INTRODUCTION

Phosphoric acid etching of enamel was introduced by Buonocore\(^1\) in 1955, which has since led to dramatic changes in the practice of orthodontics\(^2,3\). By the 1970s, the bonding of orthodontic brackets became an accepted clinical technique\(^2,4\). Adhesives for brackets have been considered as one of the most significant developments in clinical orthodontics\(^5\). Nowadays, the use of acid-etching adhesive systems when attaching brackets to the enamel surface is widely accepted by most orthodontists as a routine technique\(^5-7\). However, acid etching produces iatrogenic effects on the enamel surface, including loss of enamel\(^6-9\). The amount of enamel loss due to acid etching is reportedly 5–60 µm, depending on the etching time and variations in and between teeth\(^10-12\).

In an effort to improve the adhesion procedure, reduce loss of enamel, prevent saliva contamination, and save chair-side time, self-etching adhesive systems have been introduced on the market\(^2,8,13\). Lately, such systems have generated wide interest, leading to their increasingly popular use\(^13,14,15\), chiefly because enamel rinsing is not required after self-etching primer application. In other words, both the procedural steps and the time required to bond orthodontic brackets to teeth are reduced\(^12\).

Scanning electronic microscopy (SEM) studies have shown that self-etching adhesive systems produce a more conservative etch pattern and smaller amount of demineralization on the enamel surface than acid-etch adhesive systems\(^6,16\). However, SEM cannot provide quantitative data about surface roughness and morphology. Atomic force microscopy (AFM) has been used to study the structural topography of enamel crystals in healthy and developmentally affected enamel\(^17-19\). Its main advantage is the ability to provide quantitative data at the nanometer level in all three dimensions with their respective images, without the need for sample preparation\(^18,20,21\).

Dentin surfaces demineralized by soft drinks\(^22\) and self-etching primer\(^23\), and enamel surfaces affected by different phosphoric acids used in clinical restorative procedures\(^18\) have been already examined by AFM. To our knowledge, this is the first time AFM has been used in a comparative study of four adhesive systems that are frequently used in orthodontic dentistry.

The purpose of this study was to evaluate the erosion effects of common clinically used adhesive systems on human enamel surfaces by AFM.

MATERIALS AND METHODS

Tooth specimens

Eighty extracted human maxillary interincisors were collected and stored in a 0.1% thymol solution in a cool dark place. They were extracted for dental treatment reasons. The criteria for tooth selection included labial enamel surface unaffected by any pretreatment chemical agents, no cracks incidental to extraction, no hypoplastic enamel, and no caries.

The selected teeth were randomly divided into five experimental groups of sixteen teeth each. Their roots were cut and the crowns were embedded in a chemically cured epoxy resin (EpoFix, Struers, Ballerup, Denmark).
Table 1 Materials and procedures employed in the study

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
<th>Batch No.</th>
<th>Composition</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kurasper F (KF)</td>
<td>Kuraray Medical, Tokyo, Japan</td>
<td>K-etchant: 00471A</td>
<td>37% Phosphoric acid</td>
<td>40 s apply, 20 s wash, Strongly air-dry</td>
</tr>
<tr>
<td>Beauty Ortho Bond (BO)</td>
<td>Shofu, Kyoto, Japan</td>
<td>Primer A: 091108</td>
<td>Water, Solvent</td>
<td>3 s apply, Gently air-dry</td>
</tr>
<tr>
<td>Orthophia LC (OL)</td>
<td>Tokuyama Dental, Tokyo, Japan</td>
<td>Primer: 0021</td>
<td>Phosphoric acid monomer, Solvent, Dyes</td>
<td>3 s apply, Medium air-dry</td>
</tr>
<tr>
<td>Transbond XT (TX)</td>
<td>3M Unitek, Monrovia, California, USA</td>
<td>Transbond Plus self-etching primer: 400844C</td>
<td>Methacrylated phosphoric acid esters, Amino benzoate, Camphorquinone</td>
<td>3 s apply, Gently air-dry</td>
</tr>
</tbody>
</table>

**Adhesive systems**

Table 1 shows the adhesive systems used in this study: a phosphoric acid-etching adhesive system (Kurasper F [KF], Kuraray Dental, Tokyo, Japan) and three self-etching adhesive systems (Beauty Ortho Bond [BO], Shofu, Kyoto, Japan; Orthophia LC [OL], Tokuyama Dental, Tokyo, Japan; and Transbond XT [TX], 3M Unitek, Monrovia, CA, USA).

