Influence of Phosphoric Acid Pretreatment on Self-Etching Bond Strengths

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ABSTRACT

Purpose: The purpose of this investigation was to evaluate the influence of phosphoric acid pretreatment on shear bond strength of two self-etching bonding systems to enamel and dentin.

Materials and Methods: Forty-eight extracted third human molar teeth were mounted, embedded into polystyrene resin, polished with 600-grit aluminum oxide papers, and randomly divided into four groups ($n = 12$): group 1—Clearfil Liner Bond® 2V (Kuraray Co. Ltd., Osaka, Japan); group 2—One Up Bond F® (Tokuyama Corp., Tokyo, Japan); group 3—phosphoric acid (Condicionador Dental Gel®, Dentsply Ind. Com. LTDA, Rio de Janeiro, Brazil) and Clearfil Liner Bond 2V; group 4—phosphoric acid and One Up Bond F. In groups 3 and 4 the substrate was pre-etched for 15 seconds with 37% phosphoric acid, rinsed, and dried with an air stream. In all groups adhesive systems were applied according to manufacturers' instructions and light cured; then a restorative composite resin (TPH Spectrum®, Dentsply Ind. Com. LTDA) was placed in a polytef matrix and cured. The specimens were stored in humidity for 7 days at 37°C. The shear bond strength test was performed in a universal test machine with a crosshead speed of 0.5 mm/min. All procedures were repeated for the dentin evaluation. Mean values were analyzed with two-way analysis of variance and Duncan tests ($p < .05$).

Results: The values obtained are listed in decreasing order: enamel—group 3 = 24.6 MPa, group 4 = 23.6 MPa, group 1 = 19.2 MPa, group 2 = 8.5 MPa; dentin—group 1 = 17.2 MPa, group 2 = 16.1 MPa, group 4 = 13.1 MPa, group 3 = 11.3 MPa.

Conclusions: Under the conditions of this study, enamel etching with 37% phosphoric acid provided statistically significant higher shear bond strength values, regardless of the adhesive system. However, in dentin, for Clearfil Liner Bond 2V, phosphoric acid pretreatment negatively affected bond strength values.

CLINICAL SIGNIFICANCE

The use of self-etching systems in composite-to-enamel bonding restorative techniques still needs improvement when compared with the high bond strengths obtained with phosphoric acid treatment. However, lower shear bond strengths were observed in dentin when phosphoric acid was used in association with either adhesive system.

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The presence of a smear layer created during enamel or dentin preparation complicates resin bonding,[1] so its removal with acidic conditioners for the development of a hybrid layer is both convenient and essential to achieving optimal adhesion and improving bond strengths.[2] The smear layer varies from 1 to 10 µm in thickness and is composed mainly of hydroxyapatite, altered collagen, and bacteria,[1,2] with an external surface formed by gel-like denatured collagen.[3]

Since the introduction of the enamel acid-etch technique by Buonocore in 1955,[4] adhesion of restorative materials to the hard components of the tooth structure has been possible. Conditioning agents include phosphoric, maleic, nitric, 10% citric, and ethylenediaminetetraacetic acids,[5,6] which are used to remove the smear layer and to demineralize the underlying intact enamel and dentin, creating microporosities that retain resin materials.[1,4] The most commonly used conditioner, phosphoric acid, may denature some peptides exposed during the removal of smear layer, depending on the phosphoric acid concentration and the time of dentin surface exposure.[7,8]

Currently, the vast majority of effective dentin bonding agents involve acid-etching, rinsing, and drying steps for the removal of the bur-prepared smear layer before primer or adhesive application.[6,9] With these total-etch bonding systems, the demineralization depth may frequently be deeper than the zone of monomer diffusion and impregnation owing to a weak capability of the bonding agents to effectively wet and infiltrate into the partially demineralized superficial dental surface.[9–11]

Recent developments in bonding have reintroduced the concept of using the smear layer as a bonding substrate.[12,13] The development of self-etching primers raised the possibility of incorporating the original smear layer into the hybrid layer.[13,14] The approach to preventing the collapse of demineralized dentin is to leave the smear layer in place but to use acidic monomers—esters of bivalent alcohols with methacrylic and phosphoric acid or derivates[9]—to etch through the smear layer into the underlying enamel or dentin and to avoid rinsing the conditioned surface.[13,14] This prevents the loss of dentin mass but solubilizes enough apatite crystallites from around collagen fibrils to permit infiltration of adhesive monomers.[15] Demineralization and monomer infiltration of the dentin take place simultaneously, thereby creating a hybrid layer with no need for separately applied acid etching and priming.[14,16]

