 µm and displayed the gradation in the concentration of hydroxyapatite crystallites without any residual smear deposits on the top of the hybrid layer. For SB, the two-step etch&rinse control dentin was demineralized to a depth of 3.2-5.1 µm (Figure 2F), without morphological evidence of hydroxyapatite crystallites in the hybrid layer. A clustered precipitate of an electron-dense material was observed in the adhesive layer. The transition from hybrid layer to intact dentin did not show any gradual increase in hydroxyapatite crystals. The resin tags were filled in their entire extension with particles that did not exceed 0.035 µm when observed at 100,000x (not shown). The hybrid layer of SE (Figure 2G) was very well-defined and filled with hydroxyapatite crystals without an organized coating of smear layer on the top of the hybrid layer. The dentin was partially demineralized to a depth of 0.2-0.5 µm. Hydroxyapatite crystallites and rare residual smear residues were observed within the hybrid layer. There was a gradual transition in electrolucency from the top of the unaffected dentin to the adhesive layer.

### Conditioned Unground Enamel-FESEM

AP (Figure 3A) was able to unveil the enamel crystallites across the entire enamel surface; however, this pattern was mostly intra-prismatic, that is, without unveiling the periphery of the prisms. S3 (Figure 3B) resulted in frequent, shallow intra-prismatic etching.

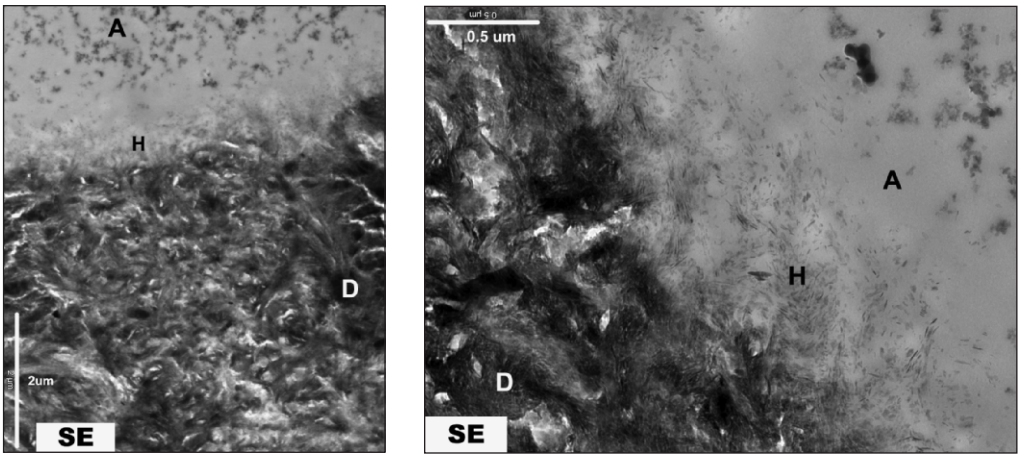


Figure 2G: *Representative interfaces formed with SE. The hydroxyapatite crystals in the hybrid layer are not surrounded by smear layer particles as in the other self-etch adhesives except for AP. Note the low concentration of hydroxyapatite crystallites in the hybrid layer (H) in the right micrograph with a gradual transition in density to the unaffected dentin (D). Magnifications—15,000x (left); 50,000x (right). (A = adhesive)*

Figure 3: *FESEM of conditioned unground enamel.*

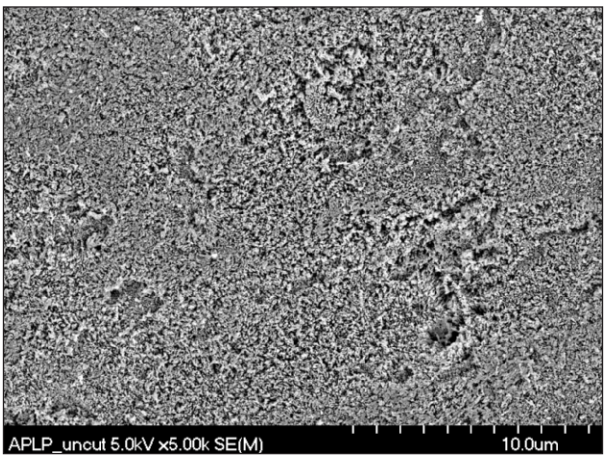


Figure 3A: *Representative morphology formed with AP on unground enamel showing a well-defined inter-prismatic etching pattern (5000x).*

