Micro-shear bond strength and morphological analysis of a self-etching primer adhesive system to fluorosed enamel

Dinesh S. Weerasinghe, Toru Nikaido, Kamal A. Wettasinghe, Janaka B. Abayakoon, Junji Tagami

Graduate School, Cariology and Operative Dentistry, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8549, Japan
Department of Restorative Dentistry, Faculty of Dental Sciences, University of Peradeniya, Peradeniya, Sri Lanka
Masirah Air Base, Royal Air Force of Oman, P.O. Box 731, PC 111-Muscat, Oman

Received 27 July 2004; received in revised form 6 November 2004; accepted 8 November 2004

KEYWORDS
Micro-shear bond strength; Enamel; Fluorosis; Self-etching primer

Summary
Purpose. The aim of this study was to evaluate micro-shear bond strength and morphological analysis of a self-etching primer adhesive system to fluorosed enamel.

Materials and methods. Extracted human molars were classified according to the severity of fluorosis using Thylstrup and Fejerskov index into four groups (TFI: 0, normal; 1-3, mild fluorosis; 3-6, moderate fluorosis; 6-7, severe fluorosis) and divided into following two sub-groups. For the first sub-group, a self-etching primer adhesive system was applied to the ground enamel surfaces and the other sub-group was conditioned with 37% phosphoric acid for 30 s prior to application of the same adhesive system. Teeth were then restored with a resin composite, stored for 24 h in water and micro-shear bond strengths were measured. After shear testing, the fracture modes were observed under a laser scanning microscope. Morphological study of etching patterns and adhesive interface was done under a scanning electron microscope (SEM). The data were analyzed using two-way ANOVA and Sheffe test (p = 0.05).

Results. No statistically significant difference was there between the different degrees of fluorosis in each sub-group. However, significant difference in the bond strengths between phosphoric acid etching and self-etching was found in moderate and severe groups. The SEM observations showed at the resin-enamel interface, thick resin tag like extensions penetrated around 3.5 μm into the enamel etched with phosphoric acid, while self-etching primer created, 1 μm lamina like penetration.

Conclusion. Severity of fluorosis affects the micro-shear bond strength of a self-etching bonding system to fluorosed enamel.

© 2004 Elsevier Ltd. All rights reserved.
Introduction

Fluorosed enamel is characterized by an outer hypermineralized, acid-resistant layer and retention of more porous enamel in the areas of the subsurface hypomineralization. The pores are occupied by water as well as enamel secretory proteins which are retained due to the effect of the excessive fluoride levels on ameloblasts.1

In the milder forms of fluorosis enamel may be characterized clinically by white opaque lines corresponding to the position of perikymata, while in more severe cases the enamel contains distinct irregular, opaque or cloudy white areas.2 As a result of post-eruptive trauma, the formation of subsurface enamel defects may appear as single pits or the surface may be chipped away particularly from the incisal edges or cusp tips.3 Therefore, these patients require tooth colored restorations such as composite veneers.4

As the fluorosed teeth may need to be restored with composite materials, bond strength between composite materials and fluorosed enamel has been investigated.5–7 Some investigators5,8 have recommended extended enamel conditioning with phosphoric acid when bonding composite resin to the fluorosed enamel. In contrast Ng’ang’ et al. and Ateyah and Akpata reported no significant differences in the bond strengths between fluorosed and normal enamel at different etching times.6,9

Unlike bonding to sound dentine, application of self-etching systems on enamel has been a controversial issue. Hara et al. reported that bonding of self-etching adhesives to ground enamel was inferior when compared with systems utilizing phosphoric acid as a separate conditioner.10 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.11,12 Pashely et al. reported that the efficacy of self-etching primers on unground enamel does not depend upon their etching aggressiveness.13

This study investigated micro-shear bond strength of a self-etching primer adhesive system to different degrees of fluorosed enamel and the influence of phosphoric acid conditioning prior to the application of self-etching primer on fluorosed enamel. In addition to the micro-shear bond test, the failure modes were observed for by using a confocal laser scanning microscope (CLSM) and adhesive interfaces were examined morphologically by a scanning electron microscope (SEM).

Materials and methods

Eighty extracted molars from subjects living in endemic areas for fluorosis in Sri Lanka 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 4 months prior to the study and belonged to subjects aged in-between 20 and 40 years.