Therefore, adhesive resin systems that require simple procedures—resulting in high bonds to any substrate, independent of depth, region, and mineralization of the substrate—remain a necessity for adhesive dentistry.[5] The development of self-etching primers brought about a new alternative in bonding systems and procedures; however, many concerns about the efficacy of these systems have arisen. Smear layers reinforced with impregnated resin, hybridized smear layers, may be too weak to provide strong durable mechanical properties.[17]

The purpose of this work was to determine the influence of phosphoric acid pretreatment on shear bond strength of two self-etching bonding systems to enamel and dentin. The null hypothesis tested was that phosphoric acid pretreatment has no effect on the bond strength of self-etching primers to enamel and dentin.

MATERIALS AND METHODS

For this in vitro study, 48 freshly extracted human molar teeth were selected. After thorough débriding and polishing with a slurry of pumice and water in a rubber prophylaxis cup at a low speed, the teeth were stored in a 0.1% thymol buffered solution until preparation. A 30 mm^2 piece from the central part of the buccal face of the crown was obtained per tooth. Each piece then was placed in a 1.9 cm diameter polyvinyl chloride ring, which was filled with self-curing polystyrene (Piraglass®, Piracicaba, São Paulo, Brazil), embedding the teeth.

The embedded teeth were ground on a water-cooled mechanical grinder (Maxigrind® Solotest, São Paulo, Brazil) using 180-, 320-, 400-, and 600-grit aluminum oxide abrasive paper (Carborundum Abrasivos, São Paulo, Brazil) to obtain flat standardized enamel surfaces. The polished surfaces were inspected with a stereomicroscope (Meiji Techno America, San Jose, CA, USA) at times 20 magnification to determine the presence of any dentin surfaces. If dentin surfaces were present, the specimen was excluded from the experiment.

Specimens were randomly assigned to four equal groups according to the materials tested ($n = 12$). Before the adhesive application, the bonding area of each tooth was demarcated by placing a piece of vinyl tape over the tooth surface, in which a 3 mm diameter hole had been punched.

The procedures used for each group are described in Table 1. For groups 3 and 4, enamel surfaces were pre-etched with 37% phosphoric acid (Condicionador Dental Gel®, Dentsply Ind. Com. LTDA, Rio de Janeiro, Brazil) for 15 seconds and rinsed with water for 15 seconds. Excess water was removed by blotting, leaving the tooth surface visibly moist. The adhesive systems' composition, batch number, and manufacturer are outlined in Table 2.

A 3 mm diameter detachable polytef ring mold with a central hole 3 mm in diameter and 5 mm deep was mounted on the pretreated enamel surfaces to apply the filling material. A composite resin (TPH Spectrum®, Dentsply Ind. Com. LTDA) was inserted in two increments each 2.5 mm high, which were both light cured in the mold

TABLE 1. ADHESIVE SYSTEMS* APPLICATION PROTOCOL PER STUDY GROUP.

Group 1: Clearfil Liner Bond 2V

1. Mix primers A and B. Apply to a moist surface with continuous scrubbing for 30 s.
2. Dry surface for 5 s with an air syringe.
3. Apply one coat of Clearfil Liner Bond 2V liquid adhesive.
4. Allow slight air dry with an air syringe for 2 s.
5. Light cure for 20 s.

Group 2: One Up Bond F

1. Mix primers A and B. Apply to a moist surface with continuous scrubbing for 20 s.
2. Light cure for 20 s.

Group 3: phosphoric acid and Clearfil Liner Bond 2V

1. Surface condition with etchant for 15 s.
2. Rinse with water for 15 s.
3. Dry with an air syringe for 5 s to remove excess water.
4. Mix primers A and B. Apply to a moist surface with continuous scrubbing for 30 s.
5. Dry surface for 5 s with an air syringe.
6. Apply one coat of Clearfil Liner Bond 2V liquid adhesive.
7. Allow slight air dry with an air syringe for 2 s.
8. Light cure for 20 s.

Group 4: phosphoric acid + One Up Bond F

1. Surface condition with an etchant for 15 s.
2. Rinse with water for 15 s.
3. Dry with an air syringe for 5 s to remove excess water.
4. Mix primers A and B. Apply to a moist surface with continuous scrubbing for 20 s.
5. Light cure for 20 s.

