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Phosphoric Acid-Etching Promotes Bond Strength and Formation of Acid-Base Resistant Zone on Enamel

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Clinical Relevance

The present study was undertaken to examine the effect of phosphoric acid (PA) etching on the bond strength and acid-base resistant zone (ABRZ) formation of a two-step self-etching adhesive to enamel. Taking both bond strength and ABRZ formation into consideration, we recommend bonding enamel by the means of directly applying Clearfil SE Bond adhesive after 35% PA etching.

SUMMARY

This study examined the effect of phosphoric acid (PA) etching on the bond strength and acid-base resistant zone (ABRZ) formation of a two-step self-etching adhesive (SEA) system to enamel. An etch-and-rinse adhesive (EAR) system Single Bond (SB) and a two-step SEA system Clearfil SE Bond (SE) were used. Human teeth were randomly divided into four groups according to different adhesive treatments: 1) SB; 2) SE; 3) 35% PA etching→SE primer→SE adhesive (PA/SEp+a); (4) 35% PA etching→SE adhesive (PA/SEa). Microshear bond strength to enamel was measured and then statistically analyzed using one-way analysis of variance and the Tukey honestly significant difference test. The failure mode was recorded and analyzed by χ^2 test. The etching pattern of the enamel surface was observed with scanning electron microscope (SEM). The

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bonded interface was exposed to a demineralizing solution (pH=4.5) for 4.5 hours and then 5% sodium hypochlorite with ultrasonication for 30 minutes. After argon-ion etching, the interfacial ultrastructure was observed using SEM. The microshear bond strength to enamel of the SE group was significantly lower ($p<0.05$) than that of the three PA-etched groups, although the latter three were not significantly different from one another. The ABRZ was detected in all the groups. In morphological observation, the ABRZ in the three PA-etched groups were obviously thicker compared with the SE group with an irregular wave-shaped edge.

INTRODUCTION

In recent years, bonding to tooth substrates with self-etching adhesive (SEA) systems has gained popularity. Because these systems contain specific acidic monomers to condition and prime tooth substrates simultaneously, separate etching and water-rinsing steps are eliminated, reducing the application procedures and technique sensitivity.[1] Furthermore, it has been shown that some functional monomers in SEA can chemically interact with the hydroxyapatite in the tooth demineralized layer within a clinically manageable time.[2–7] Hypothetically, this chemical interaction can improve the bonding performance and degradation resistance of the bonding interface.[8–10]

Nevertheless, unlike the predominant position of SEA in dentin bonding, bonding to enamel with SEA seems to be a controversial issue. The literature does not provide a straightforward answer whether SEA bonded to enamel can resist the mechanical and chemical challenges as well as the conventional etch-and-rinse (ERA) systems can. Some studies indicated that contemporary SEA could be used as a satisfactory alternative when bonding to enamel,[11–13] whereas other published work demonstrated that enamel bonding using SEA was inferior to that of ERA systems, probably related to the comparatively moderate etching capacity of most SEA systems.[14–17] They demineralized enamel mildly, resulting in shallow intercrystallite resin infiltration and a lack of interprismatic resin tag formation.[18–19]

Given that enamel bonding is believed to be primarily based on the micromechanical interlocking structure of adhesive resin into microporosities, it seems logical that the etching procedure should influence the bonding performance of an adhesive. The adjunctive use of phosphoric-acid (PA) etching is recommended to improve the bonding of some SEA systems by facilitating enamel dissolution.[20,21] It was repeatedly demonstrated that, with this method, the enamel bond strength significantly increased. In most of these studies,[20,21] the enamel surface was PA etched, rinsed, and then treated with the recommended procedures of commercial SEA systems. This procedure is beyond dispute for those single-step SEAs with a mild pH. However, questions arise for two-step SEA, which involves two application steps: the conditioning of tooth susbstrates with a self-etching primer, followed by the application of a bonding resin. It is still not clear, after enamel is PA-etched, whether a self-etching primer ought to be used and to what extent a self-etching primer contributes to the overall bond strength.