The teeth were classified into four groups according to severity of fluorosis using Thystrup and Fejerskov index14 (TFI 0-normal, 1-3 mild, 4-6 moderate, 7-8 severe). Classification according to TFI index was done by two investigators independently. Intra-examiner reproducibility gave a Cohen’s kappa statistic15 of 0.965. Teeth with TFI 9 were not available for the present study. Roots were cut and separated just below the cemento-enamel junction. Crown segments approximately 2 mm in thickness 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. Two coronal and cervical enamel regions were selected as substrate for the micro-shear bond test. The cervical region was 2 mm above the cemento-enamel junction. Bonding sites were taken as 1 mm each side to midline parallel to the long axis of the tooth.

In order to standardize the enamel reduction, 0.5 mm depth orientation pits were prepared with air-rotor bur and 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 prior to the bonding procedure to remove any remaining SiC dust particles. Teeth were divided into two subgroups and they received one of the following treatments (Details of the materials used are summarized in Table 1).

Sub-group I. Treated with Clearfil SE Bond Primer for 20 s and dried. Clearfil SE Bond Adhesive was applied; air-thinned and light-cured for 10 s.

Sub-group II. Etched with K-Etchant gel containing 37% phosphoric acid for 30 s, rinsed with water jet and spray for 30 s and then dried specimens were treated in a similar manner to sub-group I.

Prior to light-curing of the bonding resin, an iris was cut from micro-bore tygon tubing (R-3603, Norton Performance Plastic Co. Cleveland of USA) with an internal diameter of 0.8 mm and a height of 0.5 mm and mounted on enamel surface to restrict
the bonding area (Fig. 1). A resin composite (Clearfil ST; shade A2) was filled into the cylinder and a plastic matrix strip was placed over the resin composite and gently pressed flat and light-cured for 40 s using a light-curing unit (Optilux 500, Demetron, Danbury, USA). Because the tygon cylinder was bonded tightly to the tooth surface by the bonding resin simultaneously with the photo-curing of the bonding resin, no flash of resin composite extended on to the tooth beyond the base of the cylinder. In this manner, very small cylinders of resin, approximately 0.8 mm in diameter and 0.5 mm in height were bonded to the surface (Fig. 1(A)). The specimens were stored at room temperature (23°C) for 1 h prior to removing the tygon tubing, and then stored in water at 37°C for 24 h.

After the specimens had cooled to the room temperature, their shear bond strengths were measured by micro-shear testing. Before testing, all samples were checked under an optical microscope (magnification of ×30) for bonding defects. The samples showing formation of apparent interfacial gaps, air bubble inclusions or any other defects were excluded from the study and replaced with new specimens.

Fig. 1(B) shows the micro-shear bond apparatus. The tooth slice with the resin cylinders was adhered to the testing device (Bencor-Multi-T, Danville Engineering Co, San Ramon, CA 94583, USA) with a cyanoacrylate adhesive (Zapit, Dental Ventures of America, Corona, CA 92882 USA), which in turn was placed in a universal testing machine (EZ-test-500N, Shimadzu Co, Kyoto, Japan) for shear bond testing. A thin wire (diameter 0.2 mm) was looped around the resin composite cylinder, making contact through half its circumference and was gently held flush against the resin/enamel interface. A shear force was applied to each specimen at a cross-head speed of 1 mm/min until failure occurred. The resin enamel interface for test, the wire loop and the center of the load cell were aligned as straight as possible to ensure the desired orientation in the shear test force.

All de-bonded enamel surfaces after the shear bond test was examined under a CLSM (ILM 21 H/W. Laser Tec. Co. Yokohama, Japan) under ×20 and ×100 magnifications to identify defects.
the mode of failure. The failure modes were categorized into one of the five types.

- A. Adhesive failure in more than 95% of bonded area between enamel, hybrid like enamel layer or overlying adhesive resin.
- B. Cohesive failure in enamel more than 95% of bonded area.
- C. Cohesive failure in resin more than 95% of bonded area.
- D. Mixed failure, adhesive failure in more than 50% of bonded area.
- E. Mixed failure, cohesive failure in enamel more than 50% of bonded area.
- F. Mixed failure, cohesive failure in resin more than 50% of bonded area.

Six specimens (24 sites) were tested for each test group. The bond strengths were compared using enamel type as a fixed factor and bonding approach as a random factor using two-way ANOVA under general linear model. Post hoc comparison between different types of fluorosed enamel for each adhesive system was performed using Scheffe-test. The Pearson $\chi^2$ was used to explore the association between the failure modes and types of fluorosis for the each bonding approach. The failure modes were put into three groups before analysis; adhesive (A and D), cohesive in enamel (B and E) and cohesive in resin (C and F). SPSS for windows (SPSS, Inc., Chicago, IL) version 11 was used for the data analysis.