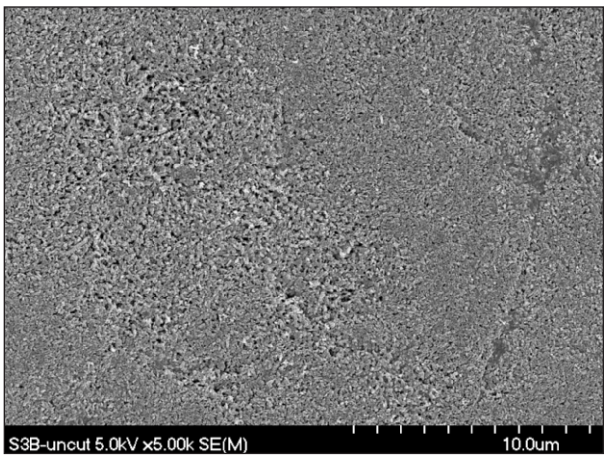


Figure 3B: *Representative morphology formed with S3 on unground enamel. S3 resulted in frequent shallow intra-prismatic etching (5000x).*

GB (Figure 3C), iB (Figure 3D) and SE (Figure 3G) resulted in a mostly featureless morphology resembling that of untreated enamel with areas in which the superficial enamel layer was removed without dissolving the subsurface enamel. For XE (Figure 3E), less than 25% of the areas showed intra-prismatic and inter-prismatic etching, while the other areas did not display any etching characteristics. Phosphoric acid in SB (Figure 3F) resulted in the deepest intra- and inter-prismatic etching.

### Conditioned Ground Enamel-FESEM

When applied on ground enamel, AP (Figure 4A) resulted in a well-defined inter-prismatic etching pattern (exposure of the periphery of the prisms with signs of hydroxyapatite dissolution). S3 (Figure 4B), GB (Figure 4C), iB (Figure 4D) and SE (Figure 4G) resulted in islands of superficially dissolved enamel within areas without evidence of enamel dissolution. However, the etching pattern of SE was better defined than that of S3, GB and iB (compare Figure 4G with Figures 4C and 4D). XE (Figure 4E) resulted in etched enamel areas with intra-prismatic exposure of crystallites and slight delimitation of the prism boundary (mild inter-prismatic etching). The etch&rinse adhesive SB (Figure 4F) resulted in deep intra- and inter-prismatic enamel etching with deep demineralization of the inter-prismatic spaces and demarcation of the prisms characteristic morphology.

## DISCUSSION

With etch&rinse adhesives, the resin monomers are applied on dentin after rinsing the phosphoric acid

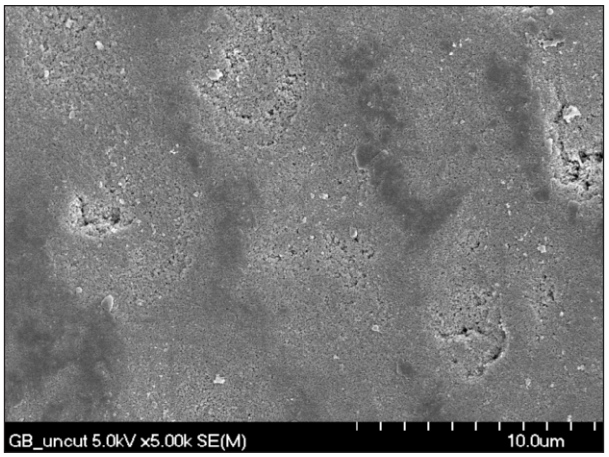


Figure 3C: *Representative morphology formed with GB on unground enamel. While there are signs of a mild etching effect, a few islands persist in which there is no evidence of acid attack (5000x).*

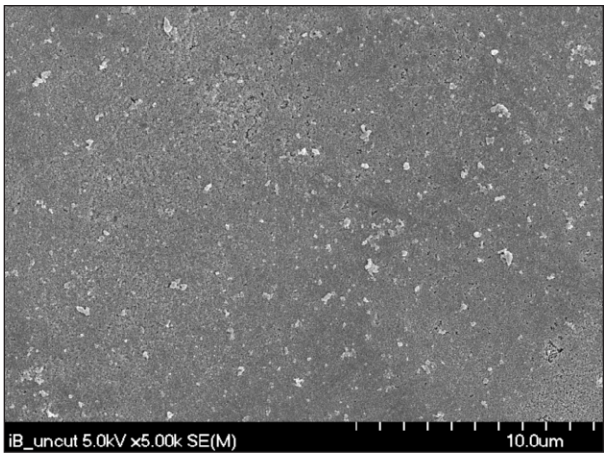


Figure 3D: *Representative morphology formed with iB on unground enamel. Although GB and iB showed a similar morphology, the etching pattern of iB was mostly featureless and less defined than that of GB (5000x).*

