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Enamel and Dentin Bond Strength, Interfacial Ultramorphology and Fluoride Ion Release of Self-etching Adhesives During a pH-cycling Regime

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Purpose: This study evaluated the effects of pH cycling on fluoride release and bond strength of two self-etching adhesive systems to both enamel and dentin. The ultramorphology of the interfaces produced by the adhesive systems were also analyzed.

Materials and Methods: The buccal surfaces of bovine incisors were flattened to expose enamel and dentin, which were bonded with either Clearfil Protect Bond (CPB) or One-Up Bond F Plus (OBP). The bonded samples were prepared for microtensile bond strength (μTBS) testing, fluoride ion release, and transmission electron microscopy. pH cycling comprised demineralization (8 h/day) and remineralization (16 h/day) cycles for 8 days. The μTBS data were analyzed by two-way ANOVA, while fluoride release was analyzed using the Friedman and Wilcoxon tests.

Results: The adhesives presented similar bond strengths to enamel. However, the dentin bond strength of CPB was higher than that of OBP. pH cycling did not influence enamel or dentin μTBS. The amount of fluoride released from the bonded enamel and dentin was low and varied among the groups. The morphological evaluation showed that the thickness of the dentin hybrid layers was similar for both adhesives.

Conclusion: The pH-cycling regime did not affect enamel or dentin bond strengths. In enamel, both the self-etching adhesives tested presented similar bond strengths, but in dentin, Clearfil Protect Bond showed higher dentin bonding than One-Up Bond F Plus.

Keywords: dental enamel, dentin, adhesives, fluoride, bond strength.

J Adhes Dent 2015; 17: 27–34.
doi: 10.3290/j.jad.a33343

Submitted for publication: 02.01.14; accepted for publication: 05.11.14

Self-etching bonding systems were developed to facilitate the clinical use of bonding agents. Enamel and dentin are not previously etched with this type of bonding agent, since they contain acidic monomers, which have the ability to simultaneously demineralize the dental tissues and chemically bond to the mineral content during adhesive resin infiltration. During bonding procedures, the smear layer is incorporated into the hybridization process, forming a thin hybrid layer and short resin tags. These systems have been classified according to the number of application steps (one- or two-step) and the pH of the solution (mild, moderate, or strong).[20,23]

It has been previously demonstrated that for bonding to dentin tissue, the self-etching primers are as effective as the etch-and-rinse adhesive systems.[3,19] However, enamel etching with phosphoric acid has been proven to result in a stronger and more durable bond than the self-etching systems. To overcome this drawback, some authors have shown that the enamel must be previously ground with a diamond bur and/or etched with phosphoric acid.[4,21,25]

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Table 1 Composition and manufacturer's instructions of the bonding agents

Bonding agent	Composition (pH) (lot number)	Manufacturer's instructions
Clearfil Protect Bond (Batch # 00042A)	Primer: 2-hydroxyethyl methacrylate (25-45%), 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), 12-methacryloyloxydodecylpyridinium bromide (MDPB), hydrophilic aliphatic dimethacrylate, water, initiators, accelerators, dyes (pH 2.0) (18B) Bond: 2-hydroxyethyl methacrylate (20-40%), sodium fluoride (<1%), bisphenol A diglycidylmethacrylate, 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), hydrophobic aliphatic dimethacrylate, colloidal silica, dl-camphorquinone, initiators, accelerators (29A)	1. Apply primer gently for 20 s. 2. Dry with mild air flow. 3. Apply bond. 4. Gently air flow. 5. Light cure for 10 s. 6. Place resin composite.
One-Up Bond F Plus (Batch # 555)	Bottle A: 11-methacryloxy-1,1-un-decanedicarboxylic acid (MAC-10) (10-30%), bisphenol A polylthoxy methacrylate (10-30%), dibutyl hydroxyl toluene (<1%), methacryloyloxyalkyl acid phosphate (phosphoric acid monomer, 30-60%), methyl methacrylate (5-20%) (pH 0.7) (678M) Bottle B: 2-dimethylaminoethyl methacrylate (1-5%), 2-hydroxyethyl methacrylate (30-60%), dibutyl hydroxyl toluene (<1%), methyl methacrylate (5-20%), fluoroaluminosilicate glass, water (5-105) (pH 7.7) (660M)	1. Dispense one drop of each bottle and mix (pH: 1.2). 2. Apply and rub for 10 s. 3. Light cure for 10 s. 4. Place resin composite.

In order to prevent caries progression around the restorations, fluoride-based materials have become an attractive option, as fluoride could inhibit demineralization and enhance remineralization of lesions close to the restoration wall.[7] Although some etch-and-rinse dental adhesives contain fluoride, they seem to have a lower ability to inhibit secondary caries development when compared to glass-ionomer cements.[6,8,13,14] On the other hand, it has been previously reported that a specific self-etching adhesive system containing sodium fluoride and MDPB has the potential for artificial secondary caries inhibition around restorations.[9,11,12,18,22,24] This is an important property for a bonding agent, particularly in caries-challenged conditions. Therefore, it is important that such studies occur under a pH regime in order to more closely simulate an oral clinical condition and verify the pH influence over the fluoride release of the adhesive systems tested.