**Morphological study using SEM**

In addition 16 enamel slices belonging to all four groups of fluorosis were conditioned or primed in the same manner as employed in the shear bond test samples. The slices were also treated with 60 s acetone rinse under ultrasonic movement for removal of any crystals or other residues from the primer. The specimens were dried using a moderate vacuum for 24 h. Finally, the surface was sputter-coated with gold and observed under a field emission scanning electron microscope, FE-SEM (S4500; Hitachi, Tokyo, Japan).

The interfacial structures between the enamel and the resin were also observed in 16 enamel slices. Eight bonded enamel-resin interfaces involving four groups were checked in the same manner as employed in the shear bond tests, cut in cross-section and then ground and polished down to 0.25 $\mu$m using abrasive diamond paste. They were etched with an argon beam; 1 kV, 1.5 mA, 40 s (Elonix, Hachioji, Japan) and the specimens were placed overnight under moderate vacuum, gold sputter-coated and then examined using a FE-SEM.

### Results

**Micro-shear bond test**

Mean shear bond strength values and standard deviations in MPa are shown in Table 2. Two-way ANOVA indicated that no statistically significant difference was there between the different degrees of fluorosis and mean micro-shear bond strengths within each bonding approach ($F=1.348$, $p=0.248$). Furthermore, Scheffe post hoc comparison showed no statistically significant difference in the bond strengths between phosphoric acid etching and self-etching bonding approaches in normal ($p=1.000$) and mild fluorosed enamel ($p=0.319$). However, a significant difference in the bond strengths was found between the phosphoric acid etching and self-etching bonding approaches in moderate ($p=0.016$) and severe fluorosis groups ($p=0.01$).

Modes of failure following the micro-shear bond test are summarized in Table 3. $\chi^2$ analysis revealed no significant association in mode of failure with the SE Bond. ($\chi^2 = 6.796$, $p=0.340$). When the previous phosphoric acid etching was done there was a significant association in the mode of failure ($\chi^2 = 16.253$, $p=0.012$). The adhesive failure was the most prevalent type of failure except in moderate and severe fluorosed groups with the previous phosphoric acid etching.

<table>
<thead>
<tr>
<th>Category of fluorosis</th>
<th>SE primer</th>
<th>37% Phosphoric acid + SE primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>$28.8 \pm 7.2$</td>
<td>$29.3 \pm 4.8$</td>
</tr>
<tr>
<td>Mild</td>
<td>$25.7 \pm 3.7$</td>
<td>$31.5 \pm 7.0$</td>
</tr>
<tr>
<td>Moderate</td>
<td>$29.6 \pm 4.2^*$</td>
<td>$35.0 \pm 4.8^*$</td>
</tr>
<tr>
<td>Severe</td>
<td>$25.1 \pm 3.5^{**}$</td>
<td>$30.2 \pm 6.0^{**}$</td>
</tr>
</tbody>
</table>

$n=24$ for each group. Same asterisk symbols show statistically significant differences.
the self-etching primer are shown in Fig. 2. The self-etching primer showed less demineralization of enamel and produced, shallower and micro-irregular etch patterns (Fig. 2(A, B)). The application of phosphoric acid for 30 s completely removed the smear layer from both normal and fluorosed enamel producing irregular type III like etching pattern (Fig. 2(C, D)). SEM images of the adhesive interface between the enamel and the SE bond adhesive after argon etching are shown in (Figs. 3(A, C) and 4(B)). Both normal and fluorosed enamel showed a 2 μm deep etched zone (Figs. 3(A) and 4(A)) with SE priming. When the enamel was etched with phosphoric acid it produced a deep etched zone around 3.5 μm (Figs. 3(C) and 4(B)) and micro-mechanical interlocking with significant resin tag like formations was obvious (Fig. 4(C)) forming an enamel hybrid like layer of approximately 3 μm in depth (Figs. 3(D) and 4(C)). Resin tag like formations in severely fluorosed enamel, under magnification of ×30,000 is shown in Fig. 4(D).

