Effect of Office Bleaching on Enamel Bonding Performance

Somayah Halabia / Naoko Matsuib / Toru Nikaidoc / Michael F. Burrowd / Junji Tagamië

Purpose: To evaluate the effect of in-office bleaching on enamel microshear bond strength (μSBS) of two self-etch adhesives (SEA).

Materials and Methods: For the bleaching group, bovine enamel surfaces were treated with a bleaching agent and divided into two main groups: one in which adhesive was applied immediately after bleaching and another in which adhesive was applied after one week. Enamel was bonded using the following procedures: group 1: application of a two-step SEA (Clearfil SE Bond 2, SE2, Kuraray Noritake); group 2: etching with 40% phosphoric acid (PA) and application of SE2; group 3: application of a one-step SEA (G-Premio Bond, GP, GC); group 4: etching with PA and application of GP. Resin composite was placed and μSBS was measured at 24 h (immediately) and 1 week after bleaching. Acid-base resistant zones (ABRZ) were observed under SEM. Three-way ANOVA was used for data analysis.

Results: Bleaching significantly decreased the μSBSs even after PA etching with both SE2 and GP (p ≤ 0.005). However, there were no significant differences in μSBS between groups with and without PA etching, except for SE2 without bleaching, in which the μSBS significantly increased with PA etching (p < 0.005). Thick ABRZs were found in SE2 and GP after bleaching, which exhibited an irregular wave-like shape for PA-treated specimens.

Conclusion: The enamel μSBSs were adversely affected by in-office bleaching. Thick ABRZs were created with bleaching and PA treatment.

Keywords: office bleaching, ABRZ, microshear bond strength.


Tooth bleaching is today a widespread esthetic dental treatment. Patients increasingly seek to have an attractive smile, as it is considered equivalent to good health and a good appearance, and is associated with professional and social benefits. Currently, dental bleaching is recognized as an effective method for the treatment of discolored teeth, and is considered a conservative and biologically safe form of therapy.

In-office bleaching is carried out before esthetic restorative procedures such as direct resin composite restorations. One of the adverse effects of in-office tooth bleaching is lower bond strength of the resin composite to enamel immediately after the bleaching procedure, which has been ascribed to the presence of oxygen ions that inhibit resin polymerization. However, an antioxidant such as sodium ascorbate has been used in various concentrations and durations to improve composite bond strength after bleaching.

Self-etch adhesives are classified according to the number of clinical application steps: one-step or two-step. One-step self-etch adhesives, the so-called all-in-one adhesives, combine etching, priming, and bonding in one procedure. These adhesives contain acidic functional monomers, hydrophilic and hydrophobic monomers, water, and organic solvents in a single solution. However, two-step self-etch adhesives contain a hydrophilic self-etching primer comprising acidic monomers which simultaneously etch and prime the tooth substrate, and after solvent evaporation, a layer of hydrophobic bonding agent seals the tooth surface. It has been reported that selective etching of enamel with phosphoric acid prior to application of a self-etch adhesive

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significantly increases enamel bond strengths. Since phosphoric acid etching is more aggressive than self-etching adhesives, resin monomers can penetrate more deeply into the etched surface.

Initially, nanoleakage was mainly described as the discrepancy between depth of demineralization of the tooth structure and subsequent resin infiltration. Using ammoniacal silver nitrate, Tay et al. defined reticular and spotted nanoleakage patterns for several adhesives. However, there is little information related to nanoleakage expression at the adhesive-enamel interface after in-office bleaching.

The acid-base resistant zone (ABRZ) for adhesive-dentin interfaces was first reported by Tsuchiya et al. The ABRZ is characterized as a structure at the resin-dentin interface remaining after an acid-base challenge. Recent studies have also demonstrated the formation of ABRZ at enamel interfaces with one-, two- and three-step self-etch adhesives. It seems to contribute to sealing of the restoration margins and therefore to promoting the durability of the restoration. However, there is no information on the effect of bleaching agents on the morphology of the enamel ABRZ.