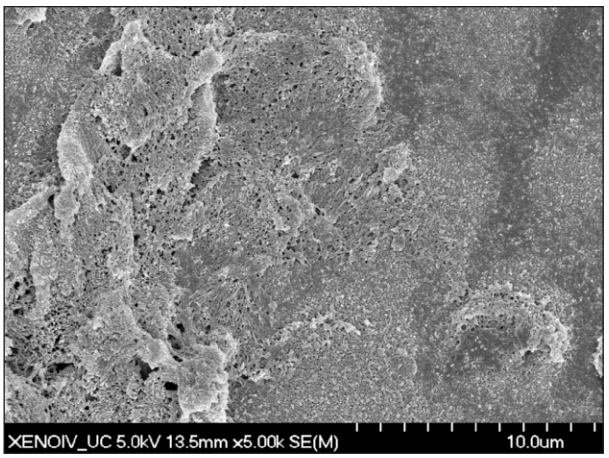


Figure 3E: *Representative morphology formed with XE on unground enamel. Enamel treated with XE displayed areas of intra-prismatic and inter-prismatic etching mixed with areas without a defined etching pattern (5000x).*

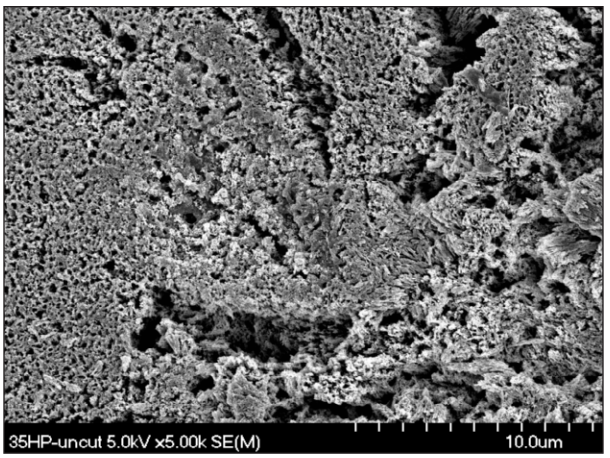


Figure 3F: *Representative morphology formed with SB on unground enamel. The phosphoric acid group resulted in the best defined etching pattern (5000x).*

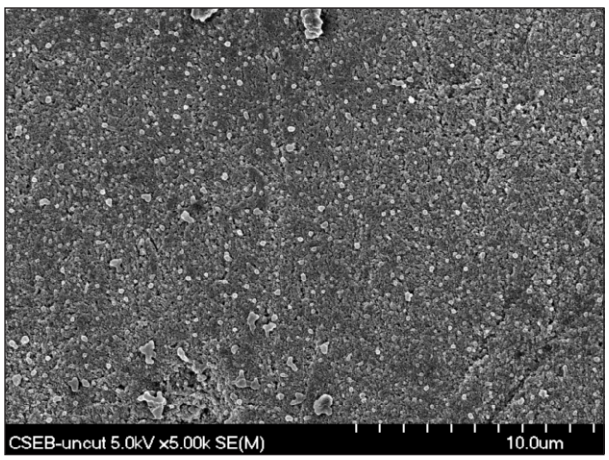


Figure 3G: *Representative morphology formed with SE on unground enamel. The etching pattern of SE on unground enamel was mostly featureless (5000x).*

etchant. These monomers permeate the water-filled spaces between adjacent collagen fibers that were formerly occupied by the dissolved hydroxyapatite crystals, resulting in a hybrid tissue composed of collagen, resin, residual hydroxyapatite and traces of water. Because of their less acidic pH, self-etch adhesives do not demineralize dentin as deep as phosphoric acid. Nevertheless, self-etch adhesives are still able to intermingle with dentin and the residual smear layer, as the depth of dentin demineralization depends on the acidity of the acidic primer.[14]

