Effectiveness of bonding resin-based composite to healthy and fluorotic enamel using total-etch and two self-etch adhesive systems

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The aim of this study was to evaluate the bond strength of three adhesive systems: Excite™, Adper Prompt L-Pop™ and AdheSE One™ to varying degrees of fluorotic enamel using micro-tensile bond strength (μTBS) tests. Human enamel was classified according to the Thylstrup and Fejerskov Index. The interface resin-enamel was observed using stereoscopic and electron microscopy. The Excite™, achieved the highest μTBS when bonded to healthy enamel and decreased as the degree of fluorosis increased (p<0.05). The Prompt L-Pop™ improved the bonding on moderate and severe fluorosis. The μTBS of the AdheSE One™, was significantly lower in all degrees of fluorotic enamel (p<0.05) indicating a very poor bonding ability to enamel. These results will provide clinicians with preliminary data to assist them in the selection of the most effective adhesive systems for treatment of fluorosis enamel, resulting in more successful restorative care.

Keywords: Microtensile, Dental adhesive, Fluorotic enamel, SEM

INTRODUCTION

The prevalence of fluorotic enamel has increased in many parts of the world7, as a result of fluoride ingestion during tooth development5. An outer hypermineralized layer with a porous hypomineralized subsurface characterizes the affected enamel. The pores are occupied by enamel secretory proteins that are retained due to the effect of the excessive fluoride level on ameloblasts8. In its milder form, fluorotic enamel is clinically characterized by white lines that are caused by accentuated perikymata; while in more severe cases, the enamel contains cloudy areas, which may be discrete or confluent4. Sometimes the subsurface hypomineralization is so extensive that the outer well-mineralized surface layer is poorly supported and becomes vulnerable to brittle fracture, and chewing forces may result in the formation of surface enamel defects4.

Fluorotic teeth sometimes need to be restored with resin-based composites for functional or aesthetic restorations, which largely depend on the micromechanical retention obtained from acid-etched enamel5. However, clinicians have found that bonding to fluorotic teeth is a clinical challenge6 because fluorapatite is more resistant to acid dissolution than hydroxyapatite7; consistent with reports, which suggest that severe dental fluorosis adversely affects the bonding of composites to enamel, thereby compromising clinical success8,9. Although phosphoric acid has been used as the gold standard for etching effectiveness in esthetic dentistry because of its effectiveness in producing microporosity in healthy enamel9,10, there is considerable uncertainty on the most suitable etching time with phosphoric acid for the different degrees of fluorosis.

Some studies have recommended that extended enamel conditioning with phosphoric acid be performed during bonding onto fluorotic enamel12,15; in contrast, other studies reported no significant differences in the bond strengths to fluorotic and normal enamel with varying etching times11,14. With the developments in adhesive dentistry, manufacturers have recently developed adhesive systems (AS's) where the etchant, primer and adhesive are combined. These (AS's) are becoming more commonly used because they require less working time10; but despite their simplicity and time savings, their effectiveness on fluorotic enamel still remains questionable15-21. Therefore, the aim of this study was to perform an in vitro evaluation of the bond strength of the total-etch and two self-etch (AS's) to varying degrees of fluorotic enamel using micro-tensile bond strength (μTBS) tests. In addition, optical microscopy and scanning electron microscopy were used to analyze and classify the failure modes of the enamel/adhesive bonds. These results will provide clinicians with preliminary data to assist them in the selection of the most effective adhesive system (AS) for treatment of fluorotic enamel, resulting in more successful restorative care.

MATERIALS AND METHODS

This study was approved by the ethics committee of the Master of Dental Science degree with Specialization in Advanced Education General Dentistry Program, Faculty of Dentistry at San Luis Potosi University.