Therefore, the present study aimed to evaluate enamel bonding properties of self-etch adhesives in one- and two-step self-etch adhesives (SEA) with and without phosphoric acid etching prior to self-etch adhesive application to enamel surfaces after in-office bleaching. An in-office bleaching material was used prior to etching or adhesive application and tested by means of a microshear bond strength (μSBS) test. Nanoleakage patterns at the adhe-
sive-enamel interface and micromorphological features of the ABRZ were assessed using scanning electron microscopy (SEM). The null hypothesis was that in-office bleaching has no effect on μSBS with self-etch adhesive application.

MATERIALS AND METHODS

Materials

The materials used in this study are described in Table 1. A two-step self-etch adhesive (Clearfil SE Bond 2, SE2, Kuraray Noritake Dental; Tokyo, Japan) and a one-step self-etch adhesive (G-Premio Bond, GP, GC; Tokyo, Japan) were used. SE2 comprises the functional monomer, 10-methacryloyloxydecyl dihydrogen phosphate (MDP), in the self-etching primer and a MDP-containing bonding resin. GP also contains MDP with water and organic solvents in the one-bottle adhesive. A 40% phosphoric acid gel (PA, K-etchant, Kuraray Noritake Dental) was used as the etchant in this study. The in-office bleaching agent selected was TiON In-Office (GC) containing 35% hydrogen peroxide (pH 6).

Specimen Preparation

Specimen preparation is illustrated in Fig 1. One hundred forty-four bovine incisors, free of structural cracks and defects, were used in this study. Roots were resected 1 mm above the cementoenamel junction using a diamond saw (Isomet 1000, Buehler; Lake Bluff, IL, USA) under water cooling. Each tooth was sectioned to obtain one fragment from the labial surface per tooth (10 x 10 mm). Enamel surfaces were wet-ground with 600-grit silicon carbide abrasive paper to create a flat surface and standardized smear layer. The labial surface of each tooth was finished to ensure it was parallel to the applied force during the μSBS test.

The enamel surfaces were treated either with or without the bleaching agent. For the bleaching groups, the enamel surface was treated with the in-office bleaching agent (TiON In-Office, GC) according to the manufacturer’s instructions. The reactor part of the bleaching agent was first applied on the experimental surface using a disposable brush, and excess reactor was removed by gentle air drying. The mixed bleaching gel was then applied to the enamel surface followed by exposure to a quartz-halogen curing light (Optilux 501, Demetron; Danbury, CT, USA) with a power density of 550 mW/cm² for 1 min. The bleaching agent was left on the specimens for 5 min. The bleaching gel was then wiped off the experimental surface using a piece of gauze. This procedure was repeated three times, and the specimen was then rinsed under water, dried gently, and divided into two main groups: adhesive materials applied immediately (immediate) and one week (1 week) after the bleaching procedure. After bonding, specimens were stored in an incubator at 37°C in distilled water. Each group was subdivided into four subgroups according to bonding procedures: group 1: application of SE2; group 2: etching with PA and application of SE2; group 3: application of GP; group 4: etching with PA and application of GP (Fig 2).

μSBS Testing

After bonding (n = 60), tygon tubes (R-3603, Norton Performance Plastic; Cleveland, OH, USA) with an internal diameter of 0.79 mm and a height of 0.5 mm were placed on the bonded enamel surface and light cured for 10 s. A resin composite (Clearfil AP-X, shade A2, Kuraray Noritake Dental)
SEM (JSM-5310LV, JEOL; Tokyo, Japan) at 200X magnification. Failure modes were classified into five types: type 1: adhesive failure at the interface between the bonding resin and enamel; type 2: mixed failure including adhesive failure and cohesive failure in the bonding resin; type 3: cohesive failure in the bonding resin; type 4: cohesive failure in enamel; type 5: mixed failure including cohesive failure in enamel and bonding resin.