*See Table 2 for details about the adhesive systems used.

TABLE 2. COMPOSITION, BATCH NUMBERS, AND MANUFACTURERS OF ADHESIVE SYSTEMS TESTED.

Adhesive System	Manufacturer	Batch Nos.	Composition
Clearfil Liner Bond® 2V	Kuraray Co. Ltd., Osaka, Japan	00073C, 00073B, 00120B	Primer A: MDP, hydrophilic dimethacrylate, CQ; primer B: HEMA, water, N,*N*-diethanol p-toluidine; bond liquid A: MDP, HEMA, hydrophobic dimethacrylate, CQ, N,*N*-diethanol p-toluidine, silanized colloidal silica
One Up Bond F®	Tokuyama Corp., Tokyo, Japan	020, 517	Primer A: adhesive phosphoric monomer MAC-10, photo initiator 1; primer B: water, glass aluminum silicate fluoride, photo initiator 2 (borate)

CQ = dil-camphorquinone; HEMA = 2-hydroyxylethyl methacrylate; MDP = 10-methacryloyloxy methacrylate.

for 40 seconds and, again, without the polytef ring mold, for 40 seconds while moving the light to ensure cure of the total cylinder. The intensity of the light unit used for all bonding procedures was periodically checked with a radiometer (Demetron/Kerr, Danbury, CT, USA) and ranged around 550 mW/cm^2. Restorative procedures were done following a random sequence. All specimens were stored in a humid environment at 37°C for 7 days prior to testing.

Shear bond strength tests were performed in a Universal Testing Machine® (EMIC Ltda, São José dos Pinhais, Parana, Brazil), with the dental surface parallel to the crosshead machine's trajectory. A steel knife-edge was placed over the specimen so that the shear force was directly on the bond interface. The specimens were loaded continuously until failure at a crosshead speed of 0.5 mm/min.[18] The force at which the composite dislodged from the enamel surfaces was recorded, and the shear bond strength (MPa) of each specimen was calculated from the cross-sectional area of the composite cylinder. The fracture sites of the debonded surfaces were determined with a stereozoom microscope under times 15 magnification.

After enamel shear tests, the same specimens previously used had dentin surfaces exposed on a water-cooled mechanical grinder using 320-, 400-, and 600-grit aluminum oxide abrasive papers. The exposed dentin surfaces were inspected with a stereozoom microscope to ensure that no enamel remained.

The specimens were randomly assigned to four equal groups (n = 12). Bonding and restorative procedures, storage interval, shear bond testing, and examination of the specimens were exactly the same as those performed for enamel evaluation.

STATISTICAL ANALYSIS

Bond strength data from enamel and dentin surfaces were subjected separately to two-way analysis of variance (ANOVA) to determine whether there were significant differences among groups ($p < .05$). Duncan's multiple range test for pairwise contrasts was used to detect differences in shear bond strength among pairs of groups. The statistical analysis was processed with STATA™ software system (STATA Corp., College Station, TX, USA).

RESULTS

Enamel

The mean shear bond strengths and SDs for enamel are displayed in Table 3. Two-way ANOVA revealed significant differences in bond strengths among the groups. Duncan's post hoc test disclosed three statistically different subsets at a 95% CI. This ranking showed that the highest mean shear bond strengths were achieved after phos-

TABLE 3. SHEAR BOND STRENGTHS OF ADHESIVE SYSTEMS TO ENAMEL AND FAILURE MODES.

Adhesive System	Mean SBS ± SD (MPa)*	Failure Mode†		
		No. of Adhesive	No. of Cohesive	No. of Mixed
CLB	19.2 ± 4.5[b]	10	0	2
PA + CLB	24.6 ± 6.2[a]	10	0	1
OUB	8.5 ± 4.9[c]	9	2	1
PA + OUB	23.6 ± 3.9[a]	9	1	2

CLB = Clearfil Liner Bond; OUB = One Up Bond F; PA = phosphoric acid; SBS = shear bond strength.
*Means with the same letter are not significantly different at $p < .05$.
†Adhesive = adhesive failure in the interface; cohesive = cohesive failure in enamel; mixed: failure in both the enamel and adhesive interface.

phoric acid conditioning independent of the adhesive system. Without phosphoric acid pretreatment, Clearfil Liner Bond 2V produced higher shear bond strength values than did One Up Bond F system, which was classified in the lowest subset.

