SHORT COMMUNICATION

Examination of enamel-adhesive interface with focused ion beam and scanning electron microscopy

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Introduction: The purpose of this study was to observe, with a scanning electron microscope, the interface between enamel and orthodontic adhesive after focused ion-beam milling. In addition, enamel etched with phosphoric acid was compared with enamel conditioned with self-etching primer. **Methods:** Four freshly extracted human premolars were collected and pumiced by using rubber cups with fluoride-free paste, washed, and dried. The enamel of 2 teeth was etched with 37% phosphoric acid for 30 seconds, washed, and dried; the enamel of the other 2 teeth was conditioned with self-etching primer for 5 seconds. Stainless steel brackets were bonded with Transbond XT adhesive (3M Unitek, Monrovia, Calif) according to the manufacturer's instructions. The specimens were milled by focused ion beam and observed under the scanning electron microscope. **Results:** The scanning electron micrographs showed that 37% phosphoric acid seemed to produce more enamel loss than the self-etching primer. Moreover, the enamel-adhesive interface was more irregular when the enamel was etched with 37% phosphoric acid. Finally, a gentler etch pattern of the self-etching primer on the enamel surface was observed, and this conditioner could be used clinically for minimal intervention in the orthodontic bonding procedure. **Conclusions:** Focused ion-beam milling to prepare samples allowed clear observation of the enamel-adhesive interface without artificial damage. (Am J Orthod Dentofacial Orthop 2007;131:646-50)

The procedure for bonding orthodontic brackets has become an interesting and challenging topic in orthodontics. The acid-etch technique for bonding orthodontic brackets reported in 1965 by Newman[1] was considered a significant development in orthodontics.[2] However, acid etching produces iatrogenic effects on the enamel surface, including the loss of enamel.[3,4]

To improve adhesion procedures, reduce enamel loss, prevent saliva contamination, and save chair time, self-etching primer (SEP) was introduced. SEPs combine the conditioning and priming agents into a single acidic primer solution.[5] Therefore, according to Cehreli et al,[6] current bonding systems can be divided into 2 categories: those with phosphoric acid etchant for enamel and adhesive resin, and those that include SEP, which combines etching and priming in a single step.

Maintaining a sound, unblemished enamel surface after debonding orthodontic brackets is a clinician's primary concern.[5] Moreover, the ideal would be minimal enamel loss at each stage of treatment,[4] and the least enamel loss occurred when SEP was used.[3]

The scanning electron microscope (SEM) is a useful device to observe the action of the conditioner agents on enamel surfaces. Nevertheless, additional sample preparation is required to observe the enamel-adhesive interface, but, unfortunately, the mechanical sectioning performed with a slow-speed diamond saw can produce microfractures at the level of the interface. On the other hand, the focused ion beam (FIB) system, commonly used as a cross-sectioning technique for failure analysis of semiconductor devices, has been applied to biologic tissues to expose their ultrastructure for examination.[7,8] Ion milling is routinely used in microscopy applications, and it was reported that FIB produced a clean cut without detectable damage beyond 10 nm.[7] FIB milling has several advantages in the preparation of specimens, including mineralized tissues such as dentin; this system is less time-consuming and allows for precise site specificity when making specimens. Moreover,

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Submitted, September 2006; revised and accepted, November 2006.
0889-5406/$32.00

doi:10.1016/j.ajodo.2006.11.017

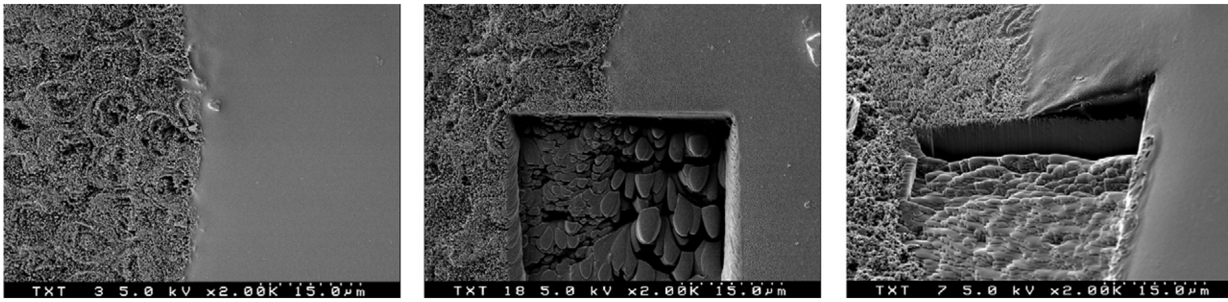

Fig 1. SEM images of specimen conditioned with 37% phosphoric acid. **A,** Junction between etched enamel and adhesive before FIB milling. Adhesive surface is smooth, but enamel surface appears etched; **B,** after FIB milling, and **C,** tilted image shows enamel-adhesive interface; penetration of conditioners can be observed (original magnification × 2000).

the use of FIB avoids mechanical sectioning, which could introduce artificial damage.[9]

In this study, we aimed to observe under SEM the interface between enamel and orthodontic adhesive after FIB milling. In addition, enamel surfaces etched with 37% phosphoric acid and those conditioned with SEP were compared.