The acid-base resistant zone (ABRZ), a structural layer formed on the tooth bonding interface, has been confirmed by many studies.[22–27] Because this layer can resist acid and base challenges, it might play an important role in the prevention of secondary caries. In previous studies, the effects of various dental materials on the formation of dentin ABRZ were assessed. It has been shown that morphology of dentin ABRZ was highly adhesive-material dependent and that dentin ABRZ formed in SEA systems but not in ERA systems.[24,25,28] This phenomenon might be related to the aggressive etching capacity of PA, demineralizing dentin deeper than the infiltration of bonding resin. The incompletely sealed interface may facilitate acid penetration and give rise to demineralization below the hybrid layer. For enamel bonding substrate, we first reported[23] the formation of ABRZ with a two-step SEA system, Clearfil SE Bond, which contains 10-methacryloyloxydecyl dihydrogen phosphate (MDP) as the acidic functional monomer. However, it has not been investigated whether there is an ABRZ when enamel is etched with aggressive PA. Concerns emerge regarding the morphology of enamel ABRZ using the combination of PA and SEA.

Based on these considerations, the present study was undertaken to examine the effect of PA etching on the bond strength and ABRZ formation of a two-step SEA to enamel. The null hypotheses tested were: 1) PA etching would not affect the bonding performance of the two-step SEA to enamel; 2) PA etching would not influence the formation and morphology of ABRZ when enamel was treated with SEA.

Table 1: *Composition and Instructions of the Adhesive Systems According to the Manufacturers*

Materials	Manufacturer	Composition		Directions
Single Bond (etch and rinse)	3M-ESPE, St Paul, MN, USA	Etchant pH = 0.7	35% phosphoric acid	Apply etchant for 15 s; water rinse and air dry; apply two coats of adhesive; blow gently for 5 s; light cure 10 s.
		Adhesive	Bis-GMA, HEMA, polyalkenoic acid copolymer, water, ethanol, dimethacrylates	
Clearfil SE Bond (self-etch)	Kuraray Medical, Tokyo, Japan	Primer pH = 20	MDP, water, HEMA, hydrophilic dimethacrylate, camphorquinone, N,N-diethanol p-toluidine	Apply primer and leave for 20 s; moderate air dry for 10 s; apply bond and leave for 10 s; gently air blow for 10 s; light cure 10 s.
		Adhesive	MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, camphorquinone, N,N-diethanol p-toluidine, silanated colloidal silica	

Abbreviations: Bis-GMA(bisphenol-A glycidyldimethacrylate), *HEMA(*hydroxyethyl methylacrylate*), MDP (*10-methacryloxydecyl dihydrogen phosphate*).*

MATERIALS AND METHODS

Twenty noncarious human third molars and 30 human premolars were collected after the individuals' informed consent was obtained under a protocol approved by the Institutional Review Board of Tokyo Medical and Dental University. The teeth were stored in water at 4°C and used within one month after extraction.

Two adhesive systems were used in this study (Table 1): an ERA system Single Bond (3M ESPE, St Paul, MN, USA) and a two-step SEA system Clearfil SE Bond (Kuraray Medical, Tokyo, Japan). For PA etching, K-etchant gel (35% PA, Kuraray Medical) was used. A conventional tungsten-halogen light-curing unit (XL3000, 3M-ESPE) at 600 mW/cm^2 power density was used for the light curing of specimens.

Microshear Bond Test

A 2-mm-thick cuspal enamel slice was sectioned from each molar using a low-speed diamond saw (Buehler Isomet 1000, Buehler Ltd, Lake Bluff, IL, USA). Flat enamel surfaces were created by wet-grinding of the slices with 240-grit SiC paper (Buehler Ecomet V, Buehler Ltd). In order to standardize the smear layer, the ground enamel surfaces were wet-polished with 600-grit SiC paper for one minute.