Collection of tooth specimens

Patients undergoing extraction of premolars for
periodontal and orthodontic reasons between 19 and 32 years old were asked to donate their caries-free extracted teeth, and informed patient consent was then obtained. A total of 120 teeth were collected from areas with different water fluoride levels, (WFLs): (1) 30 teeth from Ciudad Valles (San Luis Potosí, México), which has a WFL between 0.1 and 0.6 ppm F; (2) 60 teeth from San Luis Potosí, City (México) with a WFL between 0.7 and 2.0 ppm F; and (3) 30 teeth from Salitral de Carrera (San Luis Potosí, México) with a WFL between 2.0 and 5.0 ppm F. All specimens were cleaned and disinfected in an ultrasonic bath (Biosonic UC 300-115B, Colténe/Whaladent, Cuyahoga Falls, Ohio, USA), washed in running water, dried, and analyzed by visual observation for fluorotic severity according to the Thylstrup and Fejerskov Index (TFI)\(^ {22} \). The classification according to TFI was done independently by two investigators. Intra-examiner reproducibility gave a Cohen's Kappa Statistic of 0.98\(^ {23} \). This index allows correlation between the clinical appearance of fluorosis and the pathologic changes in human enamel and is normally the index of choice for the evaluation of fluorotic severity\(^ 6 \). This index is based on a 10-point ordinal scale, where zero represents the nonaffected tooth (TFI=0) and 9 the most severely affected tooth. The 120 specimens were first divided into four groups of 30 specimens each: the Healthy group (H, TFI=0 and three fluorotic groups, the Mild Fluorotic group (MI) [TFI=1–3], the Moderate Fluorotic group (MO) [TFI=4–5] and the Severe Fluorotic group (S) [TFI=6–9]. Prior to preparation of specimens for the \(\mu\)TBS tests, all premolars were stored for a maximum of 2 months in 1.0% aqueous chloramine solution (Sigma Chemical, St Louis, MO, USA). For each specimen, the root was removed and the crown was longitudinally sectioned to obtain buccal and lingual halves using a high-speed diamond bur (MRMC254-Brasseler, Savannah, GA, USA). Finally, in order to standardize the enamel reduction, 0.5 mm grooves were prepared on the buccal side of each specimen and then superficial enamel was ground using a 600-grit silicon carbide (SiC) disc (Buehler, Lake Bluff, IL, USA) under flowing water coolant. Only buccal surfaces of each tooth were used for the bond strength measurements in order to standardize the localized bond strength variation in enamel\(^ {24} \).

Table 1 depicts the materials used in this study, three AS’s were used: (1) Excite™ (EX™, Ivoclar Vivadent, Schaan, Liechtenstein); (2) Adper Prompt L-Pop™ (APL-P™, 3M ESPE, St. Paul, MN, USA) and, (3) AdheSE One™ (ASO™, Ivoclar Vivadent, Schaan, Liechtenstein). To perform the tests, each fluorotic teeth group was randomly divided into the three different AS’s, forming 4 subgroups in each AS. EX™ bonded to H group was selected as control group due to the effectiveness in producing microporosity in healthy enamel therefore it has been used as the gold standard\(^ {10} \). Each AS was applied according to the manufacturer’s instructions, as described below.

Protocol for the EX™ subgroup: Each specimen was conditioned with 37% phosphoric acid gel for 15 s, and rinsed with distilled water for 15 s leaving the surface slightly moist. EX™ was applied for 10 s and evaporated for 5 s, then light-cured for 10 s (bluephase C8 light, Ivoclar Vivadent, Schaan, Liechtenstein; at 800 mW/cm\(^2\)). After the bonding procedure, a composite block (Tetric N-Ceram, Ivoclar Vivadent, Schaan, Liechtenstein) was built prepared on the enamel buccal surface by adding 2-mm-thick increments to a height of approximately 4 mm. Each composite layer was light cured for 40 s.

Protocol for the APL-P™ subgroup: Bonding liquids A and B were mixed and applied by double application and scrubbed for 15 s. Volatile ingredients were evaporated with mild air and cured for 10 s (bluephase C8 light, Ivoclar Vivadent, Schaan, Liechtenstein; at 800 mW/cm\(^2\)). After the bonding procedure, a composite block (Tetric N-Ceram, Ivoclar Vivadent, Schaan, Liechtenstein) was built prepared on the enamel buccal surface by adding 2-mm-thick increments to a height of approximately 4 mm. Each composite layer was light cured for 40 s.

Protocol for the ASO™ subgroup: Each specimen was conditioned with 37% phosphoric acid gel for 15 s, and rinsed with distilled water for 15 s leaving the surface slightly moist. EX™ was applied for 10 s and evaporated for 5 s, then light-cured for 10 s (bluephase C8 light, Ivoclar Vivadent, Schaan, Liechtenstein; at 800 mW/cm\(^2\)). After the bonding procedure, a composite block (Tetric N-Ceram, Ivoclar Vivadent, Schaan, Liechtenstein) was built prepared on the enamel buccal surface by adding 2-mm-thick increments to a height of approximately 4 mm. Each composite layer was light cured for 40 s.