Given the need for further analyses of the enamel and dentin bonded interface with agents containing fluoride and MDPB, this study evaluated the fluoride release and the bond strength of two fluoride-containing self-etching adhesives bonded to either enamel or dentin under a pH-cycling regime. Additionally, the ultrastructural morphology of the resin/dentin and resin/enamel interfaces formed by the self-etching adhesives was observed. The null hypotheses tested were that (1) enamel and dentin bond strengths would not be affected by the adhesive system or pH-cycling regime used, and (2) the amount of fluoride released from the adhesive systems would be similar throughout the pH-cycling regime.

MATERIALS AND METHODS

This study was divided into four parts to evaluate the following variables: enamel and dentin bond strength as determined by the µTBS test; fracture pattern of the interface observed by scanning electron microscopy (SEM); fluoride release rate determined by ion-selective electrode; and ultramorphological analysis of the interface observed by transmission electron microscopy (TEM). Fifty-six freshly extracted bovine incisors (stored in a 0.05% thymol solution at 5°C) were used as experimental units and two bonding agents were selected: a two-step self-etching primer (Clearfil Protect Bond, Kuraray; Kurashiki, Japan) and a one-step self-etching adhesive (One-Up Bond F Plus, Tokuyama Dental: Tokyo, Japan). Composition, lot number, and manufacturer's instructions are listed in Table 1.

The bovine incisors were divided into eight groups (n = 7) according to the adhesive system used and the presence or absence of pH cycling:

1. Enamel bonded with Clearfil Protect Bond submitted to pH cycling;
2. Enamel bonded with Clearfil Protect Bond not submitted to pH cycling;
3. Enamel bonded with One-Up Bond F Plus submitted to pH cycling;
4. Enamel bonded with One-Up Bond F Plus not submitted to pH cycling;
5. Dentin bonded with Clearfil Protect Bond submitted to pH cycling;
6. Dentin bonded with Clearfil Protect Bond not submitted to pH cycling;
7. Dentin bonded with One-Up Bond F Plus submitted to pH cycling;
8. Dentin bonded with One-Up Bond F Plus not submitted to pH cycling.

Microtensile Bond Strength Testing, Fluoride Release Rate, and pH-Cycling Regime

The buccal surfaces of the bovine teeth were wet abraded with silicon carbide paper (#180) using a polishing machine (APL-4, Arotec; Cotia, SP, Brazil) to expose a 1.5- to 2.0-mm-thick flat enamel or dentin surface. Afterwards, a standard smear layer was created with a 600-grit silicon carbide paper for 10 s under running water. The adhesive systems were applied to either enamel

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or dentin according to the manufacturers' instructions (Table 1). After the application of the adhesives, resin composite blocks 6 mm in height were built up incrementally with three 2-mm-thick layers (shade A2, Filtek Supreme, 3M ESPE; St Paul, MN, USA). A light-curing unit (XL 2500, 3M ESPE) with an output of 600 mW/cm^2 was used to cure the adhesives and the composites for 10 and 20 s, respectively. The restored teeth were stored in distilled, deionized water at 37°C for 24 h and then sectioned parallel to the long axis into 1.0-mm-thick slabs using a water-cooled diamond saw (Isomet 1000, Buehler; Lake Bluff, IL, USA). Two slabs were used for TEM analysis, while the other slabs were further sectioned perpendicularly to produce 6 sticks per tooth, approximately 1.0 mm^2 in cross section. All the obtained sticks were protected with a nail varnish except for a 1-mm^2 area around the bonded interface. Before testing, half of the bonded sticks were stored in distilled water for 8 days at 37°C (non pH-cycling sticks), while the other half was submitted to pH cycling.[6]

Each bonded stick was attached to a microtensile testing device with cyanoacrylate glue (Super Bonder, Henkel/Loctite; Diadema, SP, Brazil) and tested in tension in a universal testing machine (Instron 4411; Grove City, PA, USA) at a 0.5 mm/min crosshead speed until failure. After testing, the specimens were carefully removed and the cross-sectional area at the site of fracture was measured with a digital caliper (727-6/150, Starret; Itu, SP, Brazil) to the nearest 0.01 mm. The cross-sectional area of each specimen was divided by the maximum tensile load at failure to calculate the stress at fracture (MPa). A single failure stress value was then calculated for each tooth by averaging the values of three sticks from each tooth.

Two-way ANOVA (adhesive system and pH cycling as the factors) was performed to determine the effect of the two self-etching adhesive systems, pH cycling, and their interaction on the microtensile bond strength. Tukey's post-hoc test was used to detect pairwise differences among the experimental groups. All statistical testing was performed at a preset alpha of 0.05. The fractured surfaces of the tested specimens were allowed to air dry overnight at 37°C. They were then sputter coated with gold (MED 010, Balzers-Union; Balzers, Liechtenstein) and examined by a single operator using a scanning electron microscope (VP 435 Leo; Cambridge, UK).