### Discussion

Prevalence rates ranging from 29 to 57% have been reported for dental fluorosis in Sri Lanka. The classification of fluorosis in this study was done according to the modified Thylstrup and Fejerskov index, which is based on the clinical changes in fluorosed teeth. The advantages of this classification are that it is consistent with histopathological changes in the enamel and highly reproducible. To minimize the effect of tooth types, only human molars were used in our study. Additionally, the use of a specific type of tooth (molars) avoided problems that may be associated with the variation in F content between different tooth types. Knoll, Gweintt and Wolff reported statistically significant differences in the shear bond strength of anterior and posterior teeth. It was reported that there are regional bond strength variation in enamel. To standardize the regional bond strength variation, bond strengths were

<table>
<thead>
<tr>
<th>Enamel treatment</th>
<th>Type of enamel</th>
<th>Number</th>
<th>Failure mode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td><strong>SE bond</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>Mild</td>
<td>Normal</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>Moderate</td>
<td>Normal</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>Severe</td>
<td>Normal</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td><strong>K-Etchant + SE bond</strong></td>
<td>Normal</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>Mild</td>
<td>Normal</td>
<td>24</td>
<td>14</td>
</tr>
<tr>
<td>Moderate</td>
<td>Normal</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>Severe</td>
<td>Normal</td>
<td>24</td>
<td>3</td>
</tr>
</tbody>
</table>

A, Adhesive failure in more than 95% of bonded area between enamel, hybrid like enamel layer or overlying adhesive resin; B, cohesive failure in enamel more than 95% of bonded area; C, cohesive failure in resin more than 95% of bonded area; D, mixed failure, adhesive failure in more than 50% of bonded area; E, mixed failure, cohesive failure in enamel more than 50% of bonded area; F, mixed failure, cohesive failure in resin more than 50% of bonded area.

**Table 3** Mode of failure after micro-shear bond testing.
measured in two mid coronal and two cervical sites and the average obtained. In the present study, the outer 0.5 mm of enamel was ground away to flatten the enamel surface for shear testing. This removes the outer 200 μm of hypermineralized, acid-resistant enamel and it is also consistent with clinical practice when the outer 0.5 mm of labial enamel is removed during tooth preparation for composite veneers. However, bond strengths to unground enamel may be important as it may useful to the clinicians to place the resins without tooth preparation. Sample teeth were obtained from subjects in the age group of 20–40 years. Ateyah and Akapata reported that there are differences in bond strength below and above the age of 40 years.6

Measurement of bond strength, regardless of the technique chosen is a controversial topic in dental adhesion.20 In recent times researchers have preferred to use micro-tensile method and fracture mechanics to understand the properties of the adhesive interface of dentine.21 However, in the present study micro-shear bond strength testing was used to measure bond strength, as this study is focused on enamel which is more brittle in nature compared to dentine. In this method there is no necessity for preparation of the bonding surface of the specimens which will alter its surface. If there is any specimen preparation as making it to a dumbbell shape, it may damage the arrangement and contour of enamel prisms. Therefore, the advantage of this method is that there is no necessity to alter the bonding surface. Packhaa noted that there is considerable non-uniformity of stress throughout the joint, in conventional shear tests.22 In order to address this problem, micro-shear testing was used with an ultra small bonding area.23 However, bond strengths observed were higher than conventional shear bond strengths because of the smaller surface area.

Though there are controversies regarding the bonding performance of the self-etching primer bonding systems in normal unground enamel, there is sufficient evidence to indicate that these bonding systems provide bond strengths comparable to

![Figure 3](image1.png)

Figure 3  SEM images of adhesive interface. (A) SEM image of Clearfil SE Bond to normal enamel after argon etching. Etched zone approximately 2 μm in depth (arrows). (B) SEM image of Clearfil SE Bond to normal; adhesive interface after 10% phosphoric acid rinsing for 5 s. Enamel-resin hybrid like layer could be observed to 1 μm depth (arrows). (C) SEM image of Clearfil SE Bond to normal enamel; adhesive interface after argon etching (conditioned with 37% phosphoric acid for 30 s before application of SE-primer bonding system). Etched zone/hybrid like layer is approximately 3.5 μm in depth (arrows). (D) SEM image of Clearfil SE Bond to normal enamel; adhesive interface after 10% phosphoric acid etching for 5 s (conditioned with 37% phosphoric acid for 30 s before application of SE-primer bonding system). Etched zone/hybrid like layer is approximately 3 μm in depth (arrows).

