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Scanning electron microscopy and energy-dispersive X-ray analysis of self-etching adhesive systems to ground and unground enamel

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Abstract The morphological analysis of the ground and unground enamel was treated with three different self-etching adhesive systems. Ultrastructural features were observed by using the field emission scanning electron microscope (FESEM) in combination with Energy Dispersive X-Ray Spectroscopy (EDS) analysis. Thirty extracted human molars were used for this study. Teeth were divided into two groups. In the first group unground enamel was etched with either Clearfil SE Bond (Kuraray-Japan), G Bond (GC-Japan) or Tri S Bond (Kuraray-Japan) according to the manufactures instructions. In the second group ground enamel was treated as above. In addition 24 ungrounded and grounded enamel specimens were etched and bonded with the three self-etching adhesives and restored with composite resin (Clearfil ST-Kuraray). Then they were cross-sectioned and interfacial analysis was done with the combination of EDS analysis. Etching patterns of the enamel varied according to the self-etching adhesive. Clearfil SE Bond produced micro-irregular etching pattern creating crater like area in ground enamel while other two produced mild etching pattern. All three adhesives produced incomplete etching on unground enamel. Interfacial studies showed demineralization for the bonding agent penetration and the formation of hybrid layer. The self-etching adhesives produced different specific SEM morphologies on unground and ground enamel.

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Introduction

Recently many adhesive systems such as self-etching systems have been developed and are available in the market. Two-step self-etching primers have been further simplified into one step all-in-one adhesives that etch, prime and bond simultaneously [1,2]. Unlike bonding to the dentin, the application of the self-etch adhesives to enamel has been a controversial issue, particularly when mild self-etch adhesives are used on unground enamel [3]. Kanemura et al. recommended adjunctive use of phosphoric acid-etching when bonding to enamel [4]. Pashley and Tay reported that the efficacy of self-etching primers on unground enamel does not depend upon their etching aggressiveness [5, 6].

Perdigao et al. showed that there was no correlation between the less well defined enamel etching patterns produced by a self-etching primer with its shear bond strength to ground enamel [7]. In contrast, Kanemura et al. reported that the shallow etching patterns produced by self-etching systems may account to their low bond strengths to intact, unground enamel [4].

The self-etching adhesives are documented to be producing different etching effects on intact, unground enamel [7]. Authors previously reported the use of self-etching adhesives in normal ground enamel verses ground fluorosed enamel [8]. The penetration of bonding agent in enamel which was considered as a hard structure was explained. Similar results were also reported by using phosphoric acid [9] and self-etching systems [10, 11] in some previous studies. Although well-defined enamel etching patterns and resin tag formation are not prerequisites for achieving strong initial enamel bonds [11], they are associated with the stability and improved survival rate of enamel bonds created [12]. So it may be important to understand the etching characteristics of recently developed self-etching adhesives. The objective of this study was to examine the scanning electron microscopy of three self-etching adhesives to ground and intact enamel using a field emission scanning electron microscope (FESEM) with the combination of Energy Dispersive X-Ray Spectroscopy (EDS) analysis.

Materials and methods

Thirty extracted human molars were used for this study. The extracted teeth were cleaned and stored in distilled water, separately with the details of the date of extraction and the age of the patient in a refrigerator at 4 °C. All the teeth used had been extracted 9 months prior to the study and belonged to subjects aged between 40 and 50 years. The informed consent was obtained from the subjects.

Roots were cut and separated just below the cemento-enamel junction, then approximately 2 mm thick crown segments were cut parallel to the long axis of the tooth using a slowly rotating diamond blade (Isomet, Buehler, Lake Bluff, IL, USA) under water lavage. Teeth were randomly divided into two groups.

Orientation pits of 0.5 mm in depth were prepared in the samples of the first group with air-rotor bur and the superficial enamel was removed initially using a superfine diamond bur (SF# 145, Shofu Inc., Kyoto Japan) on a high speed hand piece followed by grinding with #600-grit SiC paper under water coolant. Teeth were then ultrasonically cleaned in distilled water for 5 min. No mechanical enamel preparations were done for the second group. Randomly selected three specimens from each group were subjected to one of the following treatments.