Failure patterns were classified as: type 1, adhesive along the enamel/dentin surface; type 2, cohesive within the adhesive layer; type 3, cohesive within the enamel/dentin; or type 4, mixed, when simultaneously exhibiting enamel or dentin, hybrid and/or adhesive layer, as well as remnants of composite.

Three sticks from each single tooth were used for pH-cycling analysis. Therefore, 168 bonded sticks were individually immersed in a demineralizing solution (1.4 mM Ca, 0.9 mM P, 0.05 M acetate buffer, pH 5, 3.1 ml/mm^2) and remineralizing solution (1.5 mM Ca, 0.9 mM P, 0.1 M Tris buffer, pH 7.0, 1.6 ml/mm^2) for 8 and 16 h, respectively. This pH-cycling regime was carried out for 8 days.[6] The solutions were renewed daily, and 2 ml of each was collected on the 1st, 4th, and 8th day for the fluoride release analysis. One of the two slabs set aside for TEM analysis was also submitted to the pH-cycling regime, but the solutions were not analyzed for fluoride release. To quantify the fluoride released, TISAB III (Total Ionic Strength Adjustment Buffer, Thermo Orion; Beverly, MA, USA) was added at a 1:10 ratio (buffer:solution). The amount of fluoride released was determined by using an ion-selective electrode (96-09, Thermo Orion) connected to an ion analyzer (Thermo Orion) that was calibrated with a series of eight standard solutions in duplicate. From the fluoride released on the 1st, 4th, and 8th day, the amount of ions released in the remineralizing and demineralizing solutions was calculated. The daily amount of fluoride released by each tooth was calculated by the sum of the amount released in the demineralizing and remineralizing solutions. After pH cycling, the sticks were tested in tension.

A single μTBS value was then calculated for each tooth by averaging the values of the sticks from that tooth. The data for the fluoride release was analyzed using Friedman and Wilcoxon tests ($\alpha = 0.05$).

TEM Analysis of Bonded Interfaces

Two selected slabs from each tooth were used in this part of the study. The slabs were fixed in Karnovsky's solution, post fixed in osmium tetroxide, dehydrated in an ascending ethanol series (30% to 100%), and embedded in epoxy resin. Ultrathin sections (90 nm thick) were prepared with an ultramicrotome (UC6, Leica Microsystems; Heerbrugg, Switzerland) using a diamond knife and collected on 100-mesh Formvar-coated copper grids. The ultrathin sections embedded in epoxy-resin were observed in a TEM (Zeiss EM 900, Zeiss; Munich, Germany). Representative areas of the bonded interfaces were photographed at magnifications ranging from 700X to 85,000X.

RESULTS

Bond Strength Testing

A summary of the enamel and dentin bond strengths for the experimental groups is shown in Tables 2 and 3, respectively. Two-way ANOVA indicated that the adhesive system and pH-cycling factors ($p > 0.05$) did not change the enamel bond strength results. The same analysis indicated that only the adhesive system factor ($p < 0.05$) significantly influenced the dentin microtensile bond strength and that the interaction between the two factors ($p < 0.05$) was significant.

Comparing the data from the two adhesive systems, One-Up Bond F Plus yielded significantly lower bond strength than Clearfil Protect Bond in the dentin. In the enamel, the adhesives did not differ significantly from each other.

Fracture Pattern Analysis

Figures 1 and 2 show the proportional prevalence (%) of the failure patterns in the experimental groups for enamel and dentin, respectively. All groups bonded to enamel

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Table 2 **Means (standard deviation) of enamel bond strength of adhesive systems tested in this study (MPa)**

Adhesive system	Without pH cycling	With pH cycling
Clearfil Protect Bond	25.8 (5.0)^{Aa}	21.7 (4.5)^{Aa}
One-Up Bond F Plus	20.1 (7.1)^{Aa}	19.5 (4.4)^{Aa}

Values of groups having similar superscript letters were not significantly different (uppercase letters for columns, lowercase letters for rows).

Table 3 **Means (standard deviation) of dentin bond strength of adhesive systems tested in this study (MPa)**

Adhesive system	Without pH cycling	With pH cycling
Clearfil Protect Bond	26.4 (8.0)^{Aa}	22.5 (1.9)^{Aa}
One-Up Bond F Plus	14.9 (6.8)^{Ba}	19.6 (4.3)^{Ba}

Values of groups having similar superscript letters were not significantly different (uppercase letters for columns, lowercase letters for rows).

showed adhesive failure along the enamel surface (type 1). The type 3 failure (cohesive within the enamel) was observed only in the group where Clearfil Protect Bond was submitted to pH cycling. All the tested groups showed mixed failures when bonded to dentin (type 4). Type 1 failure was observed only in the group where One-Up Bond F Plus was submitted to pH cycling, while type 3 failure was obtained only in the Clearfil Protect Bond group.