The slices were randomly divided into four groups according to adhesive treatment as follows (Table 2): 1) Single Bond (SB); 2) Clearfil SE Bond (SE); 3) PA etching and rinsing, followed by Clearfil SE Bond primer and adhesive (PA/SEp+a); and 4) PA etching and rinsing, directly followed by Clearfil SE Bond adhesive (PA/SEa). Prior to the adhesive resin polymerization, a tygon tube (R-3603, Norton Performance Plastic Co, Cleveland, OH, USA) with an internal diameter of 0.8 mm and a height of 0.5 mm was placed on the bonded area. After the adhesive was light cured, a hybrid resin composite Clearfil AP-X (shade A3, Kuraray Medical) was injected into the tube and polymerized for 40 seconds. All bonded specimens were stored in distilled water at 37°C for 24 hours before testing.

After the storage period, the tygon tube was removed to reveal a composite cylinder with a cross-sectional area of 0.5 ± 0.02 mm^2. The specimens were examined under a stereomicroscope (Olympus, Tokyo, Japan) at 20× magnification to exclude specimens with any detectable interfacial defects. Each specimen was attached to a jig (Bencor-Multi-T, Danville Engineering Co, San Ramon, CA, USA) using a cyanoacrylate adhesive (Zapit, Dental Ventures of America, Corona, CA, USA) and placed in a universal testing machine (EZ-Test-500N, Shimadzu, Kyoto, Japan). A shear force was applied to each specimen using a thin wire loop at a crosshead speed of 1.0 mm/min until failure occurred.

The mean bond strengths were calculated then statistically analyzed using one-way analysis of variance (ANOVA) and the Tukey honestly significant difference test at the significance level of $\alpha = 0.05$ (n=10). The mode of failure was determined light-microscopically at 20× magnification using a

Table 2: *Application Procedures of the Groups Tested*

Groups	Code	Application Procedures
Single Bond	SB	As recommended by manufacturer
Clearfil SE Bond	SE	As recommended by manufacturer
PA etching/Clearfil SE Bond primer/Clearfil SE Bond adhesive	PA/SEp+a	1 Etch enamel with 35% PA for 15 s, water rinse, and air dry;
		2 Apply Clearfil SE Bond primer and leave for 20 s, moderate air dry for 10 s;
		3 Apply Clearfil SE Bond adhesive and leave for 10 s, gently air blow for 10 s, light cure for 10 s.
PA etching/Clearfil SE Bond adhesive	PA/SEa	1 Etch enamel with 35% PA for 15 s, water rinse and air dry;
		2 Apply Clearfil SE Bond adhesive and leave for 10 s, gently air blow for 10 s, light cure for 10 s.

stereomicroscope. Failure modes were categorized as three types: (A) adhesive failure; (C) cohesive failure; and (AC) mixed failure. The distribution of failure mode was analyzed by Pearson χ^2 test at a significance level of $\alpha = 0.05$.

Scanning Electron Microscope Observation of the Enamel Surface After Etching

Buccal enamel surfaces of six premolars were wet-polished with 600-grit SiC paper until a flat surface was reached. They were randomly divided into three groups, conditioned with the following procedures, respectively: 1) 35% PA for 15 seconds, rinsing; 2) Clearfil SE Bond primer for 20 seconds; (3) 35% PA for 15 seconds, rinsing, followed by Clearfil SE Bond primer for 20 seconds. For the latter two groups, the treated surface was immediately rinsed with an ascending series of ethanol (30%, 50%, 70%, and 95%) for one minute each and further cleaned ultrasonically in absolute acetone for one minute in order to completely dissolve the primer and dehydrate the specimens for scanning electron microscope (SEM) observation. After air drying, samples were coated with gold for SEM observation of the surface texture and etching pattern.

Preparation and Observation of the ABRZ

Sample preparation and observation for enamel ABRZ were performed according to our previous study.[23] Crowns of premolars were sectioned mesiodistally. Crowns with buccal surfaces facing out were embedded in epoxy resin (Epoxicure Resin, Buehler). After 24 hours, they were wet-polished with 600-grit SiC paper until flat midcoronal buccal enamel surfaces were exposed. Twenty-four of the prepared enamel discs were randomly divided into four groups. For each group, the enamel surfaces were treated according to the procedures mentioned previously (Table 2). A flowable composite Metafil Flow (shade A3, Sun Medical, Moriyama, Japan) was then applied on the top and cured for 20 seconds.