Sub group I: Treated with Clearfil SE Bond Primer for 20 s and gently air-dried.
Sub group II: Treated with Tri S Bond for 20 s and dried with high air pressure.
Sub group III: Treated with G Bond for 10 s and thoroughly air-dried.

All the sub groups were then treated with 60 s acetone rinse under ultrasonic movement for the removal of any crystals and other residues from the primer. The specimens were dried using a moderate vacuum for 24 h. Finally, the surface was coated with Platinum/Palladium (4 nm) and observed under a field emission-scanning electron microscope, FE-SEM (3 kv, 10 mA; Hitachi, S-4500, Tokyo, Japan).

The interfacial structures between the enamel and the resin were also observed. Twenty four bonded enamel resin interfaces involving three sub-groups (each sub group four specimens) were prepared in the same manner as above three sub groups. The specimens were treated according to the manufacture's recommendations in self-etching adhesive systems. Then they were filled with composite resin (Clearfil ST, Shade A2, Kuraray-Japan) and light cured for 40 s using a light-curing unit (Optilux 500, Demetron, Danbury-USA). Then they were embedded in epoxy resin (Buehler, Lake Bluff, IL, USA) and cut in cross-section, then ground and polished down to 0.25 μm using an abrasive diamond paste. They were etched with an argon beam: 1 kV, 1.5 mA, 40 s (Elonix, Hachioji-Japan). The specimens were placed overnight under a moderate vacuum, gold sputter-coated with Platinum–Carbon and then observed under a FE-SEM. EDS line scan analysis was performed at 125 cycles per second for the elements, phosphorus (P) and calcium (Ca) at the bonding enamel interface. (Horiba-Japan). For each specimen three line scan analysis was performed. In each line scan analysis graph distance between the two constant levels of Ca and P was measured manually. Then the depth of demineralization in each bonding approach was statistically analyzed by using one-way ANOVA at 95% confidence interval followed by Tukey's post-hoc comparison test.

Results

FESEM observations of the etched enamel specimens showed different morphological features after the application of the three different self-etching systems. However all self-etching adhesive systems were able to completely remove the smear layer from ground enamel.

The Clearfil SE primer showed a preferential etching of the enamel crystals in ground (Fig. 1A) and unground enamel (Fig. 2A). In ground enamel micro-irregular etch patterns exposing individual enamel crystals were clearly observed in the whole surface (Fig. 1A). Nearly 1 μm of "crater like" crystal spaces were observed (Fig. 1A). In unground enamel etched with Clearfil SE primer produced a milder etching pattern exposing individual enamel crystals

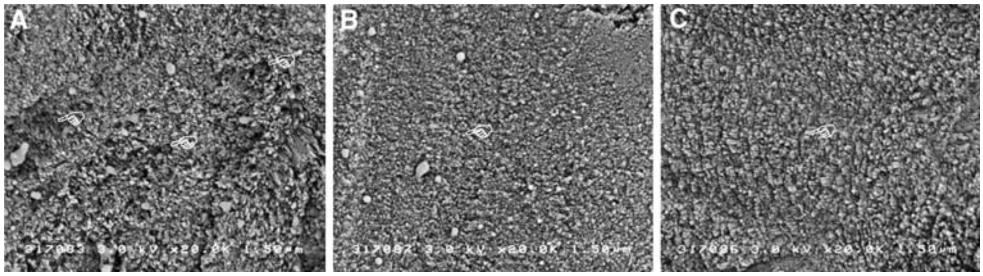

Fig. 1 Application of the self-etching adhesives on ground enamel. (**A**) FESEM micrograph showing the preferential demineralization of enamel crystals. With the application of Clearfil SE Bond. Microporosity was present along the entire surface. (**B**) FESEM micrograph showing the preferential demineralization of enamel crystals. With the application of Tri S Bond. Mild microporosity was present along the entire surface. (**C**) FESEM micrograph showing the preferential demineralization of enamel crystals. With the application of G Bond. Mild microporosity was present along the entire surface

(Fig. 2A). Tri S Bond (Fig. 2B) and G Bond (Fig. 2C) produced a similar demineralization patterns to Clearfil SE primer in ground enamel. But more densely packed enamel crystals were observed in Tri S bond and G Bond than with the Clearfil SE Bond and the crater like areas observed with Clearfil SE primer was not observed in Tri S Bond and G Bond. In unground enamel Tri S Bond could only partially dissolve the enamel crystals of the surface layer (Fig. 2B). Smear debris and undissolved bonding agent was observed in most of the surface area (Fig. 2B, C).