Table 1  Materials used in this study

<table>
<thead>
<tr>
<th>Adhesive material</th>
<th>Composition and pH</th>
<th>Lot No.</th>
<th>Restorative Resin Materials</th>
<th>Lot No.</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total-Etch System, EX™</td>
<td>Etchant: 37% phosphoric acid, pH&lt;0.6</td>
<td>M06929</td>
<td>Tetric N-Ceram</td>
<td>P46714</td>
<td>Ivoclar Vivadent, Schaan, Liechtenstein</td>
</tr>
<tr>
<td></td>
<td>Phosphoric acid acrylate, HEMA, TEG-DMA, dimethacrylate, silicon dioxide, ethanol, catalysts, stabilizers</td>
<td>M24074</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One-step self-etch, APL-P™</td>
<td>Compartment 1: methacrylated phosphoric acid, esters, bisGMA, photo initiator, stabilizer, pH&lt;1.0</td>
<td>378374</td>
<td>Z350</td>
<td>N163574</td>
<td>3M ESPE, St. Paul, MN, USA</td>
</tr>
<tr>
<td></td>
<td>Compartment 2: water, 2-HEMA, polyalkenoic acid copolymer, fluoride complex, stabilizers</td>
<td></td>
<td></td>
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<tr>
<td>All-in-one self-etch, ASO™</td>
<td>Bis-Acrylamide, water, bis-methacrylamide dihydrogen phosphate, amino acid acrylamide, hydroxyl alkyl methacrylamide, silicon dioxide, catalysts, stabilizers, pH=1.5</td>
<td>M32506</td>
<td>Tetric N-Ceram</td>
<td>P46714</td>
<td>Ivoclar Vivadent, Schaan, Liechtenstein</td>
</tr>
</tbody>
</table>
St. Paul, MN, USA) was produced as described above.

Protocol for the ASO™ subgroup: With the tip applicator, the ASO™ was left undisturbed on the enamel surface for 20 s. Volatile ingredients were evaporated with a mild stream of air and then cured for 10 s. After the bonding procedures, a composite block (Tetric N-Ceram, Ivoclar Vivadent, Schaan, Liechtenstein) was prepared for each specimen.

Microtensile Test (μTBS)
After storing the specimens for 24 h, each tooth was sectioned perpendicular to the bonded interface to obtain beam-shaped strips with a surface area of 1.0±0.2 mm². A total of 240 samples were obtained: 20 per fluorotic group in each AS. Fig. 1 shows the division of the samples. Each specimen was carefully examined under a stereo-microscope (SZ-PT Olympus, Japan, 10×, Hamburg, Germany). Specimens with irregular fracture paths that propagated away from the interface were discarded. The specimens were attached to a Universal Testing Machine (Advanced Force Gauge, Mecmesin, West Sussex, UK) with a cyanoacrylate adhesive (Zapit, DVA, Corona, CA, USA), and then subjected to tensile forces at a cross-head speed of 1 mm/min; values were calculated in MPa.

After the μTBS, a stereo-microscope was used to classify the failure mode in one of the following categories: (a) adhesive if the failure occurred entirely within the adhesive interfacial zone; (b) cohesive in composite or cohesive in enamel if the failure occurred exclusively within the composite or within the enamel; and (c) mixed if the failure continued from the adhesive into either composite or enamel.

Scanning electron microscopy (SEM)
Adhesive interfaces and morphological changes in healthy and fluorotic enamel surfaces were observed by SEM (FEI, Quanta 200, Hillsboro, OR, USA). The specimens were prepared by the same method described in the bonding procedure. They were sectioned perpendicular to the bonded interface under flowing water coolant using a high-speed diamond saw, then ground using 1000-grit silicon carbide paper (Buehler, Lake Bluff, IL, USA) and polished with 3 μm diamond paste. Polishing debris was ultrasonically removed for 15 min. And the specimens were critically dehydrated in ethanol (100%) for 2 h and coated with 10 nm of gold in a sputter coater system (Cressington Scientific Instruments Ltd., 108 Auto Sputter Coater, Watford, UK).

Statistical analysis
The Shapiro-Wilks and Brown-Forsythe methods were used to test the distribution of variables. The Student's t-test was used to compare the differences between mean bond strength among the three AS's bonded to the four groups. The Tukey test was used to compare the bond strength in each group for the different AS's. Chi-square analyses were performed to compare the failure modes in the three AS's for each of the four groups. The JMP program, version 9.0 (SAS Institute, Cary, NC, USA) and Stata version 11.0 (StataCorp LP, College Station, TX, USA) were used for statistical analysis with statistical significance set at $\alpha=0.05$.

RESULTS
Microtensile bond strength ($\mu$TBS)
Table 2 shows the mean $\mu$TBS for EX™, APL-P™ and ASO™ subgroups to H, MI, MO and S groups. The control group (EX™ bonded to H group) achieved the highest bond strength of the AS's and in all the fluorotic groups and the difference was statistically significant ($p<0.05$).