After storage in distilled water at 37°C for 24 hours, each specimen was sectioned perpendicular to the bonding interface into two halves and embedded in the epoxy resin overnight. They were wet-polished with SiC paper to 1200-grit to standardize the surface, and then subjected to an acid-base challenge to create artificial secondary caries, according to the conditions of our previous study.[23] Super Bond C&B (Sun Medical) was applied to the treated surface without acid etching, to prevent wear of the interface. Then the specimens were cut perpendicular to the interface, polished with diamond pastes and argon-ion etched in order. Following gold-sputter coating, the interface was observed using SEM (JSM- 5310LV, JEOL, Tokyo, Japan).

RESULTS

Microshear Bond Strength

The microshear bond strength values and failure modes are shown in Table 3. The SE group showed significantly lower bond strength compared with the other three groups (one-way ANOVA, $p<0.05$). For groups SB, PA/SEp+a, and PA/SEa, the bond

Table 3: *Bond Strength and Failure Mode of the Four Groups (n = 10)*

Group	Microshear Bond Strength (MPa)	Failure Mode[a]		
		A	C	AC
SB	59.61 (4.50) b[b]	0	6	4
SE	43.08 (3.73) a	2	2	6
PA/SEp+a	60.13 (6.06) b	2	4	4
PA/SEa	57.74 (5.21) b	0	5	5

Abbreviations: A, adhesive failure; C, cohesive failure in enamel or composite; AC, mixed failure.

[a] There was no significant difference in the distribution of failure modes (χ^2 test, $p>0.05$).

[b] Groups having similar letters are not significantly different (one-way ANOVA).

strength showed no significant difference ($p>0.05$). The distribution of failure mode among groups was not significantly different by the Pearson χ^2 test ($p>0.05$).

SEM Observation of Enamel Etching Pattern

Enamel etching patterns are shown in Figures 1–3. With PA etching (Figure 1), the enamel prism cores were preferentially attacked, with simultaneous conservation of the marginal area. The spatial repetition of these regular patterns created a multitude of deep pits, forming a honeycomb structure. With SE primer treatment (Figure 2), the surface exhibited a very shallow and nonuniform etching pattern. The enamel prism core was slightly dissolved. Unetched patches and polishing scratches could be clearly seen on the surface. When the enamel surface was etched with PA, followed by SE primer treatment, preferential dissolution of prism boundaries instead of the prism core was observed (Figure 3).

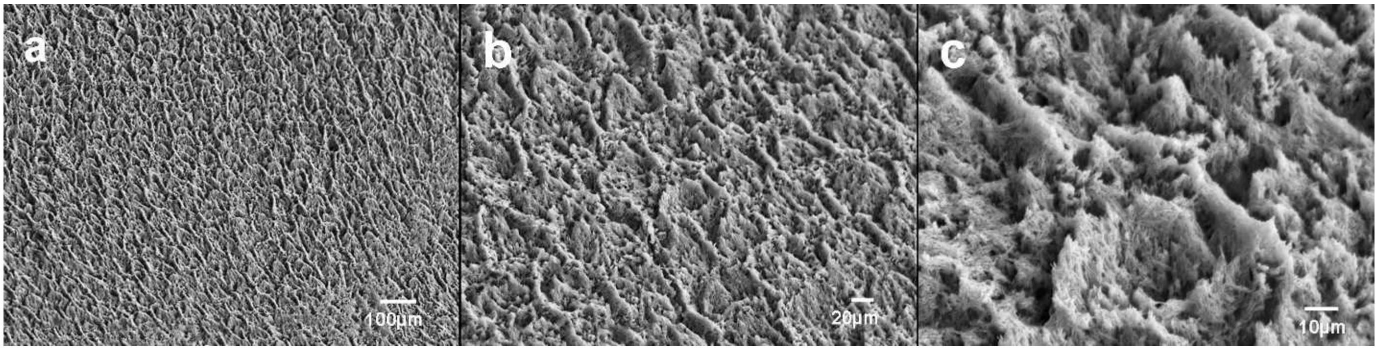

Figure 1. *SEM observation of the enamel etching pattern after 35% PA treatment. (a) Spatial repetition of regular patterns created a multitude of deep pits, forming a honeycomb structure. (b) Enamel prism cores were preferentially attacked, with simultaneous conservation of the marginal area. (c) Widely spaced crystallites were observed, forming a highly porous surface.*