Nanoleakage Expression
Sixty specimens were prepared for evaluation of nanoleakage patterns of the adhesive-enamel interface in each experimental group (n = 5). Nanoleakage patterns of the adhesive-enamel interface were evaluated on five bonded teeth from each experimental group after 24-h storage at 37°C in water. The specimen preparation for nanoleakage expression is illustrated in Fig 3. The bonded samples were sectioned vertically at the center of the specimens and divided into halves. The specimens were immersed in 50%

SEM Failure Mode Analysis
Debonded surfaces of all specimens were gold sputter-coated to evaluate the failure patterns on the enamel using SEM (JSM-5310LV, JEOL; Tokyo, Japan) at 200X magnification. Failure modes were classified into five types: type 1: adhesive failure at the interface between the bonding resin and enamel; type 2: mixed failure including adhesive failure and cohesive failure in the bonding resin; type 3: cohesive failure in the bonding resin; type 4: cohesive failure in enamel; type 5: mixed failure including cohesive failure in enamel and bonding resin.

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(wt/vol) ammoniacal silver nitrate (AgNO₃) for 24 h in darkness following the method described by Tay et al. Specimens were then thoroughly rinsed with deionized water for 5 min and placed in photodeveloping solution (acid 1L UNS Sakusan, Fuji Film; Tokyo, Japan) for 8 h under fluorescent light at ≥550 mW/cm² to reduce the silver ions into metallic silver. Following air drying and polishing with diamond pastes (Struers; Copenhagen, Denmark) down to 0.25 μm, the specimens were gold sputter-coated and examined by SEM (JSM-5310LV, JEOL) at 500X magnification.

**Acid-Base Resistant Zone**

Twenty-four specimens were used for ABRZ observation in each experimental group (n = 2) as illustrated in Fig 4. The specimens were prepared in the same manner as for the bond test. Additionally, a thin coating of resin composite (Clearfil AP-X, shade A2, Kuraray Noritake Dental) was applied on the top of the bonded specimens and cured for 40 s. After storage in distilled water at 37°C for 24 h, each specimen was sectioned into halves perpendicular to the bonded interface and embedded in slow-curing epoxy resin (Epoxide Resin, Buehler) overnight. Specimens were wet polished with SiC paper to 1200-grit to standardize the surface and then subjected to an acid-base challenge with a demineralizing solution (pH 4.5) for 2 h and 5% NaClO for 20 min. A self-curing resin, Super-Bond C&B (Sun Medical; Moriyama, Japan) was then applied to the treated surface without acid etching to prevent wear of the interface. The specimens were then cut perpendicular to the interface, polished with SiC paper to 2000 grit, and finished with diamond pastes to 0.25 μm followed by argon-ion beam (EIS-IE, Elionix; Tokyo, Japan) etching to distinguish the different zones of the interface observed with SEM (JSM-IT100LV, JEOL) at 3500X magnification.

**Statistical Analysis**

Data were analyzed for normal distribution using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Three-way ANOVA was used to show the effect of adhesive, bleaching agent, and time on shear bond strength, followed by pairwise comparison with Bonferroni correction. Statistical significance was set at p < 0.001. Statistical analysis was performed using IBM SPSS (IBM; Armonk, NY, USA) v 23 for Windows.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Effect of bleaching on μSBS to enamel with/without selective acid etching</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleaching</td>
<td>Self-etch</td>
</tr>
<tr>
<td>SE2</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>23.1 ± 6 a</td>
</tr>
<tr>
<td>Immediate</td>
<td>17.3 ± 4.4 b</td>
</tr>
<tr>
<td>1 week</td>
<td>23.5 ± 6.7 a</td>
</tr>
<tr>
<td>GP</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>18.3 ± 5.4 b</td>
</tr>
<tr>
<td>Immediate</td>
<td>10.4 ± 4.8 c</td>
</tr>
<tr>
<td>1 week</td>
<td>14.4 ± 5.1 b</td>
</tr>
</tbody>
</table>