**Pretreatment procedures**

In the KF group, the enamel surfaces were etched with 37% phosphoric acid for 40 s, rinsed with water, and air-dried. In the BO and OL groups, the enamel surfaces were pretreated with the primer, composed of phosphonous acid monomer, for 3 s and air-dried. The enamel surfaces in the TX group were pretreated with Transbond self-etching primer (3M Unitek, Monrovia, CA, USA), containing hydrophilic methacrylated phosphoric acid esters, for 5 s and air-dried. After the pretreatment in all the self-etching adhesive groups, the enamel surfaces were rinsed with acetone for 30 s and distilled water for 30 s, and gently air-dried. The pretreatment procedures were performed by the same operator according to the instructions of each manufacturer.

**Measurement of pH**

The pH in each group was measured under room temperature with a pH meter (Twin pH, Horiba, Kyoto, Japan).

**SEM evaluation**

One pretreated specimen per group was dehydrated and sputter-coated with silver for SEM evaluation (S-4800 scanning electron microscope, Hitachi, Ibaraki, Japan; ×5,000 magnification) to observe the effects of the pretreatments.

**AFM evaluation**

Fifteen pretreated specimens per group were selected for AFM evaluation (SPM-9600 atomic force microscope, Shimadzu, Kyoto, Japan). AFM were performed under atmospheric conditions at 25°C. Images were acquired at 512×512 pixels and a scan rate of 1 Hz in the direct mode. All topographic analyses of the enamel surfaces were conducted by using silicone cantilever tips (OMCL-AC160TS-C2, Olympus, Tokyo, Japan; spring contact and resonance frequency of 42 N/m and 300 kHz, respectively) with a scanner having a 20×20×2.5 µm (x, y, z) scale range.

The enamel surface roughness (ESR), absolute depth profile (ADP), and surface hardness were measured. The ESR was quantified in terms of Ra (roughness mean), representing the arithmetical mean of the absolute values of the scanned surface profile. The ADP was calculated in terms of Rz (mean peak-to-valley height), representing the average of the absolute values of the heights of the five highest profile peaks and the depths of the five deepest profile valleys within the sampling length on the scanned surface.

**Force-distance curve analysis**

For force-distance curve analysis, cantilever tips (OMCL-AC200TS-C2, Olympus, Tokyo, Japan) with a spring contact and resonance frequency of 9 N/m and 150 kHz, respectively, were used. An AFM force-distance curve is a plot of tip-sample interaction forces versus tip-sample distance. To obtain such a plot, the sample (or the tip) is ramped along the vertical axis (z-axis) and the cantilever deflection is determined. In the graph of force-distance curves (Fig. 1), the pull-in force is represented by a red line and the pull-out force...
is represented by a blue line; the former represents the force from the cantilever tip to the enamel surface and the latter represents the adhesive force exerted by the enamel surface. In addition, mechanical properties such as surface hardness can be derived from the slope of the red line. The surface hardness was calculated by dividing the tip-sample distance with the tip-sample interaction forces.

**Statistical analysis**

All data are expressed as the mean and standard error of the mean. Differences were evaluated by analysis of variance (ANOVA) with Scheffe’s $F$-test. Differences were considered significant at $p<0.05$.

**RESULTS**

**pH**

The pH values were 0.5, 1.8, 1.6, and 1.1 for the KF, BO, OL, and TX groups, respectively. All the self-etching adhesive groups showed a mild etching effect on intact enamel. On the other hand, the KF group showed the strongest etching effect on intact enamel, as expected from the low pH value.

**SEM findings**

Figure 2 shows scanning electron micrographs of the untreated and pretreated enamel surfaces. The untreated enamel surfaces showed a smear layer with some scratch lines (Fig. 2(a)). However, on all the pretreated enamel surfaces, the smear layer was absent. The KF group showed a slight change with shallow depressions compared with the control group (Fig. 2(b)). In the BO and OL groups (Fig. 2(c) and 2(d)), enamel prisms and micro-irregularities of hydroxyapatite crystals were less prominent compared with TX. The TX group had a completely different etch pattern compared with the BO and OL groups: interprismatic enamel seemed to be selectively decalcified and enamel prisms

![Fig. 1 Typical force curve characterization.](image)

- **Surface hardness of a sample is calculated from the straight part of the red line (i.e., between A and B).**

![Fig. 2 SEM photographs of the enamel surfaces.](image)

- **(a) control group, (b) KF group, (c) BO group, (d) OL group, and (e) TX group.** Original magnification ×5,000.
were not decalcified (Fig. 2(e)). Further, a less uniform etch pattern and less frequent shallow depressions were noted in the TX group than in the KF group.