Fluoride Release Rate

The fluoride release profiles during the pH-cycling regime of the bonded sticks at each period are shown in Figs 3 (enamel) and 4 (dentin). For the enamel, lower concentrations of fluoride ions were detected on the 4th day for Clearfil Protect Bond, while higher amounts of fluoride were measured on the 8th day for One-Up Bond F Plus ($p < 0.05$). In the dentin, Clearfil Protect Bond showed the opposite behavior, releasing more fluoride on the 4th day of pH cycling ($p > 0.05$). One-Up Bond F Plus adhesive released more fluoride ions on the last day (8th) compared to the first day ($p > 0.05$).

TEM of Bonded Interfaces

Figures 5 through 12 depict the structures of resin/tooth interfaces for the self-etching adhesive systems tested. TEM images show the enamel and dentin tissues, enamel crystallites, dentin hybrid layer, and adhesive layer. The pH-cycling regime produced more porous enamel than was evident in the non-cycled groups (Figs 6 and 10), while no significance difference was noted in the dentin.

The adhesive systems produced thin hybrid layers with a similar thickness, ranging from 0.8 µm to 1.2 µm (Figs 7, 8, 11, and 12). The glass fillers from both self-etching adhesives can be seen in the adhesive layers (Figs 6 through 8 and 10 through 12). Only Clearfil Protect Bond exhibited sodium fluoride crystals within the adhesive layer (Figs 6 through 8).

DISCUSSION

The bond strength of both self-etching adhesives to enamel was statistically similar, even when submitted to pH cycling. According to the TEM micrographs, both self-etching systems were properly bonded to the enamel, although the pH cycling created some demineralized areas. These regions do not seem to interfere with the enamel bond strength. At higher magnifications, it is possible to see the continuous contact of adhesives with the enamel crystallites[5] as a separate step for the bonding procedures (Figs 5 and 9), even in the absence of phosphoric acid etching.

pH cycling simulated a cariogenic challenge,[6] and instead of using the restored teeth, the microtensile specimens (bonded sticks) were submitted to the demineralizing and remineralizing solutions. This allowed the solutions to stay in contact with the entire tooth/adhesive interfaces as well as the enamel and dentin areas adjacent to the composite restorations before being tested for microtensile bond strength. The demineralizing and remineralizing solutions may have affected only the superficial area of the specimens, since no reduction in enamel or dentin bond strength was observed vs the groups not subjected to pH cycling. Conversely, Peris et al[17] evaluated the microtensile bond strength and caries formation at bonded dentin/resin interfaces submitted to pH cycling and found that the artificial caries model chosen decreased the dentin bond strength of the adhesives tested, including Clearfil Protect Bond. In that experiment, the hourglass-shaped slabs were subjected to four de/remineralization cycles consisting of 4 h immersion in demineralizing solution followed by 20 h immersion in remineralizing solution. The de/remineralizing solutions used by Peris et al had the same components as those used in the present study, but the concentration of Ca, P and buffer solutions were considerably different. However, the pH of the demineralizing solution was 4.3 and the authors used a greater amount of demineralizing solution per mm^2 of exposed dentin, exposing the bonded interface to a higher cariogenic risk, which could explain why

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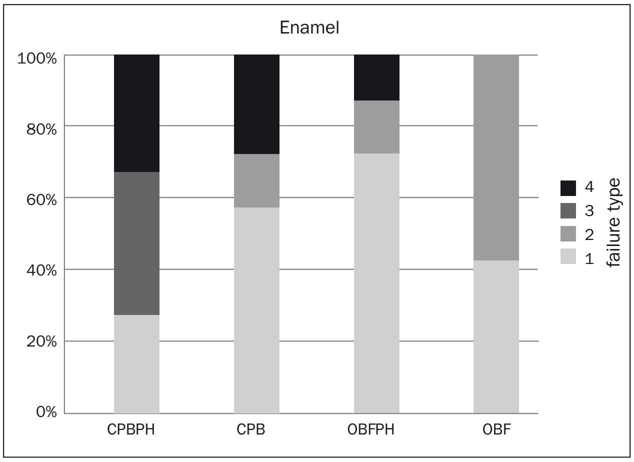

Fig 1 Distribution of failure modes among experimental groups for enamel. Type 1: adhesive along the enamel surface; type 2: cohesive within the adhesive layer; type 3: cohesive within the enamel; type 4: mixed, when simultaneously exhibiting adhesive layer and remnants of composite). CPB: Clearfil Protect Bond; CPBPH: CPB submitted to pH cycling; OBF: One-Up Bond F Plus; OBFPH: OBF submitted to pH cycling.