![Figure 4](image2.png)

Figure 4  (A) SEM image of Clearfil SE Bond to moderate/severe fluorosed enamel; adhesive interface after 5% phosphoric acid rinsing for 5 s. Enamel-resin hybrid like layer could be observed to 1 μm depths (arrows). (B) SEM image of Clearfil SE Bond to moderate/severe fluorosed enamel; adhesive interface after argon etching (conditioned with 37% phosphoric acid for 30 s before application of SE-primer bonding system). Etched zone/hybrid like layer is approximately 3.5 μm in depth (arrows). (C) SEM image of Clearfil SE Bond to moderate/severe fluorosed enamel; adhesive interface after 10% phosphoric acid etching for 5 s (conditioned with 37% phosphoric acid for 30 s before application of SE-primer bonding system). Etched zone/hybrid like layer is approximately 3 μm in depth (arrows). (D) Bonding resin penetration (arrows) through enamel in ×30,000 magnification.
bonding systems that use phosphoric acid in ground/cut enamel.\textsuperscript{11,24} Self-etching adhesives systems use weak acidic monomers to condition the enamel/dentin substrate totally.\textsuperscript{25,26} In this study, K-etchent gel (37\% phosphoric acid) produced typical etching patterns consistent to phosphoric acid regardless of severity of fluorosis. Etching pattern observed by self-etching primer was shallower to K-etchent, which use MDP as the etching agent.

Pashley et al. reported that the efficacy of self-etching primers on unground enamel does not depend upon their etching aggressiveness.\textsuperscript{13} Al-Sugair and Akpata reported that depths of etch in 37\% phosphoric acid for mildly fluorosed enamel (TFI 1-3) were generally dependent on etching time and were not significantly different from the depth obtained for non-fluorosed specimens.\textsuperscript{27} Also, it was said that severe fluorosis (TFI 4) has no correlation with the etching time. Thirty seconds of etching showed a mean depth of etch around 8.02 \pm 0.38 \mu m which is closer to non-fluorosed enamel. In this study, K-etchant gel (37\% phosphoric acid) was used to etch the specimens in which the etching time was 30 s. Depth of etch in all the specimens treated with previous phosphoric etching were around 3.5 \mu m (Figs. 3(C) and 4(B)). SE-Primer could only produce around 2 \mu m of etching depth in all the groups which is consistent with a previous study.\textsuperscript{28}

The ability of dental adhesive resins to penetrate into the subsurface micro-porosities created in etched ground enamel was initially reported by Gwinnett and Matsui\textsuperscript{29} and Buonocore et al.\textsuperscript{30} The enhancement in surface area and energy that is associated with the altered topography\textsuperscript{31} as well as the underlying layer of hybrid like enamel tissue\textsuperscript{32} has been accredited with the strong resin-enamel bond. It is possible that more hydrophilic resins that are employed in contemporary dentine adhesive systems produce deeper penetration into the interprismatic substance of the etched enamel tissue and allow formation of optimal enamel hybrid like layer.\textsuperscript{33,34} As shown in the (Fig. 3(C)), it can be seen that bonding agent has penetrated extensively with previous phosphoric acid etching. However, phosphoric acid produced a deep etched zone nearly 3.5 \mu m in depth in which bonding agent was unable to penetrate into the whole depth (Fig. 3(D)).

Based on micro-shear bond strength results, the self-etching bonding system investigated is as effective as phosphoric acid etching when bonding is performed on normal and mild fluorosed enamel. The aggressiveness of the etchant may not have a relationship with the bond strength to enamel.\textsuperscript{13,35} But the self-etching bonding system is inferior to phosphoric acid etching when bonding is performed on moderate and severe fluorosed enamel. These results are consistent with the previous studies.\textsuperscript{5,7,8} When the fractured specimens were observed by confocal laser microscope, cohesive failure was the most prevalent mode of failure in moderate and severely fluorosed teeth with previous phosphoric acid etching. This should be taken into consideration as the self-etching primer adhesive system produces a predominant adhesive failure. Hence it will not damage the enamel surface even if the restoration fails. This may be important for patients with severe fluorosis to preserve the remaining tooth/enamel substance.

As the restoration ages, bond strength may deteriorate and further studies are needed to evaluate long term durability.

Acknowledgments

This work was supported by a grant for Centre of Excellence Program for Frontier Research on Molecular Destruction and Reconstruction of Tooth and Bone in Tokyo Medical and Dental University. The authors are grateful to Dr Shizuko Ichinose and Dr Sattabanasuk Vanthana for their kind assistance and invaluable suggestions.

References

\begin{enumerate}
\item Denbesten PK, Thariani H. Biological mechanisms of fluorosis and level and timing of systemic exposure to fluoride with respect to fluorosis. \textit{Journal of Dental Research} 1992;71: 1238.
\item Fejerskov O, Richards A, Denbesten P. \textit{Fluoride in dentistry}. 2nd ed. Copenhagen: Munksgaard; 1996 p. 112.
\item Ng'ang'a PM, Ogaard B, Cruz R, Chinidia ML, Aastrum E. Tensile strength of orthodontic brackets bonded directly to
\end{enumerate}