**Three-dimensional topographic findings**

Figure 3 shows three-dimensional topographic images obtained by AFM. In the control (untreated) group, some scratch lines were observed on the ground surfaces (Fig. 3(a)). On the other hand, phosphoric acid etching produced severe etch patterns on the enamel surfaces in the KF group (Fig. 3(b)). Again, the BO and OL groups showed less prominent enamel prisms and micro-irregularities of hydroxyapatite crystals (Fig. 3(c) and 3(d)). Although the TX group showed a more conservative etch pattern than the KF group, it had a more severe etch pattern than the BO and OL groups (Fig. 3(e)).

**ESR and ADP**

Figure 4 depicts the mean (standard deviation) ESR and ADP values. Significant differences in the ESR and ADP were observed between the KF and the three self-etching adhesive groups ($p<0.05$). However, no significant difference was noted between the control and the self-etching adhesive groups excluding the TX group or between the BO and the OL groups.
To determine the surface hardness, AFM force curves of the enamel surfaces were recorded (Fig. 5). A gentle slope, as in the KF and control groups (Fig. 5(a) and 5(b)), means a relatively soft surface. On the other hand, a steep slope, as in the BO, OL, and TX groups (Fig. 5(c)–5(e)), means a relatively hard surface.

Figure 6 shows the surface hardness obtained from the force-distance curve analysis. The surface hardness of the KF group was lower than that of the control group and the lowest among all the samples tested. The TX group showed the highest surface hardness among the self-etching adhesive groups.

**DISCUSSION**

In restorative dentistry, adhesive materials are generally bonded to teeth permanently\(^{12}\). In orthodontics, however, attachments are bonded for a limited time only\(^{12}\). Therefore, sufficient bond strength, ease of debonding, and limited risk of permanent damage to the enamel surface are critical in orthodontics\(^{12}\). Alternatives to acid etching have been introduced in orthodontics to reduce enamel loss and simplify bonding procedures. These methods include the use of self-etching primers, which perform simultaneous etching and priming. However, their use to etch enamel is controversial. In this study, three self-etching adhesive systems were compared with the traditional phosphoric acid-etch technique by AFM.

Recently, more attention has been focused on the application of AFM in dental research to explore biomaterial surfaces\(^{26-28}\). AFM is useful because it has a higher resolution than SEM and three-dimensional images can be obtained\(^{26}\). However, the use of both SEM and AFM may improve the observation of morphological changes in enamel by achieving the advantages of each technique.

The effects of acids on human enamel depend on nanochemical and nanophysical interactions between the acids and enamel\(^{27,28}\). These interactions can lead to loss of hydroxyapatite crystals at the surface and an ensuing erosion process\(^{27}\). The SEM images showed different etch patterns between the phosphoric acid-etching and the self-etching adhesive systems: all the self-etching adhesive systems caused a more conservative action. These observations are in agreement with previous results\(^{6,7}\). Endo et al.\(^{29}\) reported that enamel treated with 35% phosphoric acid becomes very porous,
and numerous enamel crystallites and honeycomb structures are observable after phosphoric acid etching. The pretreatment with TX resulted in more frequent porosities on the enamel surfaces than the BO and OL pretreatments, although the enamel surfaces in all the self-etching adhesive groups were generally not very demineralized, as observed in previous studies\(^6,30\).

Loyola-Rodriguez et al.\(^{18}\) compared the ESR and ADP of enamel surfaces by AFM after using four different concentrations of phosphoric acid, and the values obtained by 37% phosphoric acid etching support our data very well. However, to the best of our knowledge, this is the first time that AFM has been applied in a comparative study of self-etching and phosphoric acid-etching adhesive systems used in orthodontics. All the self-etching adhesive systems produced a more conservative etch pattern than the phosphoric acid-etching adhesive system. BO and OL did not alter the fundamental configuration of enamel; however, TX produced different effects on the enamel structure. The AFM images of the TX-pretreated enamel surfaces showed enamel prism terminations as seen in the KF-pretreated enamel surfaces. This result can be explained by the fact that prisms do not end directly on the outer enamel surface. Acidic functional monomers could decalcify interprismatic enamel only but simultaneously improve resin monomer penetration into the porous enamel substrate\(^12,31\). However, the lower pH value of TX may be responsible for the difference in the erosion effects.