Mean ± standard deviation with the same letters (a, b, c or d) within each column indicates no significant difference (NS) (p > 0.001). * Significant difference. Number of specimens: 45 for each group.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Fracture mode analysis of debonded specimens after μSBS test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleaching</td>
<td>Self-etch %</td>
</tr>
<tr>
<td>SE2</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>80/20/0/0/0/0</td>
</tr>
<tr>
<td>Immediate</td>
<td>62/38/0/0/0/0</td>
</tr>
<tr>
<td>1 week</td>
<td>82/18/0/0/0</td>
</tr>
<tr>
<td>GP</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>22/62/16/0/0/0</td>
</tr>
<tr>
<td>Immediate</td>
<td>31/51/18/0/0</td>
</tr>
<tr>
<td>1 week</td>
<td>24/18/58/0/0</td>
</tr>
</tbody>
</table>

Failures by type: type 1/type 2/type 3/type 4/type 5. Type 1: adhesive failure at the interface between the bonding resin and enamel; type 2: mixed failure including adhesive failure and cohesive failure in the bonding resin; type 3: cohesive failure in the bonding resin; type 4: cohesive failure in enamel; type 5: mixed failure including cohesive failure in enamel and bonding resin.
RESULTS

μSBS

The μSBSs are listed in Table 2. Three-way ANOVA followed by pairwise comparison with Bonferroni correction indicated that bleaching, time, and adhesive type as well as the interaction between them influenced the μSBS (p ≤ 0.001).

The μSBSs of SE2 were significantly higher than those of GP for each bonding strategy. For SE2, its μSBS to enamel without bleaching was 23.1 MPa, while the μSBS significantly decreased in the “immediately after bleaching” group (p < 0.001). However, the bond strength recovered to the original level 1 week after bleaching. In the phosphoric acid etching groups of SE2, the SBS was 40.7 MPa in the control without bleaching, which was significantly higher than that of the self-etch group (p < 0.01). After bleaching, the μSBS was significantly lower than the immediate non-bleached (control) group, and recovered slightly 1 week after bleaching without achieving the same strength as the original value (40.7 MPa).

The μSBS of GP to enamel without bleaching was 18.3 MPa, which significantly decreased immediately after bleaching (p < 0.001). The bond strength recovered to the original value 1 week after bleaching. In the phosphoric acid etching groups for GP, the μSBS was 16.8 MPa without bleaching, which was not significantly different from that of GP without phosphoric acid etching. However, the μSBS significantly decreased immediately after bleaching, but recovered 1 week after bleaching to the original value.

Failure Mode Analysis

The fracture mode results are shown in Table 3. For SE2, in the self-etch mode, type 1 (adhesive between enamel and resin) was the main failure mode in the no-bleaching and 1-week groups; however, type 2 (mixed failure including adhesive and bonding resin cohesive failure) increased in the “immediately after bleaching” group. In the acid-etch groups, types 4 (cohesive failure in enamel) and 5 (mixed failure including cohesive failure in enamel and bonding resin) were mainly observed in the no-bleaching and “immediately after bleaching” groups. However, the failure mode changed significantly to type 1 and type 2 in the 1-week group.

For GP in the self-etch mode, types 1, 2, and 3 were observed in the no-bleaching, immediately after bleaching and 1-week groups, while in the acid-etched groups, types 3 and 4 were observed in the no-bleaching and immediately after bleaching groups, and types 2 and 3 were observed at 1 week.

Nanoleakage Expression

Representative images of the ammoniacal AgNO₃-challenged specimens of the adhesive-enamel interface are illustrated in Figs 5 (SE2) and 6 (GP). Nanoleakage was not observed in the SE2 groups. A distinctive silver pattern of nanoleakage was apparent only in the control group of GP at the adhesive-enamel interface to a depth of approximately 50 μm following the enamel prism orientation, while no silver deposition was observed in the other groups.
Acid-Base Resistant Zone
The typical SEM images of the adhesive-enamel interface for each group after acid-base challenge are shown in Figs 7 (SE2) and 8 (GP). The outer lesion (OL), created by dissolution of enamel due to the acid-base challenge, was observed in each group; the depth of the OL ranged from 10 to 15 μm. ABRZ was observed at the demineralization front of OL in all groups.