Figure 3A shows the interface SEM micrograph of the Clearfil SE Bond. Interface studies showed a 2 μm argon etched zone in ground (Fig. 3A) and unground (Fig. 3B) enamel. The argon etched zone observed in ground enamel (Fig. 3A) was more clear and demarcated than in the unground enamel (Fig. 3B), but no clear differences were observed in the argon etched zones of the three sub groups of self-etching systems. Figure 4 shows the EDS line scan analysis of the Clearfil SE Bond enamel interface. Clearfil SE Bond showed 2.4 μm of demineralization into the ground enamel (Fig. 4A) and 1.12 μm into the unground enamel (Fig. 4B).

EDS analysis results of the mean depths of demineralization are summarized in Table 2. From post hoc comparisons it was reveled that there were no statistically significant differences of the mean depths between self-etching systems. Statistically significant differences were observed in ground and unground enamel in each self-etching system.

Discussion

The ability of dental adhesive resins to penetrate the subsurface microporosity created in etched ground and unground enamel has been reported by many investigators [5, 6, 8, 9]. In this study we observed the surface etching characteristics and the depth of demineralization at the interface with currently used three self-etching adhesives to ground and unground enamel. Self-etching adhesive system involves either a two or one step application procedure. It no longer needs ‘‘etch & rinse’’ step, which lessens the clinical application time. Unlike bonding to the sound dentine, the application of self-etching systems on the enamel has been a controversial issue. Some investi-

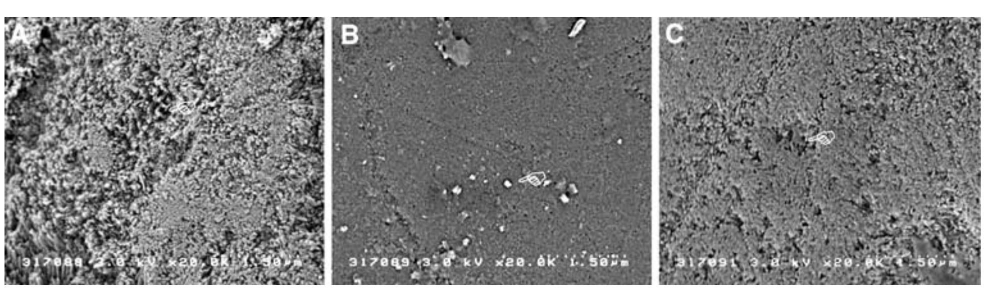

Fig. 2 Application of self-etching adhesives on unground enamel. (**A**) FESEM micrograph showing the incomplete demineralization of surface enamel crystals in unground enamel with the application of Clearfil SE Bond. (**B**) FESEM micrograph showing the incomplete demineralization of surface enamel crystals in unground enamel with the application of Tri S Bond. Residual bonding agent and the smear layer debris are present on the surface. (**C**) FESEM micrograph showing the incomplete demineralization of surface enamel crystals in unground enamel with the application of G Bond