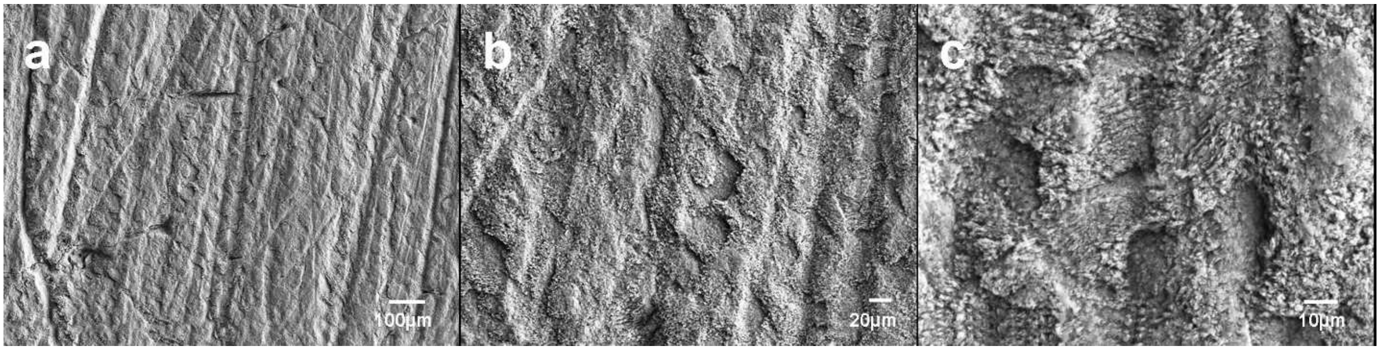

Figure 2. *SEM observation of the enamel etching pattern after SE primer treatment. (a) The surface exhibited a shallow and nonuniform etching pattern. Unetched patches and polishing scratches could be seen clearly. (b) Predominant dissolution of enamel prism core was observed. (c) Densely arranged crystallites were identified on the surface.*

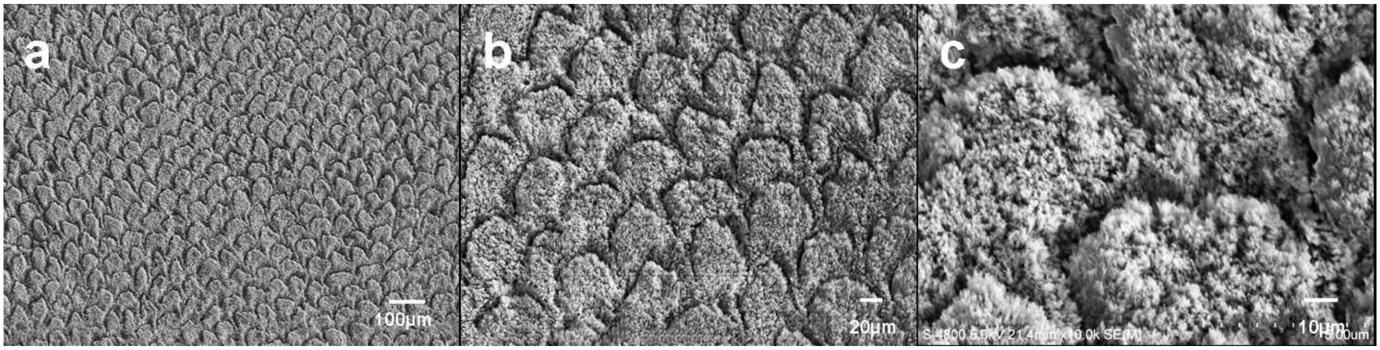

Figure 3. *SEM observation of the enamel etching pattern of PA/SEa group. (a) Spatial repetition of regular patterns created a multitude of deep grooves on the entire surface, forming a fish-scale structure. (b) Preferential dissolution of prism boundaries was observed. (c) Crystallites could be identified on the surface.*