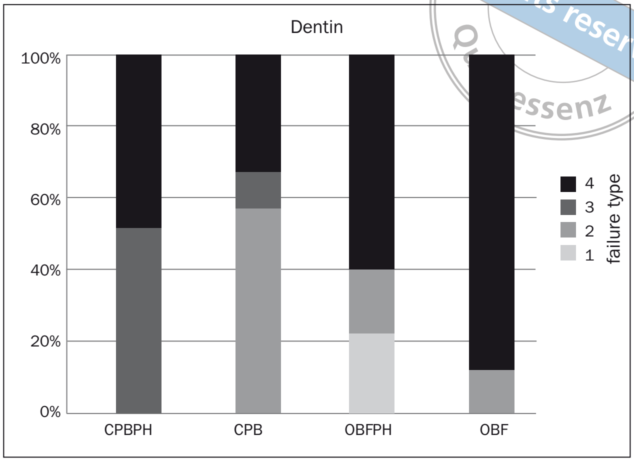

Fig 2 Distribution of failure modes among experimental groups for dentin. Type 1: adhesive along the dentin surface; type 2: cohesive within the adhesive layer; type 3: cohesive within the dentin; type 4: mixed, when simultaneously exhibiting adhesive layer and remnants of composite). CPB: Clearfil Protect Bond; CPBPH: CPB submitted to pH cycling; OBF: One-Up Bond F Plus; OBFPH: OBF submitted to pH cycling.

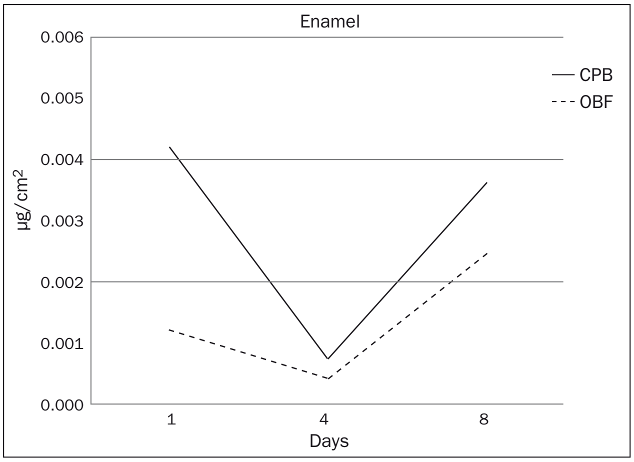

Fig 3 Fluoride-releasing behavior in enamel for adhesive systems during the pH-cycling regime (fluoride released [µg/cm^2] as a function of elapsed time for up to 8 days). CPB: Clearfil Protect Bond; OBF: One-Up Bond F Plus.

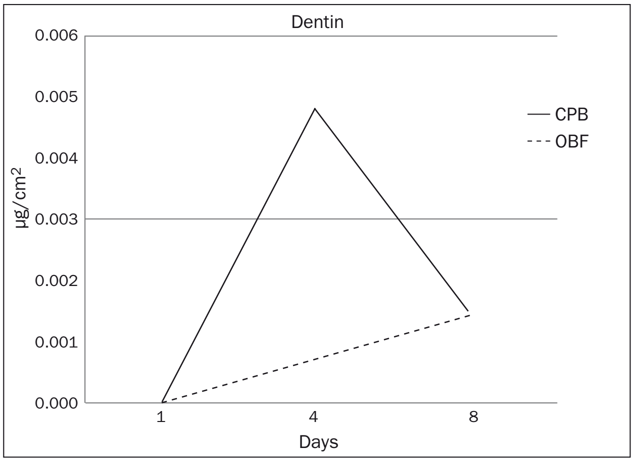

Fig 4 Fluoride-releasing behavior in dentin for adhesive systems during the pH-cycling regime (fluoride released [µg/cm^2] as a function of elapsed time for up to 8 days). CPB: Clearfil Protect Bond; OBF: One-Up Bond F Plus.

the caries model chosen by those authors significantly decreased bond strength.[17]

The fluoride ions released during the immersion of specimens in the demineralizing and remineralizing solutions following pH cycling were detected by an ion-selective electrode. The source of fluoride for the Clearfil Protect Bond self-etching adhesive is the sodium fluoride salt solution present only in the bonding resin bottle at a concentration < 1%. Sodium fluoride was added to this more hydrophobic adhesive, while an antibacterial component (bromide) was introduced into a monomeric composition of the acidic primer. Crystals of sodium fluoride salt can be observed at the adhesive layer in the TEM micrographs (Figs 6 through 8). The ionization of fluoride and the release of ions occurs when water is in contact with the salt, as sodium fluoride is soluble in water.