Dentin

The mean values obtained in each dentin experimental group are listed in Table 4. The two-way ANOVA test revealed statistical differences ($p < .05$). The Duncan test was also applied at the significance level of 95% to identify statistically significant groups. Phosphoric acid pretreatment adversely affected resin-dentin bond strength for Clearfil Liner Bond 2V. This group showed statistically lower shear bond strength when compared with non-pretreated Clearfil Liner Bond 2V and One Up Bond F groups. Clearfil Liner Bond 2V was affected the most by phosphoric acid etching, whereas One Up Bond F presented statistically similar values independent of the pretreatment.

The fracture patterns of the specimens in both enamel and dentin are given in Tables 3 and 4, respectively. Adhesive failure was the prevalent type of failure independent of the substrate and the surface treatment. In enamel, cohesive failures were only present in groups in which phosphoric acid was applied. Five of the 47 specimens had cohesive failures of dentin, with most of these occurring when Clearfil Liner Bond 2V was used in the absence of phosphoric acid pretreatment.

DISCUSSION

The adhesion mechanism of self-etching bonding agents is based on smear layer penetration, demineralization of the superficial underlying substrate, and monomer diffusion enhancement into the demineralized dentin, facilitating hybrid layer formation.[9,16,19] Therefore, fewer steps are necessary to perform an adhesive restoration. Multiple treatment steps are routinely required with many dental adhesive systems. The combining of the conditioning and priming steps into a single treatment step for both enamel and dentin is an obvious improvement in adhesive dentistry.[5] The time saving and clinical efficiency of the combined conditioner and primer is certainly a significant advantage when compared

TABLE 4. SHEAR BOND STRENGTHS OF ADHESIVE SYSTEMS TO DENTIN AND FAILURE MODES.

Adhesive System	Mean SBS ± SD (MPa)*	Failure Mode†		
		No. of Adhesive	No. of Cohesive	No. of Mixed
CLB	17.2 ± 6.8[a]	7	2	2
PA + CLB	11.3 ± 2.9[b]	10	1	1
OUB	16.1 ± 3.3[a]	11	1	0
PA + OUB	13.1 ± 4.2[ab]	10	1	1

CLB = Clearfil Liner Bond; OUB = One Up Bond F; PA = phosphoric acid; SBS = shear bond strength.
*Means with the same letter are not significantly different at $p < .05$.
†Adhesive = adhesive failure in the interface; cohesive = cohesive failure in enamel; mixed: failure in both the enamel and adhesive interface.

to the many multiple-step adhesive systems currently available.[3]

The aim of the present study was to evaluate the influence of phosphoric acid pretreatment on shear bond strengths of one self-etching primer and one self-etching adhesive system, Clearfil Liner Bond 2V and One Up Bond F, in enamel and dentin. This investigation revealed the potential of self-etching agents in creating strong resin-enamel bonding.

Adhesion of composite resin materials to enamel has become a routine and reliable aspect of restorative treatment since Buonocore proposed that phosphoric acid could be used to transform the surface of enamel "to render it more receptive to adhesion."[4,20] Subsequent research indicated that the removal of the smear layer and the creation of enamel microporosities allowed the formation of tag-like resin extensions, which is the paramount mechanism of bonding of resin to phosphoric acid–etched enamel.[6,21]

Combining the phosphoric acid preconditioning and the acidic self-etching monomers' effects afforded high-quality hybridization and reliable bond strength on grounded enamel surfaces. Results of the present study revealed that this association is promising for bonding resin to human enamel. However, an additional procedure is not a real advantage since the main role of modern adhesive dentistry is to provide strong reliable bond strengths with simplified operatory steps. The inclusion of one more step for self-etching agents defeats the goal of these systems, which includes combining the conditioning and priming steps.

The shear bond strength results of the present study suggested that one- and two-step self-etching systems—One Up Bond F and Clearfil Liner Bond 2V, respectively—could achieve a relatively high bond strength (about 24 MPa) to ground enamel after phosphoric acid application. Nevertheless, the bond strength without this extra step was significantly lower for both adhesive systems. Additionally, One Up Bond F presented statistical values even lower than did Clearfil Liner Bond 2V when no pretreatment was conducted.