Self-etch adhesives are composed of aqueous mixtures of acidic functional monomers, generally phosphoric acid- or carboxylic acid-esters, with a pH higher than that of phosphoric acid gels.[8] Water is a very important component of self-etch adhesives, as it participates in the ionization of the acidic moieties of the self-etching adhesives. Self-etch adhesives are classi-

Figure 4: *FESEM of conditioned ground enamel.*

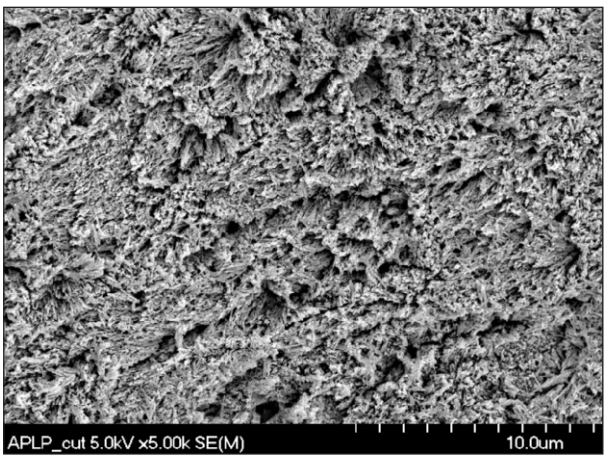


Figure 4A: *Representative morphology formed with AP on ground enamel showing a very well-defined inter-prismatic etching pattern (5000x).*

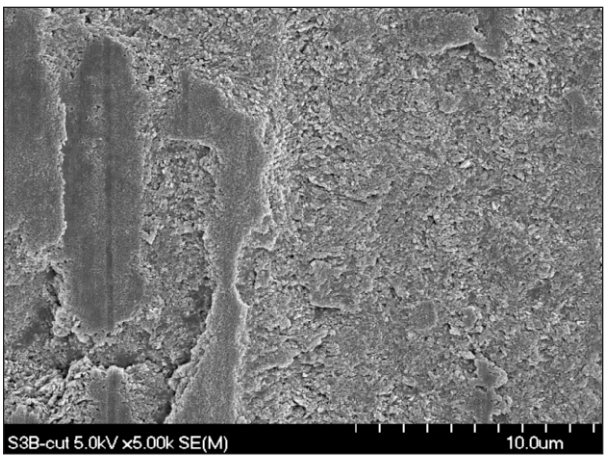


Figure 4B: *Representative morphology formed with S3 on ground enamel showing areas of etched enamel intermingled with areas of featureless enamel (5000x).*

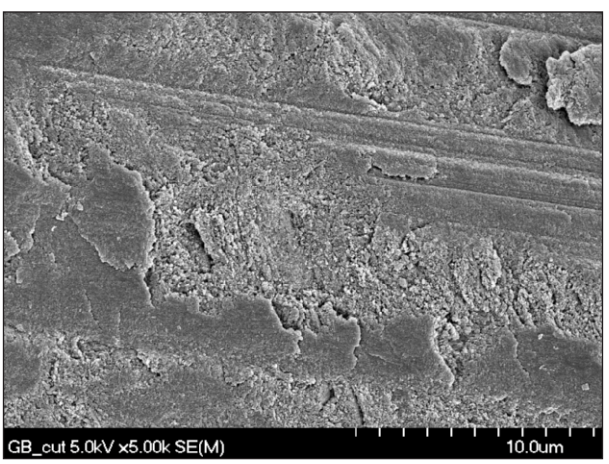


Figure 4C: *Representative morphology formed with GB on ground enamel showing areas of etched enamel intermingled with areas of featureless enamel (5000x).*

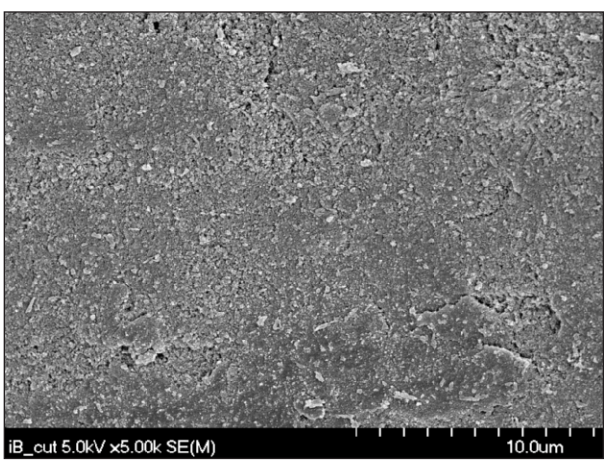


Figure 4D: *Representative morphology formed with iB on ground enamel showing areas of etched enamel intermingled with areas of featureless enamel (5000x).*