The component responsible for fluoride release in One-Up Bond F Plus is the fluoro-aluminosilicate glass solution that is present in bottle B. In general, One-Up Bond F Plus seemed to release less fluoride than Clearfil Protect Bond, especially in the enamel on the first day of evaluation and in the dentin on the fourth day. The reason for the profile release of fluoride for Clearfil Protect Bond during pH cycling may be the higher concentrations of the ion within the adhesive solution or the fluoride source, which for this adhesive is the sodium fluoride salt. In this study, the amount of fluoride released was low because the specimens were the bonded sticks used for the micro-

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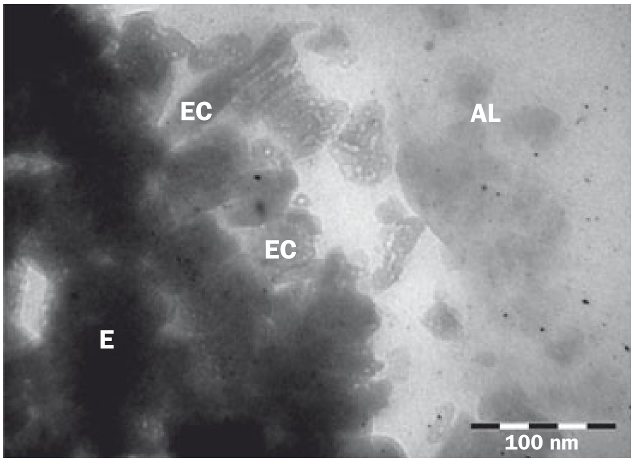

Fig 5 Representative TEM micrograph of the resin/enamel interface produced by Clearfil Protect Bond without pH cycling (original magnification 85,000X). E: enamel; EC: enamel crystallites; AL: adhesive layer.

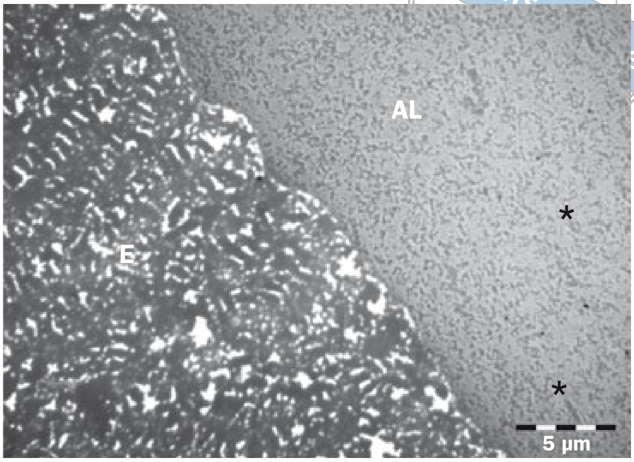

Fig 6 Representative TEM micrograph of the resin/enamel interface produced by Clearfil Protect Bond with pH cycling (original magnification 1100X). The enamel submitted to pH cycling showed porosities that are represented by white spaces among the crystals at the interface and in the inner portion of enamel. E: enamel; AL: adhesive layer; asterisk: sodium fluoride crystals.

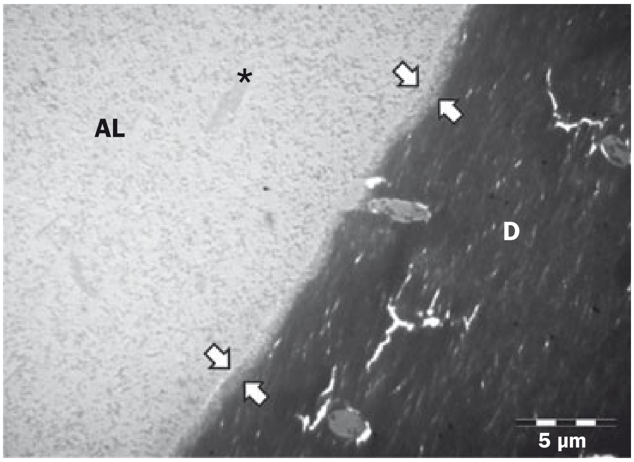

Fig 7 Representative TEM micrograph of the resin-dentin interface produced by Clearfil Protect Bond without pH cycling (original magnification 1100X). D: dentin; AL: adhesive layer; asterisk: sodium fluoride crystals.

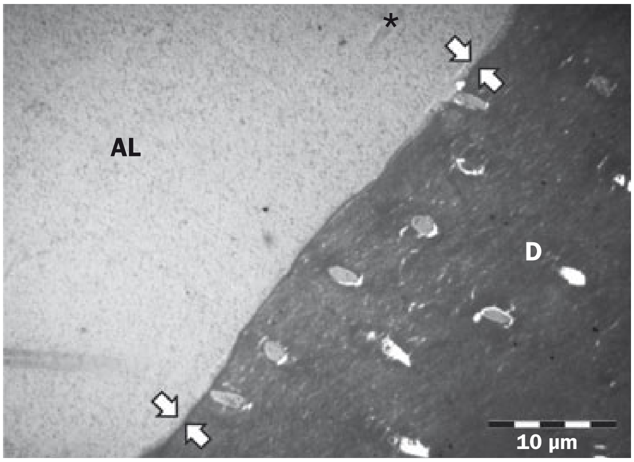

Fig 8 Representative TEM micrograph of the resin-dentin interface produced by Clearfil Protect Bond with pH-cycling (original magnification 700X). Arrows indicate the hybrid layer. D: dentin; AL: adhesive layer; asterisk: sodium fluoride crystals.