The main advantage of AFM over other technologies is that it provides quantitative data on roughness\(^15\). Recently, changes in enamel surfaces as a function of in situ surface treatment have been reported\(^16,32\). The present study showed changes in the morphology and ESR and ADP of the pretreated enamel surfaces. The erosion effects of different acidic functional monomers in relation to the bond strength of restorative materials in enamel should be tested in vitro to guarantee clinical success.

Surface hardness can be determined by AFM from force curves\(^33,34\). The elastic properties of bone and bone marrow\(^35\) and gelatin\(^36\) have been determined by AFM. Determination of surface hardness can be most easily performed by obtaining force curves while the tip is raster-scanned across the sample\(^37,38\). According to the surface hardness obtained from the force-distance curves, the KF group had softer enamel surfaces than the control group. Beyer et al.\(^{27}\) investigated the erosion effects of various acids on human enamel and showed that phosphoric acid induces surface hardness loss. The pH value alone, however, does not represent the erosive potential of acids on human enamel\(^27\). Several parameters have been identified as influencing the erosion process of acids\(^27\). Kitayama et al.\(^3\) showed that the self-etching adhesives did not significantly differ in bond strength before and after thermal cycling (TC), while the phosphoric acid-etching adhesives showed a significant reduction in bond strength after TC. Water diffusion by TC into the bonded interface between the adhesive and the tooth surface was found to reduce enamel hardness because of loss of surface calcium\(^9\). On the other hand, the surfaces of the self-etching adhesive groups were harder than those of the control group. Hannig et al.\(^{29}\) reported that individual hydroxyapatite crystallites on enamel surfaces are intimately encapsulated in self-etching adhesive systems at the nanometer level. Tanaka et al.\(^{30}\) also reported that reaction products with apatite are formed after the application of self-etching adhesive systems on enamel surfaces. Soft surfaces generally have poor durability\(^40\). In this study, the enamel surfaces treated by self-etching adhesive systems was harder than that by a phosphoric acid-etching adhesive, thus, enamel treated by self-etching may be more durable.

The majority of the orthodontic bonding materials available for clinical use have been widely tested in vitro. Kitayama et al.\(^2\) found that the shear bond strength of brackets bonded with self-etching adhesive systems (BO and TX) is lower than that of brackets bonded with 37% phosphoric acid-etching adhesive systems (KF). Zeppieri et al.\(^{41}\) also observed that TX pretreatment results in significantly lower bond strength than the acid-etch technique. On the other hand, Shinya et al.\(^{42}\) found that the shear bond strengths of self-etching adhesive systems (BO and TX) and 37% phosphoric acid-etching adhesive systems are not significantly different. Buyukyilmaz et al.\(^{43}\) found that TX provides significantly greater bond strength than etching with phosphoric acid. Moreover, Bishara et al.\(^{44}\) reported that a shear bond strength of 7 MPa is clinically acceptable for bonding to enamel surfaces in orthodontic treatment. Recently, Romano et al.\(^45\) performed a 6-month clinical assessment of the failure rate of brackets bonded with 37% phosphoric acid-etching adhesive and TX systems and reported no significant differences between these systems. As described above, the bond strength of brackets bonded by some self-etching adhesive systems were lower than that of bracket bonded with a phosphoric acid-etching adhesive system. The duration of the brackets bonding is limited and the data from the studies described above thus suggested that the self-etching adhesive systems evaluated were acceptable for routine clinical use.

It is important that the brackets used for orthodontic treatment be strongly bonded to avoid detachment; however, excessive bond strength can sometimes cause pain in the tooth or cracks in the enamel when the brackets are removed at the end of the treatment\(^45\). Three-dimensional topographic images obtained by AFM of the enamel surfaces (Fig. 3) reveal that the self-etching adhesive groups showed a more conservative action than the etching agent. It is known that self-etching adhesive groups that give enamel an appearance more similar to that of untreated enamel show less damage to enamel in the adhesive remnant index evaluation\(^6\). Thus, BO and OL might produce less damage to enamel than the other systems—in line with the principle and concept of minimal intervention.

In conclusion, the present in vitro study showed that the four commercially available adhesive systems had different erosion effects on enamel surfaces. BO and
OL caused significantly lower enamel loss and ensured higher surface hardness than KF, which may provide new approaches for formulation of acidic monomers in self-etching adhesive systems. At present, the self-etching technique produces weaker bond strength, although it is clinically acceptable comparison with the phosphoric acid-etching technique. However, it is advantageous because less time is needed to clean the teeth after debonding. The results suggest that the use of a self-etching primer for enamel conditioning could prevent decalcification produced by phosphoric acid etching.

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REFERENCES