In the SE2 groups, the ABRZ was approximately 0.5 μm thick in SE2 without bleaching (Fig 7a); however, the ABRZ increased to a thickness of about 2 μm in the immediately after bleaching group, and was approximately 3 μm thick with an irregular appearance in the 1 week after bleaching group (Figs 7b and 7c). For the phosphoric acid etched groups (Figs 7d to 7f), the ABRZ had an irregular, wavelike shape of about 3 to 5 μm in thickness.

For the GP groups, the ABRZ was 1 μm thick without bleaching (Fig 8a), which was thicker than for SE2 (Figs 7a). However, a funnel-shaped lesion (lightning symbol) was formed at the base of the OL in the no-bleaching group (Fig 8a). On the other hand, in the bleaching groups (Figs 8b and 8c), the ABRZ was 2 μm thick in the immediately after bleaching group with the presence of an ill-defined, funnel-shaped lesion. The 1 week after bleaching group showed an ABRZ approximately 3 μm thick, but the funnel-shaped lesion could not be observed. In the phosphoric acid etching groups (Figs 8d to 8f), the ABRZ formed an irregular, wavelike shape ranging between 3 to 5 μm in thickness. Funnel-shaped lesions were not observed under the ABRZ in any of the etched groups.

DISCUSSION
Enamel predominantly consists of an inorganic matrix of hydroxyapatite crystals (96%, w/w) with the organic constituents (ie, proteins and lipids) and water (4%, w/w) occupying the gaps among the apatite crystals. In order to simulate the clinical situation, an intact enamel surface should be used. However, the enamel surfaces in this experiment were ground flat for better standardization and to understand the effect of the bleaching agent on bond strength and the ABRZ.

The mechanism of action of bleaching enamel is based on hydrogen peroxide, a strong oxidizing agent which removes staining within the enamel by its oxygen-releasing action, resulting in oxidation of the stain pigments. Hydrogen peroxide is a low molecular-weight molecule and is able to denature proteins, which increases tissue permeability and allows the movement of ions through the enamel. Tooth bleaching agents can produce detrimental effects on the mechanical properties of enamel, possibly as a consequence of damaging or denaturing the proteinaceous components. The bleaching material, TiON In-Office, used in this study consists of a reactor and syringes A and B. The reactor contains a visible light photocatalyst (VL-TiO2),
which is applied on the enamel surface prior to application of the bleaching agent. Syringes A and B contain 35% H$_2$O$_2$ and 30% carbamide peroxide, respectively. The breakdown reaction of carbamide peroxide releases hydrogen peroxide and free oxygen radicals.$^{3,34}$ Mixing the contents of the two syringes results in an approximately 23% H$_2$O$_2$ gel (pH 6.0), which was applied on the enamel surface.

In-office bleaching is often carried out prior to other aesthetic restorative procedures, such as direct resin composite restorations or veneer placement.$^2$ A decrease in bond strength immediately after bleaching is a concern and may affect long-term clinical outcomes.$^{28}$ The μSBS test was selected to assess enamel bonding of resin composite after bleaching in this study. In contrast to the microtensile bond strength (μTBS) test, specimen preparation for the μSBS test is simpler and less affected by prismatic orientations of enamel.$^{15}$ The current μSBS results showed that in-office bleaching significantly decreased the μSBSs of the self-etch adhesives to enamel. The lower bond strengths to bleached enamel are believed to be mainly due to an imbalance in the redox potential caused by the bleaching agents. The residual oxygen from the bleaching agent interferes with resin polymerization. However, the two-step self-etch adhesive showed less decrease in bond strength than did the one-step self-etch adhesive. The two-step self-etch adhesive proved to have a high polymerization rate in the adhesive layer, while the polymerization of the one-step self-etch adhesive was compromised due to its greater hydrophilicity.$^1$ Several other studies have also shown similar results using different adhesives to bleached enamel.$^{1,2,14}$ A previous study showed that higher concentrations of hydrogen peroxide and carbamide peroxide produced greater reductions in bond strength and may also influence the strength of the enamel.$^{2,15}$