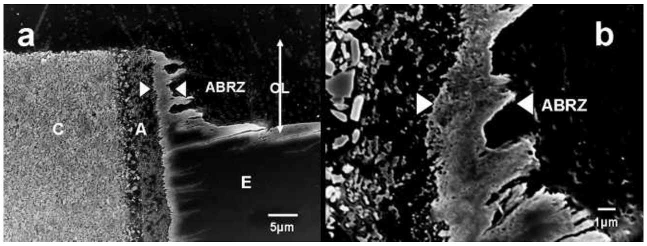

Figure 4. *Morphology of enamel-adhesive interface after acid-base challenge in SB group. OL, outer lesion; C, resin composite; A, adhesive layer; E, enamel; ABRZ, acid-base resistant zone. The ABRZ was pointed between triangles. The edge of the ABRZ toward the top of the OL showed an irregular and wavelike shape. Thickness of the zone was approximately 3 μm.*

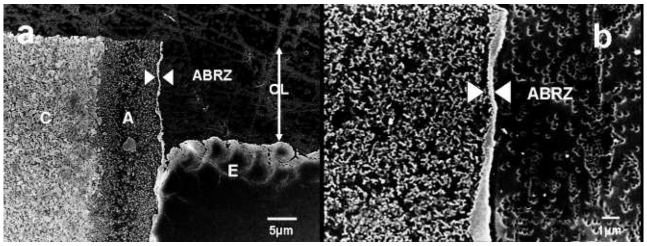

Figure 5. *Morphology of the enamel-adhesive interface after acid-base challenge in the SE group. The ABRZ was pointed between triangles. The edge of the ABRZ was relatively regular. Thickness of the zone was about 0.5 μm.*

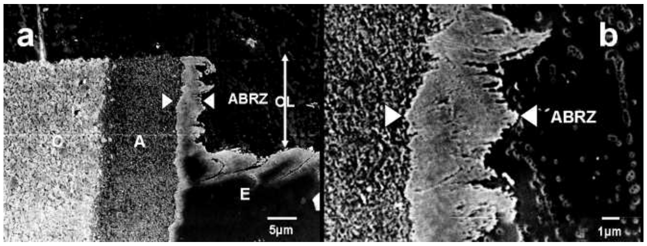

Figure 6. *Morphology of enamel-adhesive interface after acid-base challenge in PA/SEp+a group. The ABRZ was pointed between triangles. The edge of the ABRZ showed an irregular and wavelike shape, similar to that of SB. However, thickness of the zone was increased approximately 5 μm.*

SEM Observation of ABRZ Morphology

SEM observation of the enamel-adhesive interface after the acid-base challenge is shown in Figures 4–7. The top surface of the composite and adhesive was not remodeled in all specimens. An outer lesion (OL), defined as the mineral loss due to the acid-base challenge, ranged from 10 to 15 μm; and an electron-dense zone along the bonding interface beneath the adhesive resin at the OL front, coincident with enamel ABRZ, was observed in all groups.

The ABRZ in the SE group was the thinnest (Figure 5), about 0.5 μm thick. The edge of this zone toward the overlying OL was relatively regular. Under a higher magnification (60,000×), the zone consisted of densely arranged grainlike crystals (Figure 8b). For the other three groups (SB, PA/SEp+a, and PA/SEa) (Figures 4, 6, and 7), the edge of the ABRZ toward the top of the OL showed an irregular and wavelike shape. The thickness of the ABRZ was obviously increased and reached approximately 3, 5, and 5 μm at the wave trough for SB, PA/SEp+a, and PA/SEa, respectively. Examination under 30,000× magnification revealed that the ABRZ consisted of densely arranged crystals (Figure 8a).