MATERIAL AND METHODS

Four freshly extracted maxillary premolars were collected and stored in a solution of 0.1% (wt/vol) thymol. The criteria for tooth selection included intact buccal enamel, not subjected to pretreatment chemical agents such as hydrogen peroxide or enamel conditioner, no cracks from the extraction forceps, and no caries. The teeth were cleansed and pumiced by using rubber cups with fluoride-free paste (10 seconds), washed with water (30 seconds), and air dried. The enamel of 2 teeth was etched with 37% phosphoric acid for 30 seconds, washed with water (30 seconds), and dried to a chalky white appearance; the sealant (Transbond XT adhesive primer, 3M Unitek, Monrovia, Calif) was applied to the etched surfaces. The enamel of the other 2 teeth was conditioned with SEP for 5 seconds according to the manufacturer's instructions (Transbond Plus, 3M Unitek). After that, stainless steel premolar brackets (0.018 in, standard edgewise Dyna-Lock, 3M Unitek, Seefeld, Germany) were bonded to all teeth with Transbond XT and light cured for 30 seconds (Coltolux 4, Coltène/Whaledent, Cuyahoga Falls, Ohio).

The samples were stored in distilled water at room temperature for 7 days. The specimens were mounted in aluminum stubs, coated, milled with the FIB system (FB-2100, Hitachi, Tokyo, Japan), and observed under SEM (S-4500 and S-4700, Hitachi).

RESULTS

Figure 1 shows representative images of the junction between the etched enamel and the adhesive before and after FIB milling. The enamel-adhesive interface can be seen in detail when the image is tilted (Fig 1, *C*).

According to the electron micrographs of the enamel-adhesive interfaces (Fig 2), the 37% phosphoric acid seems to show greater destruction of the enamel than the SEP, and the enamel seemed porous when it was conditioned with this agent. Although both conditioners produced some enamel loss, the etch pattern of the SEP conditioner was more conservative. Figure 2, *C* and *D,* shows scanning electron micrographs at higher magnification; the penetration of the 37% phosphoric acid was considerably deeper than the SEP.

The back-scattered images in Figure 3 show an irregular interface line of the specimens conditioned with 37% phosphoric acid, in contrast to the more regular enamel-adhesive interface of the specimens treated with the SEP.

DISCUSSION

The application of phosphoric acid for bonding orthodontic brackets has the advantage of increasing bond strength, but it can cause more enamel loss.[10] The enamel loss during acid etching has been found to depend on the acid; the most commonly used is 37% phosphoric acid with etch times of 15 to 30 seconds per tooth. Wide variations in enamel surface loss from as little as 10 μm to 30 μm to as much as 170 μm have been reported.[3,10]

FIB milling allowed clear examination of the enamel-adhesive interface without any artifact such as those when the procedure is performed mechanically, including microfractures, separations, and debonding.