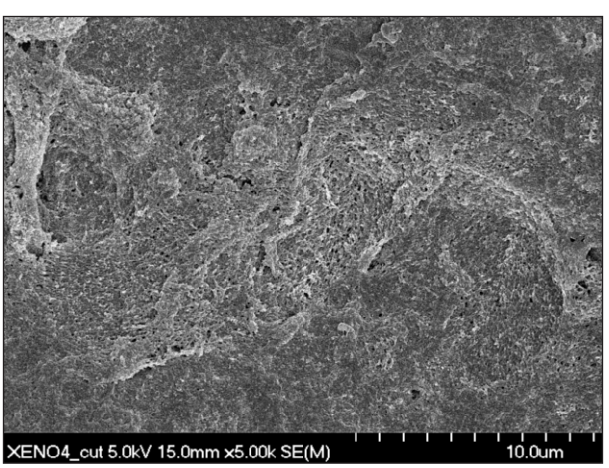


Figure 4E: *Representative morphology formed with XE on ground enamel. XE resulted in generalized intra-prismatic etching with areas displaying mild inter-prismatic etching (5000x).*

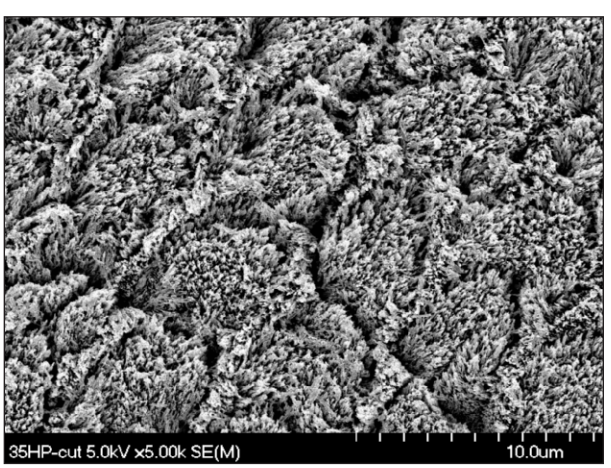


Figure 4F: *Representative morphology formed with SB on ground enamel showing the classical enamel etching pattern created by phosphoric acid (5000x).*

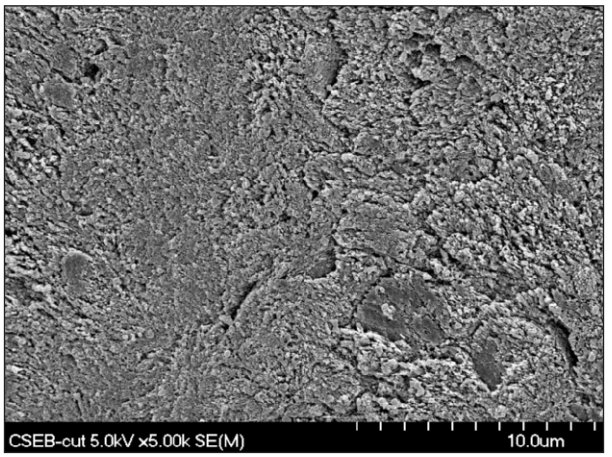


Figure 4G: *Representative morphology formed with SE on ground enamel showing areas of etched enamel intermingled with areas of featureless enamel. However, the etching pattern was better defined than that of S3, GB and iB (5000x).*

fied in three categories according to their acidity: mild, moderate and aggressive.[8] In the study, AP is considered an aggressive self-etch adhesive (pH=0.9-1.0, Table 1), while the other all-in-one adhesives are mild or moderate (pH>1.5, Table 1). Although some of the manufacturers recommend a separate etch of unground enamel, in a clinical setting, the adhesive is extended past the boundaries of the preparation, forming composite flashes. Bonding to this uninstrumented surface may be crucial to resisting marginal staining. The manufacturers of AP and iB do not recommend enamel pre-etching. The manufacturers of S3 and SE recommend phosphoric acid etching of unground enamel, while the manufacturer of GB recommends a separate acid step even for ground enamel, if additional enamel bond strength is required. One might argue that additional enamel bond strength is always required to prevent marginal gaps and leakage. For XE, the respective manufacturer recommends beveling the enamel margins for all procedures, which may be difficult to achieve in the cervical margin of Class II preparations.