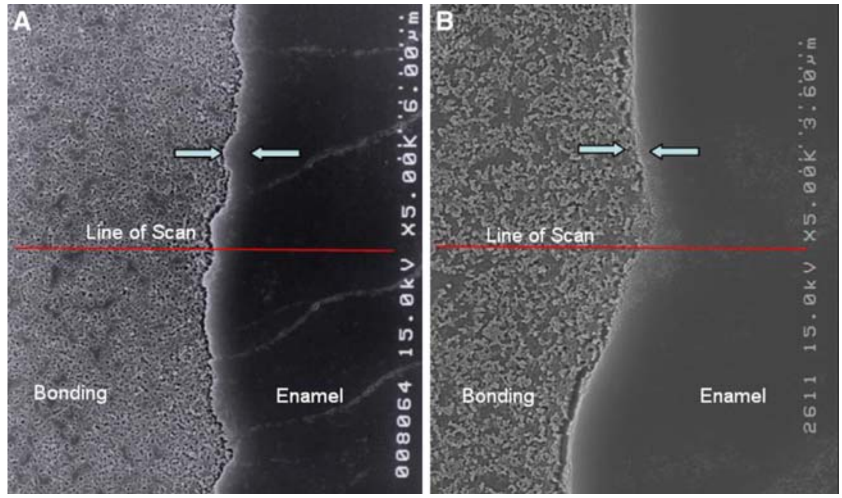

Fig. 3 FESEM images of the adhesive interface. (**A**) FESEM image of Clearfil SE Bond to ground enamel. Depth of the Enamel-resin hybrid like layer is 2 μm (*arrows*). (**B**) SEM image of Clearfil SE Bond to unground enamel. Depth of the Enamel-resin hybrid like layer is 1 μm (*arrows*)

gators have reported that the bonding of the self-etching adhesives to ground enamel was inferior to the systems utilizing phosphoric acid as a separate conditioner [13, 14]. On the other hand, other studies showed that the self-etching systems might be used as a satisfactory alternative to phosphoric acid conditioning of the ground enamel [15, 16].

Clearfil SE Bond is a two step self-etching adhesive which is consisted of water based self-etching primer and a bonding system. Tri S Bond (water and alcohol based) and G Bond (acetone based) are single step self-etching adhesive systems. Clearfil SE primer has the pH of 1.9, Tri S Bond has a pH of 2.7 and G Bond has a pH value of 2. Although the pH values of these three systems were around 2, SEM micrographs revealed that they have produced their own specific etching pattern in unground and ground enamel.

When the etching patterns were observed by the SEM micrographs all three self-etching systems showed a preferential demineralization of the enamel crystals in ground enamel. Tri S Bond and G Bond produced more densely packed etching patterns compared to Clearfil SE Bond. Crater like areas observed in Clearfil SE Bond were not observed in Tri S Bond and G Bond. In unground enamel all three self-etching adhesives could not dissolve the outer boundaries of the individual enamel crystals in the whole surface. The presence of a surface aprismatic layer in outer enamel has been well documented [17]. This aprismatic layer was reported to be less conductive to bonding [18]. Acid conditioning and grinding of this layer to expose the underlying prismatic enamel prior to acid-etching has been recommended [19].

The penetration of adhesive resins into porous enamel creates a new structure that is partly enamel and partly resin. Nakabayashi and Pashley [20] consider this structure to be the hybrid layer. Thus, hybrid layers do not need to contain collagen fibrils. Ca and P were used as EDS markers to understand the depth of demineralization and bonding agent penetration in to the enamel to form the hybridized enamel layer. As shown in Table 1 each self-etching system has its unique composition and in this study we used the depth of demineralization as a bonding agent penetration marker as the acidic monomers may have penetrated through the demineralized enamel crystals [21].

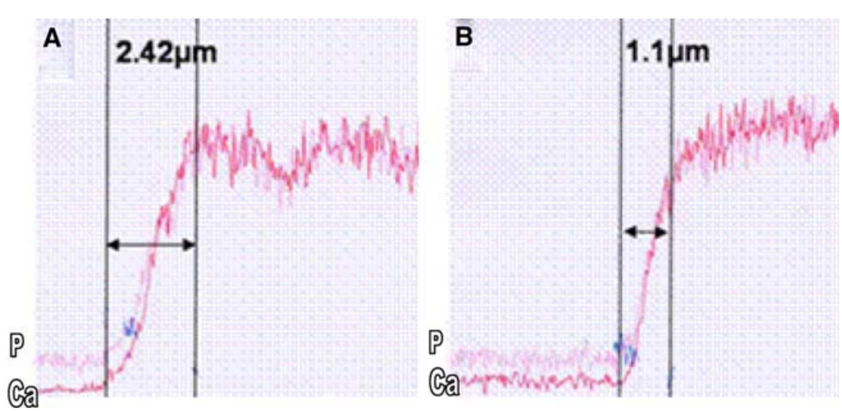

Fig. 4 EDS analysis on enamel bonding interface. (**A**) Ca and P analysis of bonding enamel interface of ground enamel treated with Clearfil SE Bond self-etching system. (**B**) Ca and P analysis of bonding enamel interface of unground enamel treated with Clearfil SE Bond self-etching system