In self-etch adhesives, the functional monomer etches the enamel, thus allowing resin monomers to penetrate into the demineralized enamel and chemically interact with hydroxyapatite crystals.$^6$ SE2 and GPB contain 10-MDP as functional monomer. The ability of 10-MDP to chemically bond to HAp has been demonstrated. Since MDP is strongly acidic in aqueous solution, HAp is superficially dissolved, and then MDP-calcium salts deposit onto the modified HAp surface. Self-etching adhesives mildly demineralize enamel, resulting in shallower intercrystallite resin infiltration and less interprismatic resin tag formation, compared with phosphoric acid etch adhesives.$^{26,27}$

The two-step self-etch adhesive, Clearfil SE Bond 2, showed a statistically significantly higher bond strength to enamel than the one-step self-etch adhesive, G-Premio Bond, when tested alone and after selective etching.$^{23}$ In the control group without bleaching, selective etching with phosphoric acid led to a significant increase in the μSBS for SE2 but not GP. The different components of SE2 and GP seemed to have caused the difference in μSBS. SE2 is composed of a separate primer and bonding resin. The primer contains water to ionize MDP. Excess water on the enamel surface was sufficiently removed with a strong air
stream before application of the bonding resin. The bonding resin component comprises a microfilled resin containing bis-GMA-based hydrophobic dimethacrylates. On the other hand, GP is an all-in-one adhesive, comprised of a combination of resin monomers with water and an organic solvent. Previous studies demonstrated that the presence of water-rich phases in the adhesive can be detrimental to bond strengths and stability of all-in-one adhesives.4

The SE2-bleached groups showed that the majority of failures were types 1 and 2, due to lower bond strengths, whereas in SE2-PA groups, the majority of failures were types 4 and 5. One week after bleaching, SE2 showed type 1 failure in the control groups, but with PA, type 2 failures increased, which was different from the failure modes of the other PA-treated groups. Failures at the resin composite-enamel interface for GP, the control and immediate groups were types 1, 2, and 3. The phosphoric acid selective-etched groups for GP showed type 4 and 5 failures, as well as type 3, which increased compared with the other groups. This might be related to the low bond strength of this adhesive compared to SE2. For GP 1 week after bleaching, type 3 formed the majority of failures in those groups with and without phosphoric acid etching. Cohesive failure (type 3) was observed for all GP groups (with and without PA), as the mechanical properties of the material itself are low, which affected the μSBS test results.27

Nanoleakage has been described as leakage within nanometer-sized channels along an adhesive interface.19 In the case of an adhesive-dentin interface, nanoleakage can occur within/beneath the hybrid layer and/or in the adhesive layer in the absence of marginal gaps between resin composite and dentin.16,25 This phenomenon has been widely implicated as an important factor associated with how an adhesive interface may degrade.16,19 In the case of enamel, nanoleakage may be caused by insufficient infiltration of resin into the demineralized enamel or by incomplete polymerization of hydrophilic monomers in the nanometer-sized interfacial spaces.16,19 In the current study, no nanoleakage was observed at the interface in the SE2 groups, suggesting the occurrence of monomer penetration into the full depth of the etched enamel and also sufficient polymerization in situ. Nanoleakage was observed along the enamel prismatic planes only in the control group of GP, in which the silver deposits were scattered in the superficial enamel up to an approximate depth of 50 μm from the interface (Fig 5a). There may be a gap between the depth of demineralization and depth of monomer penetration and its polymerization, resulting in the creation of nanospaces. Interestingly, nanoleakage was not observed in the bleached specimens of GP, indicating that the bleaching procedure may influence the adhesive-enamel interface. As described above, a bleaching agent is able to denature enamel proteins, which may create macrospaces for more effective penetration of the adhesive monomer into enamel. The pH value of the TiON In-Office is around 6.0 (manufacturer’s data), which is sufficiently above the critical pH value of enamel dissolution (ca pH 5.5).