Comparison of the $\mu$TBS of the control group and the three AS's on the different groups was performed with the Student's $t$-test. In particular, the results show that the bond strength achieved in the EX™ subgroup was influenced by the degree of fluorosis ($p<0.05$). While the differences between mean bond strength resulting from the use in the APL-P™ subgroup were statistically significant lower ($p<0.05$) when compared to the control group, the bond strength remained practically the same regardless of the degree of fluorosis. Finally, the bond strength obtained in the ASO™ subgroup had
a significantly lower bond strength for H group as well as for all the fluorotic enamel groups (p<0.05), as compared with the other experimental groups and AS's subgroups.

Analyzing the results for the different groups, in H and MI groups the highest bonding strength was obtained in the EX™ subgroup; however the APL-P™ subgroup showed low values but they remained close; ASO™ subgroup exhibited the lower bond strength in all the groups (p<0.05). For the MO group, APL-P™ subgroup showed the highest bond strength compared to the EX™ and ASO™ subgroups (p<0.05). For the S group, APL-P™ subgroup yielded a greater bond strength compared with the results of EX™ and ASO™ subgroups (p<0.05 only for APL-P™ subgroup).

**Failure mode**
The classification of failure modes is summarized in Table 3. Most failures were recorded as adhesive failures, S: severe fluorosis; H: Healthy enamel; MI: mild fluorosis; MO: moderate fluorosis; S: severe fluorosis. There was a statistically significant difference between all fluorotic groups (Chi-square test).

<table>
<thead>
<tr>
<th>Table 2 Mean microtensile bond strength of the three ASs to healthy and fluorotic enamel groups</th>
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<tbody>
<tr>
<td>Group (Enamel)</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>H</td>
</tr>
<tr>
<td>MI</td>
</tr>
<tr>
<td>MO</td>
</tr>
<tr>
<td>S</td>
</tr>
</tbody>
</table>

n=240. Results are expressed in MPa. EX™: Excite; APL-P™: Adper Prompt L-Pop; ASO™: AdheSE One VivaPen. H: Healthy enamel; MI: mild fluorosis; MO: moderate fluorosis; S: severe fluorosis. *: Control group, EX™ bonded to H group. Student’s t-test: showed statistically significant difference (p<0.05) in all the groups when comparing with the control group. Symbols ± and ± ± ± represent the statistically significant difference in each enamel group with the different adhesive system using Tukey test.

**SEM observations**
Figure 2 shows a representative image of H group etched with phosphoric acid and bonded using EX™ (a). The acid produces a deep etched pattern in enamel with well-defined tag-like formations in the intercrystalline spaces (indicated by white arrows); APL-P™ subgroup (b), showed shallow depressions but the enamel surface was not etched properly (indicated by a white arrow). When ASO™ (c) was used, no etch patterns were observed (indicated by a white arrow). Figure 3 shows the MI group: The EX™ (a) and the APL-P™ (b), as seen in the SEM micrographs, both produced a mild etch pattern with short tags (indicated by white arrows), but the ASO™ (c) did not show dissolution of the enamel surface (indicated by a white arrow).

The results shown in Fig. 4 for the MO group were as follows: The EX™ (a) revealed a very slight demineralized pattern with poorly defined tags (indicated by a white arrow). In contrast, the APL-P™ (b) produced a hybridized enamel layer with typical resin penetration into the demineralized zone (indicated by white arrows), while the ASO™ (c), presented no demineralization of the enamel surface (indicated by a white arrow). Figure 5 depicts the S group for the EX™ (a), treated with the phosphoric acid revealed poor etched surface and the AS showed a porous structure (indicated by a white arrow). The APL-P™ (b) produced a thick enamel-resin hybrid layer without pores (indicated by white arrows) and the ASO™ (c) did not demineralize the enamel surface (indicated by a white arrow).

**DISCUSSION**
Success in bond durability of esthetic treatments depends on adequate bond strength of resin composite to bonded to fluorotic groups. For the EX™ subgroup, specimens exhibited primarily a cohesive-type failure, and frequency of this type of failure decreased with increasing degree of fluorsis (p<0.05). For the APL-P™ subgroup, specimens failed adhesively, nevertheless; in the MI group cohesive failure was more frequent (p<0.05). The ASO™ subgroup, exhibited more adhesive failures (p<0.05) than the rest of the AS’s.