Table 1 Materials used

Product	Components
Self-etching primer adhesive system	
Self-etching primer (SE Primer) (Lot # 011320) Bonding resin (SE Bond) (Lot # 011320)	MDP, HEMA, Hydrophilic dimethacrylates, Photo-initiator, Water MDP, Bis-GMA, HEMA, Photo-initiator, Silanated Silica, water
G Bond	
All in one adhesive resin	4-MET, UDMA, Silica, Photoinitiator, Acetone, Water, Phosphate esters
Tri S Bond	
All in one adhesive resin	10-MDP, Bis-GMA, HEMA, Hydrophobic dimethacrylate, di-Camphorquinone, Ethyli alcohol, Water, Silanated colloidal silica
Restorative material	
Clearfil ST-Shade A2 (Lot # 0026BA)	Silanated barium glass, Silica, Colloidal silica, Bis-GMA, TEGDMA, Photo initiator

Bis-GMA: Bisphenol A-diglycidylmethacrylate, HEMA: 2-hydroxyethyl methacrylate, MDP: 10-methacryloloxydecyl dihydrogen phosphate, TEGDMA: triethyleneglycol dimethacrylate. UDMA: urethane dimethacrylate

Although three self-etching systems formed almost similar hybrid layer thicknesses (Table 2) when this was compared with the surface etching characteristics it can be speculated that the amount of bonding agent that has been facilitated to penetrate in to enamel may differ from each other. From this study a statistically significant difference of the depth of demineralization was observed in unground and ground enamel. Comparison of the interface SEM photographs of unground and ground enamel (Fig. 3A, B) reveals a clearer and well demarcated hybrid layer in ground enamel. Inability to dissolve the aprismatic layer may be the reason for the above mentioned differences. This may be the reason for the lower bond strength produced by the self-etching system in intact enamel compared to ground enamel [22]. In addition incomplete etching of the unground enamel in self-etching adhesives may lead to poor adaptation [23].

Authors have previously reported that the bonding agent may penetrate around 4 μm deep in to the enamel when 37% phosphoric acid is used to etch the enamel [8]. Phosphoric acid etching facilitated the bonding agent to penetrate deeper than the self-etching adhesives. This may contribute to a strong enamel bond strength [24, 25]. In contrast, some investigators have reported no bond strength difference with phosphoric acid using system (total etch) and self-etching system [11].

Table 2 Average depth of demineralization to enamel (μm ± SD)

Self-etching system	Unground enamel	Ground enamel
Clearfil SE Bond	1.10 ± 0.11*	2.42 ± 0.10*
Tri S Bond	1.09 ± 0.12**	2.39 ± 0.12**
G Bond	1.07 ± 0.11***	2.40 ± 0.13***

n = 12 for each group

Same asterisk symbols show statistically significant differences

The bond strengths may not simply depend on their etching aggressiveness or the pH but may depend on its specific composition [5, 6]. Even though etching effects observed in self-etching systems were very mild, when the self-etching primers were used relatively high bond strength values were reported [11]. This may be attained particularly by an adhesive monomer, MDP which was confirmed to create a more stable salt with calcium in hydroxyapatite. Thus the adhesive monomer may be considered as a significant factor for bonding, especially when the enamel etching effect is very mild as observed in this study [26].

Although well-defined enamel patterns and deeper resin penetration are not prerequisites for achieving a strong initial enamel bond strength [7, 11] they have been associated with stability [27].

In this study the authors used horizontal sections and this may differ from other sections. Because of micro structural anisotropy, orientation and density of enamel prisms may vary according to the site. Shimada et al. reported the differences in bond strength with regional prism orientation [28].

Within the limits of this study, it may conclude that the three self-etching adhesives produced different specific SEM morphology in unground and ground enamel. From EDS analysis it can be conclude that the hybridization takes place in enamel-adhesive interface.

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