When applied to enamel, a more mineralized and acid-resistant substrate, phosphoric acid might remove the smear layer, lowering its buffering capacity and leaving the enamel surface more receptive to self-etching primer diffusion. The low pH of acidic conditioners (1.2 from One Up Bond F, 2.8 from Clearfil Liner Bond 2V, and 0.6 from phosphoric acid, according to the manufacturers) had no detrimental effect on bond strength values of either adhesive systems. On the other hand, in dentin, this tendency was not clear among the systems studied.

In dentin, acidic agents remove the smear layer, demineralize the dentin surface, open the dentin tubules, and increase the microporosity of the intertubular dentin.[1] The interaction of the etching agents with dentin is limited by the buffering effect of the hydroxyapatite, smear layer, and protein components.[22] A layer of denatured collagen and residual smear layer particles may form on the dentin surface and prevent the collagen network from being completely exposed.[23,24] Bonding to dentin represents a great challenge as dentin is an intrinsically wet organic tissue penetrated by a tubular labyrinth containing the odontoblastic process, which communicates with the pulp.[19,25]

Concerning the dentin-adhesive resin bonding via hybridization, the depth of demineralization and that of monomer diffusion have to be considered.[9] Self-etching agents are very effective in penetrating the dentin while simultaneously promoting monomer impregnation at the same depth.[5,14,16,19]

Hybrid layers produced by these systems are, however, usually thinner, with a limited resin-infiltrated dentin surface layer.[10,12,14] As a result, transition from resin to nondemineralized dentin is assumed to be free from defects, in comparison to the results with total-etch agents.[9,10,12,14] Ferrari and colleagues,[26] evaluating the formation of a hybrid layer, resin tags, and adhesive lateral branches by three different enamel dentin bonding systems, reported that the lengths of resin tags produced by Prime & Bond® 2.0 (Dentsply Petropolis, Rio de Janerio, Brazil) and Scotchbond Multi-Purpose Plus® (3M ESPE, St. Paul, MN, USA) groups (total-etch systems) were longer than those found when the Clearfil Liner Bond 2V self-etching primer was applied for 30 or 60 seconds.

In a study investigating the sealing ability of Clearfil Liner Bond 2V on enamel and dentin Class V cavities under different etching times, Ferrari and colleagues found that the 60-second application time of the self-etching primer seemed to be more reliable than did a shorter conditioning time in terms of leakage and scanning electron micrographic evaluation.[27]

Despite these results, when the self-etching bonding systems tested in this study were used associated with phosphoric acid pretreatment in dentin, significant lower shear bond values and morphologic changes were found. Excessive etching of the dentin surface might have decreased bond strength owing to an incomplete infiltration of the adhesive to the base of the overetched demineralized collagen network.

The lowest shear bond strength found for both adhesive systems may have been caused by the strong acidity of conditioners when applied to a less mineralized substrate like dentin. Removal of dentinal smear layer by phosphoric acid application might have disabled the buffering ability of the demineralized dentin matrix relative to the acidity of the primer, resulting in an overetched dentin surface without complete infiltration of the monomers through this demineralized subsurface.

One Up Bond F yielded a small decrease in dentin bond strength after phosphoric acid pretreatment, and no significant difference was found among the groups that used this adhesive system. Increased etching seemed to have a negligible effect on bond strength of Clearfil Liner Bond 2V to dentin, resulting in an average value of 11 MPa.

Results of the present in vitro study indicated that the use of self-etching bonding agents in composite-to-enamel bonding restorative techniques still needs improvements as the highest bond strength values were obtained with the phosphoric acid pretreatment. In dentin the changes in bond strength after phosphoric acid application were different between the bonding systems, and the Clearfil Liner Bond 2V system was adversely affected.

Bond strength data derived from the present study warranted rejection of the hypothesis advanced, that phosphoric acid pretreatment would have no effect on the bond strength of self-etching systems to enamel and dentin. More research is required to improve the bonding efficacy of self-etching primer and self-etching adhesive systems to different clinical substrates and to evaluate the long-term success of these materials.

CONCLUSIONS

Under the conditions of the present study, it was found that both self-etching bonding systems produce good adhesion in dentin, but that bonding to enamel is still unreliable and should be improved. The use of One Up Bond F and Clearfil Liner Bond 2V on enamel, according to manufacturers' directions, resulted in low shear bond strengths. Dentin shear bond strengths of these self-etching systems might be negatively influenced by the phosphoric acid prior to acidic primer application.

DISCLOSURE

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