<p>| Table 3 Failure modes of specimens after microtensile bond test in the study groups |
|-------------------------------|----------------|-----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Cohesive</th>
<th>Adhesive</th>
<th>Mixed</th>
<th>Cohesive</th>
<th>Adhesive</th>
<th>Mixed</th>
<th>Cohesive</th>
<th>Adhesive</th>
<th>Mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>18</td>
<td>90%</td>
<td>—</td>
<td>2</td>
<td>10%</td>
<td>7</td>
<td>35%</td>
<td>8</td>
<td>40%</td>
</tr>
<tr>
<td>MI</td>
<td>11</td>
<td>55%</td>
<td>2</td>
<td>10%</td>
<td>7</td>
<td>35%</td>
<td>10</td>
<td>50%</td>
<td>6</td>
</tr>
<tr>
<td>MO</td>
<td>7</td>
<td>35%</td>
<td>8</td>
<td>40%</td>
<td>5</td>
<td>25%</td>
<td>6</td>
<td>30%</td>
<td>12</td>
</tr>
<tr>
<td>S</td>
<td>4</td>
<td>20%</td>
<td>12</td>
<td>60%</td>
<td>4</td>
<td>20%</td>
<td>2</td>
<td>10%</td>
<td>12</td>
</tr>
</tbody>
</table>

n=240. EX™: Excite; APL-P™: Adper Prompt L-Pop; ASO™: AdheSE One VivaPen. H: healthy enamel; MI: mild fluorosis; MO: moderate fluorosis; S: severe fluorosis. There was a statistically significant difference between all fluorotic groups (Chi-square test).
enamel. There are controversies regarding the bonding performance of AB's bonded to fluorotic enamel\(^7,8,11-13,26,27\), it has been reported that fluorosis significantly reduces the bond strength in enamel\(^8,9\); this decrease is thought to be associated to the acid-resistant outer layer of the fluorotic enamel\(^11,13\).

In this study, the μTBS of three AS’s that were bonded to fluorotic and nonfluorotic ground enamel was determined. 0.5 mm of enamel was ground to be consistent with clinical practice where 0.5 mm of labial enamel is removed during tooth preparation for composite veneers. Teeth were obtained from subjects in the age group of 19–32 years, due to it has been reported\(^13\) that there are differences in bond strength below and above the age of 40 years. Several previous studies of bonding to fluorotic enamel surfaces have led to the preferred use of the μTBS test and fracture mechanics to understand the properties of the adhesive interfaces\(^28\).

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**Fig. 2** SEM images of H enamel (E) and resin (R) interfaces in a cross-sectioned specimen treated with: (a) EX™; (b) APL-P™ and (c) ASO™, arrows indicate the penetration of the AS's.

**Fig. 3** SEM images of the MI enamel (E) and resin (R) interfaces in a cross-sectioned specimen treated with: (a) EX™; (b) APL-P™ and (c) ASO™, arrows indicate the penetration depth of the AS's.

**Fig. 4** SEM images of the MO enamel (E) and resin (R) interfaces in cross-sectioned specimen treated with: (a) EX™; (b) APL-P™ and (c) ASO™, arrows indicate the penetration depth of the AS's.

**Fig. 5** SEM images of the S enamel (E) and resin (R) interfaces in cross-sectioned specimen treated with: (a) EX™; (b) APL-P™ and (c) ASO™, arrows indicate the penetration depth of the AS's.
EX™ is a total-etch AS that uses a preliminary etching step with a strong phosphoric (pH<0.6) that effectively etches the H group. This AS produced the highest bonding strengths, 21±3.8 MPa and 13±2.8 MPa, to H and MI groups, respectively. In these groups it was observed that the acid produced a well-etched enamel surface with well-defined tag-like formations in the intercrystalline spaces. These structures were associated with the highest percentage of cohesive failures. According to previous bond strength studies, when the adhesive system contains the bond orientation, the bond strength of enamel is dependent on the prismatic zone but also for the parallel zone less influenced by prism orientation

failures, which are indicative of poor mechanical retention.

CONCLUSIONS

The results of this study indicate that the level of enamel fluorosis significantly decreased the bond strength to composite for the traditional total-etch AS. It also suggests that the EX™ is the best AS to be used in H and MI groups. For MO and S groups the self-etch AS APL-P™ improves bonding. The self-etch AS ASO™, yielded the lowest bond strength. It is important to note that this is the first study that tests different types of adhesive systems with all the varying degrees of fluorosis. The findings of this study will better prepare clinicians to select adhesive system according to or restoring enamel with different degrees of fluorosis, resulting in more durable and cost-effective treatments.

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REFERENCES