tensile test, which are smaller than the usual specimens tested in other studies. Hara et al[6] evaluated the fluoride release of adhesive systems and glass-ionomer cement using disk-shaped specimens 10.0 mm in diameter and 0.5 mm in thickness, and the cumulative fluoride released ranged from 3.6 to 19.2 mgF/cm^2. Furthermore, Aguiar et al[1] measured the fluoride released from self-adhesive resin cements using disk-shaped specimens of the same size as Hara et al, but the cumulative fluoride amount ranged from 1.3 to 13.6 mgF/cm^2.[1] Peris et al[17] found instead that the fluoride release of the dentin-bonded interface of hourglass-shaped specimens was lower than 0.03 ppm F$^-$. In the current study, the fluoride released was also low, not stable for either adhesives, and dependent on the evaluation time. The highest fluoride concentration released from self-etching adhesives was approximately 0.005 mgF/cm^2 for Clearfil Protect Bond and less than 0.003 mgF/cm^2 for One-Up Bond F Plus. It is possible that the low concentration found in the current study and elsewhere[17] is related to the dimensions of the specimen evaluated: disk-shaped specimens would release a higher amount of fluoride.[1,6]

Regarding dentin bond strength, Clearfil Protect Bond – a two-step self-etching primer adhesive – showed higher tensile bond strength than One-Up Bond F Plus, a one-step self-etching primer adhesive. Clearfil Protect Bond contains 12-methacryloyloxydodecylpyridinium bromide (MDPB) and 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) as functional monomers.[22,26] 10-MDP is able to form a strong chemical bond to the hydroxyapatite of the enamel or

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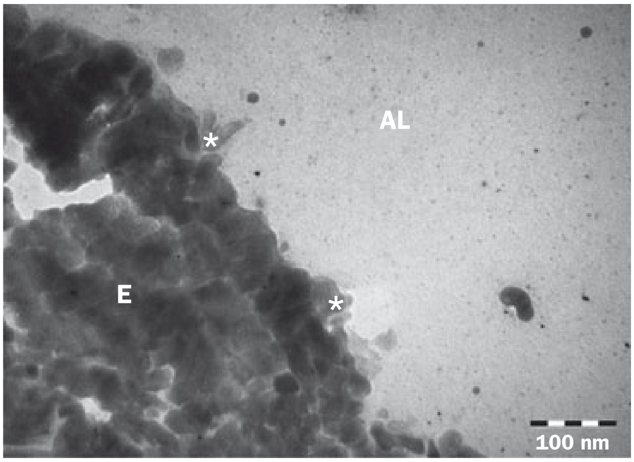

Fig 9 Representative TEM micrograph of the resin/enamel interface produced by One-Up Bond F Plus without pH cycling (original magnification 50,000X). E: enamel; asterisk: enamel crystallites; AL: adhesive layer.

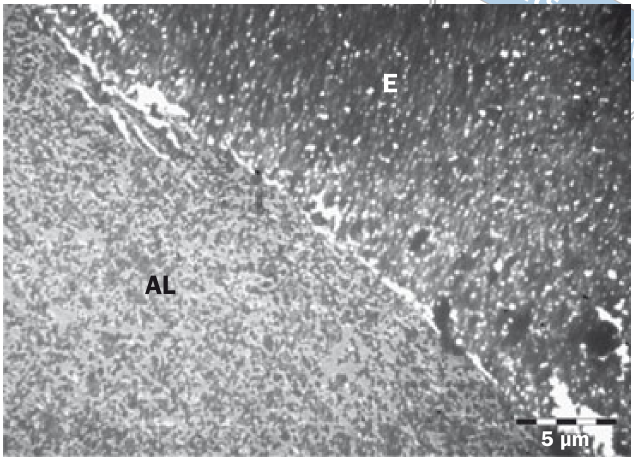

Fig 10 Representative TEM micrograph of the resin/enamel interface produced by One-Up Bond F Plus with pH cycling (original magnification 1100X). The enamel showed porosities that are represented by white spaces among the crystals at the interface and in the inner portion of enamel. E: enamel; AL: adhesive layer).

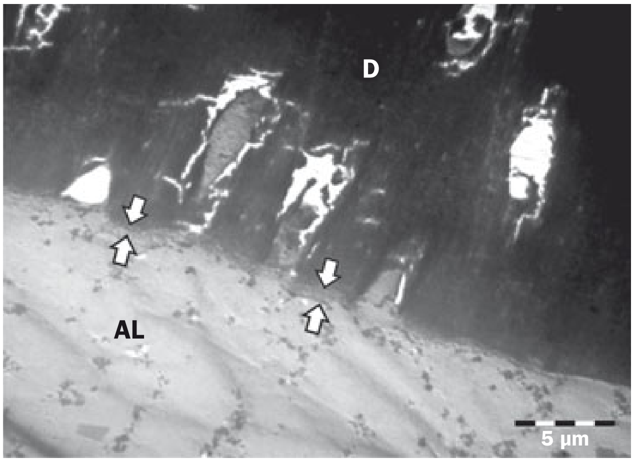

Fig 11 Representative TEM micrograph of the resin/dentin interface produced by One-Up Bond F Plus without pH cycling (original magnification 1100X). Arrows indicate the hybrid layer. D: dentin; AL: adhesive layer.