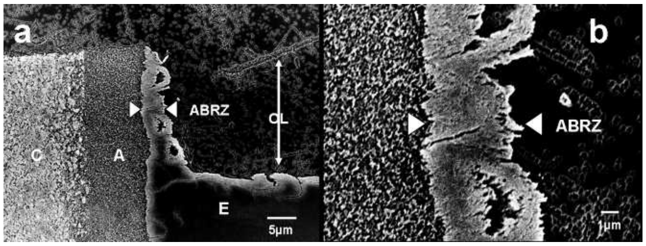

Figure 7. *Morphology of the enamel-adhesive interface after acid-base challenge in the PA/SEa group. The ABRZ was pointed between triangles. ABRZ morphology was similar to that of the PA/SEp+a group.*

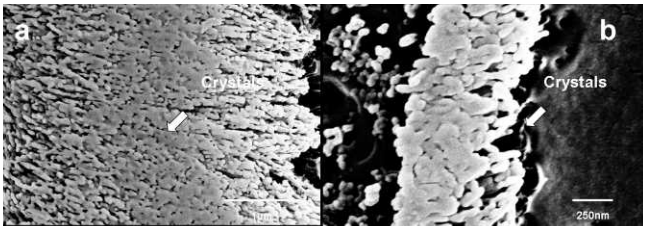

Figure 8. *Ultrastructure of the ABRZ under high magnification. (a) SB group (30,000×); (b) SE group (60,000×). It revealed that the ABRZ consisted of densely arranged crystals.*

DISCUSSION

PA etching is recommended by some manufacturers to improve the bonding performance of SEA systems to enamel. However, after enamel is etched with PA, the resistance of the bonding interface to mechanical and chemical challenges has not been fully elucidated. From this perspective, microshear bond strength and ABRZ formation of the enamel bonding interface were investigated in the present study. Two different adhesive systems were selected: an ERA system SB and an SEA system SE. SB is a two-step ERA system that requires pre-etching with 35% PA and water rinsing to remove the smear layer and demineralize the underlying substrates. SE is a two-step self-etching primer adhesive system. An acidic functional monomer, MDP, is present in the primer, which demineralizes the smear layer and underlying substrates simultaneously. Moreover, with this system, the formation of the ABRZ along the enamel bonding interface had been confirmed in our previous research.

In this study, the SE group showed lower bond strength than the PA-etched groups, which is in agreement with several other studies.[14,15] The most plausible explanation might be related to the different etching capacities of SE primer and 35% PA. Compared with the mild etching pattern of the SE primer, PA etching promotes the porosity of the demineralized enamel, resulting in increased resin interlocking and micromechanical retention. In spite of the weak correlation between enamel etching depth and bond strength found in the literature, the particular etching pattern achieved by 35% PA was believed to play an important role in enamel bonding. Erickson and others[14] showed that when enamel is etched with PA, the following adhesive resin penetration is fairly extensive, the structure is quite three dimensional, and the transition from resin to sound enamel is extended over a few microns. Such an interface may be more resistant to crack propagation than the relatively planar interface obtained with SEA systems. However, it should also be noted that only crosscut enamel was evaluated in the current study. Shimada and others reported[29] that, although the bond strength of SB to crosscut enamel was significantly higher than that of SE, its bonding performance was drastically influenced by the enamel prism orientation. On the contrary, the enamel bonding of SE is stable for any orientation of enamel. They suggested that PA etching was too aggressive for the parallel-cut enamel. Therefore, the results in this study may not be extrapolated to an experimental setup with a different enamel prism orientation.

Because PA etching could increase the bond strength of SEA to enamel, some researchers[21] recommended extending a two-step SEA into a three-step adhesive. However, as far as this study was concerned, after enamel was etched with PA (groups PA/SEp+a and PA/SEa), whether a SE primer was applied or not, neither the bond strength nor the ABRZ morphology were affected. The primary functions of primer in two-step SEA systems include two aspects.[6] One is to condition the surface of bonding substrates, creating spaces for resin penetration; the other is to prime the demineralized layer, promoting the infiltration of hydrophobic monomers into tooth tissue. Etching with PA could substitute for the former function of self-etching primer. Because enamel contains a very small amount of water and organics, the hydrophobic resin monomers could deeply and completely infiltrate into the demineralized layer without primer application. In addition, it was suggested that the bonding agent alone may have better mechanical properties compared with the mixture of the water-containing primer and the bonding agent, which may affect the durability of the bond.[30] However, whether eliminating the step of a self-etching primer on etched enamel is effective for the other two-step SEA systems should be further investigated.