A recent SEM study reported that the etching pattern on Adper Prompt, the bottle version of Adper Prompt L-Pop, is similar to that of phosphoric acid.[15] This finding partially corroborates the authors of the current study's enamel morphological results, as AP resulted in an etching pattern more defined than that of other self-etch adhesives, although not as deep as that formed by phosphoric acid. The enamel bond strengths of AP are usually higher than those of other self-etch adhesives,[16] which may have to do with the relatively low pH of AP. In the current study, AP hybridized dentin to an average depth more than 2 µm, which is in the range of that provided by some etch&rinse adhesives. In spite of this thick hybrid zone, a few areas of debonding were still observed for AP. AP has resulted in a wide variation in dentin bond strengths, which may be a result of variations in the application method.[17] When the predecessor of AP, Prompt L-Pop, was applied strictly per the manufacturer's directions, dry spots remained on the dentin surface. Application of the adhesive in multiple layers resulted in higher bond strengths and a better infiltrated hybrid layer.[18-19] For the current version, the manufacturer recommends rubbing the adhesive continuously for 15 seconds, followed by the application of a second coat. This extra layer may result in better impregnation of the monomers into the hybrid layer.

GB and iB did not result in a defined etching pattern on unground enamel; whereas on ground enamel, both resulted in islands of superficially dissolved enamel within areas without evidence of enamel dissolution. This was somewhat predictable, as other authors have reported that iB and GB result in severe enamel microleakage and debonding following thermal stresses and may be unable to penetrate through smear layers.[19-21] Very low bond strengths of iB to enamel and dentin have been reported by other authors when the adhesive is applied per the manufacturer's directions.[11,22] The behavior of iB and GB may also be a result of the presence of 4-META, which does not bond very strongly to hydroxyapatite.[23] The inadequate enamel etching pattern of GB and iB in the current study may be a result of their relatively high pH. The inability of iB to etch enamel[24] may be the source of massive marginal failure in a recent clinical study.[21] At one-year, only 7.4% of the restorations were rated *alfa* for marginal adaptation and 3.7% for marginal staining.[21]

The interfacial gaps and very thin dentin hybrid layer containing a residual smear layer observed for GB, iB and XE under the FESEM may suggest that these materials do not result in a magnitude of dentin bond strengths that are able to withstand polymerization stresses. One recent study reported that iB was not able to withstand polymerization shrinkage stresses nor thermocycling when applied to one-surface occlusal preparations, resulting in a high percentage of pre-testing debonds.[11] Several mechanisms may account for this poor performance as compared with other adhesives. The magnitude of dentin bond strengths, and therefore the quality of resin-dentin bonds, depend on the degree of infiltration of the resin monomers into the collagen pre-treated with an acidic conditioner or with phosphoric acid.[24-25] The application of an additional layer of adhesive might have sealed the non-polymerized oxygen layer and allowed it to cure.[18] A thicker adhesive layer might also have improved the stress distribution due to an increased deformability of the adhesive layer.[26]

Degradation of the dentin bonding interface is caused by the availability of exposed collagen fibrils at

the base of the hybrid layer[27] or by hydrolytic degradation of resin components in the hybrid layer.[27-29] Water can also plasticize the resin matrix, which decreases the mechanical properties of the polymer.[30] There may be other factors responsible for the inadequate penetration of the acetone-based iB and GB into enamel and dentin. Porosities (or blisters) occur at the bonding interface, because most simplified all-in-one adhesives behave as semi-permeable membranes.[5,31-32] These interfacial defects are usually observed when interfaces are stained with heavy metals. The porosities may be a result of water accumulation either caused by an osmotic gradient or by monomer-solvent phase separation upon evaporation of the acetone.[33-34] The number and size of these blisters may also depend of the intensity of the air-drying step.[31]

The morphological evaluation of etched enamel showed that XE is slightly more aggressive to unground or ground enamel than either GB or iB. This feature was unexpected, as the pH of XE is relatively high (>pH=2.0, Table 1). The role of XE's penta-phosphate monomer in chemical interaction with hydroxyapatite-rich enamel is a possible explanation for this apparent discrepancy between acidity of the material and respective bond strengths obtained with the precursor Xeno III.[36] On dentin, XE resulted in interfacial gaps in spite of the abundant resin tags. These interfacial gaps, along with a low conversion rate,[36] may be responsible for XE's low dentin bond strengths compared to those of SE and AP.[37] Also, due to its high hydrophilicity and consequent higher water sorption, the elastic modulus of XE may decrease substantially after water sorption due to the plasticizing effect of water on this adhesive.[38] If contraction forces stress the interface, the bonding may fail, because the stresses cannot be transferred across the adhesive joint if the adhesive has been plasticized by water.