![Fig 8](image-url) SEM images of enamel-adhesive interface after acid-base challenge in GP. OL: outer lesion; B: bonding; E: Enamel; R: resin; ABRZ: acid-base resistant zone. a: control; b: immediately after bleaching B0; c: one week after bleaching B1; d: PA group; e: bleaching B0 and PA; f: bleaching B1 and PA. ABRZ was detectable in all groups.
Application of self-etch adhesives to enamel has been much debated.\cite{26} In self-etch adhesives, the acidic functional monomer behaves as the etching agent, but also allows resin monomers to penetrate into the demineralized enamel and chemically interact with HAp crystals. In this way, these crystals are protected from further acid attack, thereby allowing an ABRZ to form. Selective enamel etching has been considered a strategy for optimizing enamel bond strength of self-etch and universal adhesives. Since phosphoric acid etching is more aggressive than self-etching adhesives, resin monomers penetrate more deeply into the etched layer.\cite{26} The current results confirmed the formation of the enamel ABRZ in all adhesive groups (Figs 7 and 8). This is supported by Li et al,\cite{18} who were the first to report formation of an enamel ABRZ using an MDP-containing two-step self-etch adhesive. However, the morphological features were influenced by the adhesive, method of enamel etching, and bleaching. The SEM images demonstrated that SE2 created a durable adhesive-enamel interface, which is similar to that found in previous studies.\cite{8,32,33} GP created a thick (about 1 μm) ABRZ, and a funnel-shaped lesion adjacent to the ABRZ was observed.\cite{8,32,33} The lesion suggested a weak zone less able to resist acid-base attack beneath the bonding interface (Figs 8a and 8b).

Application of the bleaching agent created a thick (about 1 to 2 μm) ABRZ. This fact suggested that bleaching enamel may increase enamel porosity, leading to deeper penetration of the primer; however, residual oxygen from the bleaching agent may hinder polymerization of the adhesive, causing a decrease in the microshear bond strength. After 1-week storage of the bleached enamel, the lesion in the GP group was not observed at the interface, presumably because residual bleaching agent was no longer present in the bleached enamel.

In both SE2 and GP, similar images were observed with/without bleaching in the acid-etch groups. Li et al\cite{17} reported that phosphoric acid selective etching improved the enamel bonding performance of two-step self-etch adhesives. The present study confirmed that selective phosphoric acid etching was useful for bonding to enamel after bleaching. Especially for GP adhesive, phosphoric acid etching created a thicker ABRZ, about 5 μm thick, without a funnel-shaped erosion being created at the interface despite the effect of bleaching (Figs 8d to 8f). Sato et al\cite{26} also reported that the thickness of the ABRZ increased, that its edge showed an irregular wave-like shape when enamel was etched with PA, and enabled deeper penetration of GP adhesive into enamel to provide sufficient interaction with HAp. Those authors suggested that the penetration of resin monomers into the etched enamel surface may have encapsulated its crystal-light components to provide an effective seal and protect the outermost enamel from dissolution.

Based on the current results of this study, the null hypothesis was rejected. The enamel μSBSs were adversely affected by in-office bleaching followed immediately by bonding, but the μSBS recovered after 1-week storage.

**CONCLUSION**

In-office bleaching adversely affected the μSBS to enamel; however, the μSBS values could recover after 1-week of storage. Selective acid-etching may be effective to enhance enamel bond strength of the SE2 adhesive.

**ACKNOWLEDGMENTS**

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**REFERENCES**

Clinical relevance: The results of this study indicated that a one-week delay after the bleaching treatment in addition to selective phosphoric-acid etching promotes stable adhesive-enamel interfaces when applying two-step self-etching adhesives to enamel.