The dentin ABRZ was confirmed with SEA systems only. In previous studies, [24,25,28] when dentin was treated with SB, no ABRZ could be distinguished. Researchers presumed that it might be related to the aggressive etching capacity of PA in SB. After PA etching and rinsing, the dentin surface gets completely demineralized to a certain depth. However, due to the hydrophilic property of bonding resin and the existence of water in dentin, the bottom of the demineralized dentin could not be completely infiltrated by hydrophobic resin, creating a weak collagenous band at the base of the hybrid layer. During the acid-base challenge, the weak area is vulnerable to acid-base attack, promoting the demineralization of dentin below the hybrid layer. On the contrary, in the present study, when enamel was treated by PA (groups SB, PA/SEp+a, and PA/SEa), the ABRZ could be clearly detected beneath the bonding interface. As is known, enamel has a higher mineral content with a matrix structure different from a dentin collagen network. Even after etching with a strong acid like PA, what remains is not a collagen matrix but rather a mineral framework. Hydroxyapatite (HAp) crystals are distributed all over the demineralized layer. These crystals either may be directly wrapped and protected by polymerized resin components or may chemically interact with specific functional monomers of the adhesive systems, producing insoluble calcium salts. As a result, a layer resistant against an acid-base attack forms on the PA-etched enamel bonding interface.

In the SE group, the ABRZ was the thinnest, and the edge of the ABRZ toward OL showed a relatively regular shape. This may be explained by the mild etching pattern produced by the SE primer. As shown in SEM micrographs, the SE primer etched enamel moderately, leaving shallow prismatic depressions and some intact unetched areas. When the bonding resin was applied to the poorly etched surface, monomers penetrated superficially. On the other hand, when enamel was PA etched, the thickness of the ABRZ was obviously increased, and its edge showed a wavelike shape. SEM examination confirmed that PA produced an aggressive etching pattern. The resin monomers could penetrate deeper into the demineralized layer, resulting in thicker ABRZ formation.

In the three PA-etched groups (SB, PA/SEp+a, and PA/SEa), the ABRZ of group SB was the thinnest. As confirmed by our former work,[23] acidic functional monomers in adhesive systems may play an important role in the formation and morphology of the ABRZ. The functional monomer contained in SE is MDP, which has been found to chemically interact with HAp intensively and stably, forming MDP-calcium salt with a low solubility. For the SB group, polyalkenoic acid copolymer was used. The relatively larger molecular size may prevent the deep penetration of resin monomers into a demineralized layer, partially contributing to the thinner ABRZ morphology. Furthermore, the chemical bonding capacity of polyalkenoic acid copolymer with HAp has been shown.[31] The carboxyl groups of the polyalkenoic acid can replace phosphate ions of the substrate and make ionic bonds with calcium ions of HAp. This mechanism is similar to 4-MET, for which the chemical interaction capacity with HAp has proved to be inferior to that of the MDP.[3,9] Therefore, it is reasonable to assume that the crystals in the SB group are less protected and the resulting polyalkenoic acid copolymer-calcium salts are prone to faster dissolution during the acid-base challenge. Further chemical analysis is necessary to prove these speculations.

CONCLUSION

In the present study, the null hypotheses were both rejected. With 35% PA etching, the bond strength of SEA system SE to enamel was significantly increased, comparable with the EAR system SB. Also, the formation of the ABRZ on enamel was promoted. Taking both bond strength and ABRZ formation into consideration, we recommend bonding enamel by the means of directly applying SE adhesive after 35% PA etching. Further studies are necessary to evaluate the effect of PA etching on one-step SEA systems to enamel.

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Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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