In this study, the enamel loss produced by the

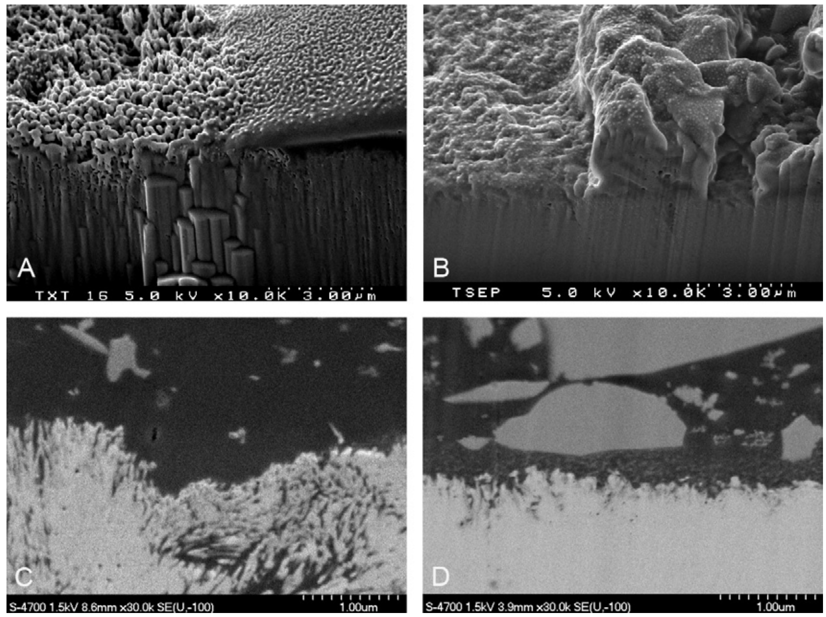

Fig 2. SEM images of enamel-adhesive interfaces. Areas milled by FIB are illustrated in lower portion of each image, and interfaces are clearly seen. **A,** Enamel conditioned with 37% phosphoric acid; **B,** enamel conditioned with SEP. Etch pattern with SEP appears to be more conservative (original magnification × 10,000.); **C** and **D,** SEM images of enamel-adhesive interfaces at higher magnification (original × 30,000); **C,** enamel conditioned with 37% phosphoric acid; irregular enamel surface is joined to adhesive primer; **D,** enamel conditioned with SEP, containing microfillers, which are visible next to enamel surface. Macrofillers of adhesive paste are also visible.

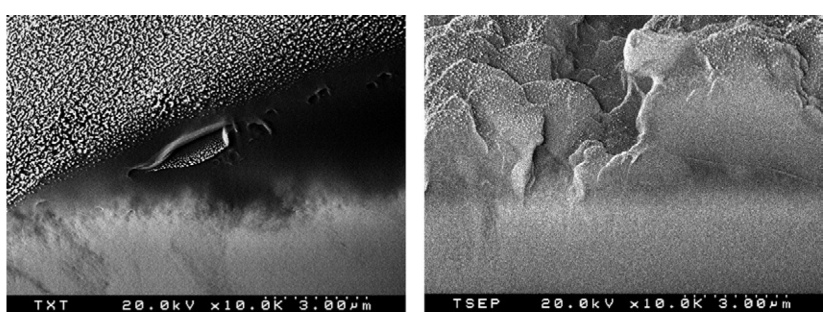

Fig 3. Back-scattered micrographs of enamel-adhesives interfaces. Enamel and adhesive are intimately united; contrast offered by back-scattering allows identification of both. **A,** Enamel conditioned with 37% phosphoric acid; **B,** enamel conditioned with SEP (original magnification × 10,000).

conditioners was observed with a SEM. The manufacturer's instructions recommend etching the enamel surface for 15 seconds, but the phosphoric acid was applied for 30 seconds; this could exaggerate the results. Nevertheless, 37% phosphoric acid seemed to produce greater enamel loss than SEP, and these findings agree with other studies.[3,11-14]

Bonding orthodontic brackets to tooth surfaces is a temporary procedure because the brackets are removed after active treatment.[14,15] According to the amount of enamel loss, clinicians should consider routinely using SEPs for minimal intervention and more conservative clinical treatment. Therefore, the use of phosphoric acid might be indicated when increased bond strength is necessary to achieve treatment goals.[16] Increased bond strength is essential when there is patient noncompliance to prevent excessive bond failure.[17] Moreover, deciduous teeth and fluorosed or hypocalcified enamel surfaces produce inconsistent results when conditioned conventionally, and new products have been introduced to achieve adequate bond strengths for atypical enamel surfaces.[18] However, enamel fracture on debonding can occur more often when bond strength increases,[4,19,20] and an ideal orthodontic adhesive should have adequate bond strength while maintaining unblemished enamel.[21] It must be sufficient to withstand functional forces but allow bracket debonding without causing damage to the enamel.[4]

Although low bond strength of SEPs has been reported,[6,22] some recent studies showed no significant differences of bond strengths between SEPs and conventional etching agents.[23-25] From a clinical standpoint, the use of SEPs can be desirable because they reduce clinical steps, save chair time, prevent saliva contamination, improve adhesive procedures, and reduce the risk of decalcification or white-spot formation.[26,27] In addition, SEPs have significantly lower adhesive remnant index scores,[24] and this could make it easier and faster for cleanup after debonding.[28] The SEP used in this study (Transbond Plus) has been shown to provide lower bond strength than Transbond XT, but it is clinically acceptable.[5,29] It seems to fulfill the requirements for clinical efficiency[30] and is potentially adequate for orthodontic bonding needs.[12] Moreover, this SEP can be safely used for bonding orthodontic brackets, because it provides higher survival rates than those of conventional acid etching, when the self-etch is applied for a longer time.[31] However, longer application time might cause greater enamel loss. A gentler etch pattern of SEP on the enamel surface was observed, and this conditioner could be used clinically for minimal intervention in the orthodontic bonding procedure.

In any case, too much enamel conditioner might unnecessarily etch some areas of enamel; to prevent this undesirable effect, the application of enamel conditioner should be only as large as necessary.

CONCLUSIONS

The application of FIB milling to prepare samples allowed clear observation of the enamel-adhesive interface without artificial damage such as that produced by mechanical sectioning.

We thank Kouichi Kurosawa, Akinari Morikawa, Toru Kumazawa, and Yukari Dan, Hitachi High-Technologies, Tokyo, Japan; their kind support in operating the FIB system made this study possible.

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