SB, an etch&rinse two-step adhesive, has been shown to result in high enamel and dentin bond strengths. In this study, the enamel etching pattern and penetration into the dentinal substrate reinforce the idea that etch&rinse adhesives are still the benchmark for other adhesives when it comes to laboratory performance.[2-3,16] However, concerns have been raised regarding the degree of dentin infiltration by Single Bond, the unfilled version of Adper Single Bond Plus.[39] There is also evidence that the hybrid layer of Single Bond undergoes degradation in the form of increased porosity up to one year,[40] which might lead to clinical degradation of the interface. Once the hybrid layer and the adhesive interface undergo degradation, one may raise the question: how relevant is the depth of adhesive permeation into the hybrid layer? Nonetheless and in spite of all the debate about the difficult penetration of the polyalkenoic-acid copolymer into the hybrid layer, clinical studies with the unfilled version of SB have demonstrated an excellent clinical behavior up to five years.[41-44] This excellent clinical behavior may be a result of the resistance to mechanical fatigue of SB.[45] One study, however, reported a retention rate at 18 months below ADA acceptance levels.[46]

The version of SB used in the current study contains nanofiller, according to the respective manufacturer. The TEM observations in the current study confirmed the presence of filled resin tags in all specimens, along with polyalkenoic-acid copolymer globules in the adhesive layer. The filler may reinforce the bonding interface and decrease the stresses associated with resin shrinkage. The inclusion of filler in the adhesive may slightly increase bond strengths, as the cohesive strength of the adhesive itself may play a role in the fracture resistance of dentin-adhesive interfaces.[47] Nonetheless, recent studies with filled vs unfilled resins have not found differences in bond strengths.[48-49]

One of the most relevant morphological findings in the current study was the lack of dentin interfacial gaps with either Clearfil adhesive. When compared to Adper Prompt L-Pop in a laboratory study,[37] S3 resulted in similar unground enamel bond strengths, in spite of a very different pH (Table 1). On ground enamel, the bond strengths of S3 are statistically higher than those of other self-etch adhesives.[50] This apparent paradox may be a result of the presence of 10-MDP, a molecule with chemical affinity for dental tissues.[23] The two hydroxyl groups in 10-MDP chelate to calcium.[7] As S3 only decalcifies enamel and dentin superficially, as observed in the FESEM and TEM images, the availability of calcium and the chemical affinity of 10-MDP for calcium may explain the relatively high bond strengths, sealed interfaces and clinical behavior of the material.[21]

The pH of S3 is less acidic than that of SE primer (2.4 vs 1.8, respectively, Table 1). The manufacturer claims that the difference between the two 10-MDP-based adhesives may lie in the "molecular dispersion technology" that keeps the homogeneity of S3 adhesive and prevents phase separation, which occurs with acetone-base "all-in-one" adhesives.[34] A recent study showed that S3 is more resistant to mechanical stress than its two-bottle predecessor,[52] which is surprising, taking into consideration the excellent retention rates (above 90%) of SE Bond in Class V clinical studies at two-to-three years.[53-54] This excellent clinical behavior of SE denotes a good resistance to clinical fatigue of 10-MDP-containing adhesives.

## CONCLUSIONS

The interfacial morphology created by the more aggressive all-in-one adhesive AP resembles that of etch&rinse adhesives. The newest all-in-one adhesives that do not require mixing do not infiltrate enamel and

dentin to the level observed for the etch&rinse two-step control. On the other hand, the depth of infiltration of the newest all-in-one adhesives is similar to that obtained for SE, the two-step self-etch control. These findings corroborate the idea that the depth of dentin hybridization is not a predictor for magnitude of dentin bond strengths, but depth of enamel demineralization may correlate well with enamel bond strengths. At the magnifications used in this study, some self-etch adhesives do not cause visible dissolution of enamel hydroxyapatite when applied to intact enamel. Further studies are needed to assess the long-term clinical behavior of the newest simplified adhesives.

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