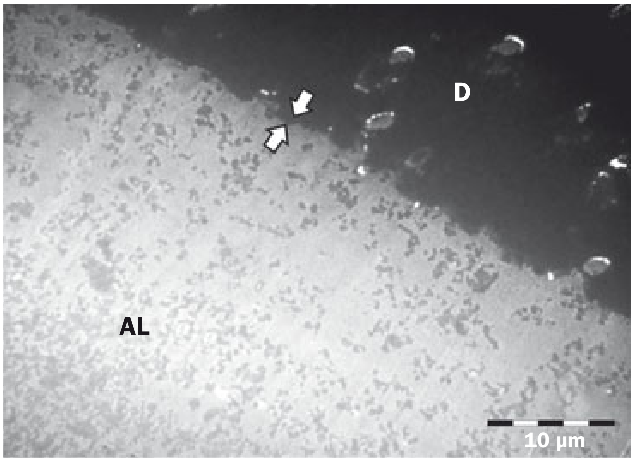

Fig 12 Representative TEM micrograph of the resin/dentin interface produced by One-Up Bond F Plus with pH cycling of the bonded stick (original magnification 700X). Arrows indicate the hybrid layer. D: dentin; AL: adhesive layer.

dentin, contributing to the improvement of the adhesion.[27] In addition, the hydrophobic layer applied over the primed dentin surface reduces the hydrophilicity of the hybrid layer, increasing the polymerization rate of the adhesive systems as well as increasing the dentin bond strength.[2,3]

One-Up Bond F Plus self-etching adhesive contains six monomers, some hydrophobic and others hydrophilic. The hydrophilic functional monomers are 11-methacryloxy-1, 1-un-decanedicarboxylic acid (MAC-10), 2-hydroxyethyl methacrylate (HEMA), and methacryloyloxyalkyl acid phosphate. However, MAC-10 is the adhesion-promoting monomer and, like 10-MDP, its molecular structure has ten carbon atoms at the spacer group. Both monomers (10-MDP and MAC-10) are hydrolytically stable, since this type of spacer group makes these monomers hydrophobic. The chemical structures of monomers, especially those of the spacer group, determine the properties and characteristics of the adhesive systems, such as viscosity, solubility, hydrolysis susceptibility, wetting, and penetration behavior.[26] These might substantially influence the bond strength and fluoride-release profile. In addition to the positive outcome of MDPB-containing adhesive for dentin bond strengths observed in this study, another important aspect of this monomer is its reported long-lasting antibacterial effect. MDPB is incorporated and immobilized in the hybrid layer network and it cannot be leached away.[10,16]

In the present study, bonding to enamel and dentin resulted in different failure patterns. The adhesive failure along the surface was prevalent for the adhesives tested on the enamel. As the TEM images showed only a superfi-

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cial interaction between adhesives and enamel, the tested specimens tended to fracture adhesively (type 1) with a low incidence of mixed and cohesive failures (types 2 and 4).[16] Conversely, the tested self-etching adhesives formed a distinct hybrid layer when bonded to the dentin, ranging from 0.8 to 1.2 µm in thickness. The hybrid layer can affect the type and complexity of the fracture mode, resulting in a higher incidence of mixed fractures,[26] involving all the components of tooth/composite bonded interfaces investigated in this study. The first null hypothesis was partially accepted, as the bond strength was influenced by the type of adhesive system used when tested on dentin, but pH cycling did not influence the enamel or dentin bond strengths. As the amount of fluoride released was not constant from the first to the last day of pH cycling, the second hypothesis was accepted.

CONCLUSION

The type of self-etching adhesive system tested influenced the dentin bond strength. Eight days of pH-cycling regime did not change the enamel or dentin bond strengths for either adhesive. Further studies are recommended to clarify the effects of longer pH-cycling regimes on the bonding performance of self-etching adhesives to enamel and dentin. The resin-dentin interdiffusion zone and the superficial interaction between the enamel and adhesives were observed in TEM images.

ACKNOWLEDGMENTS

This investigation was supported by Research Grants 06/53828-2 from the State of São Paulo Research Foundation, FAPESP, São Paulo, Brazil, and Research Grants 303587/2007-5 from the National Council for Research and Development, CNPq, Brasília, Brazil.

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Clinical relevance: In dentin, Clearfil Protect Bond presented higher bond strengths than One-Up Bond F Plus, but the amount fluoride released from the bonded enamel and dentin was low and varied among the groups. The chemical structure of the self-etching adhesives determines their properties and these might substantially influence the bond strength behavior and fluoride-release profile.