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Bonding Durability of Single-Step Adhesives to Previously Acid-Etched Dentin

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Clinical Relevance

To achieve optimum bond strengths, acid etching of dentin prior to the application of single-step self-etch adhesive systems should be avoided.

SUMMARY

This study investigated the effect of phosphoric acid etching on the dentin bond strength of five single-step self-etch adhesive systems; Absolute, Clearfil tri-S Bond, Fluoro Bond Shake One, G-Bond and One-Up Bond F Plus. Bovine mandibular incisors were mounted in self-curing resin and the facial surfaces were wet ground with #600 SiC paper. Adhesives were applied on the prepared dentin surfaces with and without prior phosphoric acid etching and light irradiated. Resin composite was condensed into a mold (ø4x2 mm), light irradiated and stored in water at 37°C. Four groups (n=10) were made per adhesive system: with and without prior acid etching and with and without thermal cycling between 5°C and 55°C for 10,000 cycles. The specimens were tested in a shear mode at a crosshead speed of 1.0 mm/minute. Two-way ANOVA, Student *t*-test and Tukey HSD test at a level of 0.05 were done. For specimens without prior acid etching, the mean bond strengths to bovine dentin ranged from 12.8 to 17.1 MPa and ranged from 6.7 to 13.3 MPa for specimens with prior acid etching after 24 hours storage in water. When the specimens were subjected to thermal cycling, the mean bond strengths ranged from 10.7 to 24.8 MPa for the specimens without prior acid etching and 4.6 to 13.9 MPa for the specimens with prior acid etching. The changes in dentin bond strength were different among the

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adhesive systems tested. Failure modes were commonly adhesive failure associated with mixed failure for specimens with prior acid etching. For specimens without prior acid etching, failures in composite and dentin were increased. From the results of this *in vitro* study, prior acid etching might be not acceptable for increasing the dentin bond strengths of single-step self-etch adhesive systems.

INTRODUCTION

To reduce technique sensitivity that affects the bonding ability of adhesive systems, steps required for bonding procedures have been reduced.[1] New approaches for bonding restorative materials to tooth substrates without phosphoric-acid etching, such as self-etch systems, have been introduced. Recently, single-step self-etch adhesive systems that combine the functions of a self-etching primer and a bonding agent have been developed.[2] The use of single-step self-etch adhesives may eliminate technique-sensitive factors that negate the bonding ability of the restorations. The single-step self-etch adhesive is applied to the tooth surface prior to resin composite placement to ensure maximum adhesion through the mechanism of improved monomer penetration into the tooth substrate as well as improved wettability of the tooth surface via the resin components. However, the etching effect of mild self-etch adhesives has been reported to interact less effectively with thicker smear-layer covered dentin.[3] The residual smear layer disturbs monomer infiltration into underlying dentin, leading to a more degradation-sensitive interface.[4] This raises the question whether the creation of a dentin/adhesive interaction zone is sufficient to create stable adhesion after thermal cycle stress.[5]

Evaluation for bonding durability is important, since stability of the bond between the restoration and tooth substrate may be related to the long-term clinical success of tooth-colored restorations.[6] Although the most reliable conclusions about the performance of dental adhesive systems in the oral environment are derived from long-term clinical trials, long-term aqueous storage of the bonded specimen or subjecting it to thermal cycling may give some information about degradation of the material.[7] A thermal-cycling test is the process of subjecting specimens to an extreme temperature that simulates intraoral conditions.[8] Also, this test induces stress between the tooth substrate and the restorative material due to differences in the coefficient of thermal expansion. It has been reported that the effect of thermal cycling on the bond strength of multi-step bonding systems depended on the bonding system used and the number of thermal cycles.[9-11]

There are studies reporting different conclusions about the bonding effectiveness to dentin of a two-step self-etch adhesive system when placed either with or without prior phosphoric acid etching. A study was conducted to test the effect of an initial phosphoric acid etch on the bond strength of a two-step self-etch adhesive to dentin, which concluded that acid etching should be limited to enamel, because of impaired dentin bond strengths.[12] Another study reported no significant differences among the different smear layer treatments with the same adhesive system.[13] As the components of single-step self-etch adhesives differ from those of two-step self-etch adhesives, the effect of prior acid etching may also vary. For single-step self-etch adhesives, little information is available regarding removal of the smear layer with a previous phosphoric acid etch to facilitate adhesive diffusion through the dentin.

The current study determined the effect of prior acid etching on the dentin bond strengths of single-step self-etch adhesive systems to bovine dentin by means of measurement of the shear bond strength, fracture mode and field-emission scanning electron microscopy (FE-SEM) observation of the treated dentin surfaces and resin-dentin interface. The effect of thermal cycling on the dentin bond strengths of single-step self-etch adhesive systems to bovine dentin was evaluated. The hypothesis tested was that prior acid etching would increase the bond strength to bovine dentin.

METHODS AND MATERIALS

Materials Tested

Single-application self-etch adhesive systems, with the combination of resin composite evaluated, included: Absolute/Esthet•X (Dentsply Sankin, Tokyo, Japan), Clearfil tri-S Bond/Clearfil AP-X (Kuraray Medical Inc, Tokyo, Japan), Fluoro Bond Shake One/Beautifil (Shofu Inc, Kyoto, Japan), G-Bond/Gradia Direct (GC Corp, Tokyo, Japan) and One-Up Bond F Plus/Estelite ∑ (Tokuyama Dental Corp, Tokyo, Japan). They are listed in Table 1. All adhesive systems were used in combination with the manufacturers' restorative resins. Application protocols suggested by each manufacturer are listed in Table 2.

The visible-light activating unit, Optilux 501 (sds Kerr, Danbury, CT, USA), was used, and the power density (800 mW/cm^2) of the light was checked with a dental radiometer (Model 100, sds Kerr) before making the specimens.

Bond Strength Test

A total of 200 mandibular incisors extracted from cattle and stored frozen (-20°C) for up to two weeks were used as a substitute for human teeth.[14-15] After removing the roots with a slow-speed saw using a diamond-impregnated disk (Isomet, Buehler Ltd, Lake Bluff, IL, USA), the pulps were removed and the pulp chamber of each tooth was filled with cotton to avoid penetration

Table 1: *Materials Tested*

Code	Adhesive System (Manufacturer)	pH	Main Components	Lot #	Composite (Shade)	Lot #
AB	Absolute (Dentsply Sankin)	0.8	4-MET, PPTM, PEM-F, UDMA, acetone, initiator	393-016	Esthet•X (Y-E)	0501132
CT	Clearfil tri-S Bond (Kuraray Medical)	2.7	MDP, bis-GMA, HEMA, initiator, ethanol, water, stabilizer, filler, hydrophobic dimethacrylate	040219	Clearfil AP-X (A2)	00987A
FB	Fluoro Bond Shake-One (Shofu)	2.2	4-AET, 4-AETA, bis-GMA, water, PRG, fluoroaluminosilicate glass, initiator, solvent	A: MS-13 B: MS-13	Beautifil (A2)	020135
GB	G-Bond (GC)	2.8	4-MET, UDMA, acetone, water, silanated colloidal silica, initiator	031015	Gradia Direct (A2)	0312121
OF	One-Up Bond F Plus (Tokuyama Dental)	1.4	MAC-10, HEMA, MMA multifunctional methacrylic monomer, fluoroaluminosilicate glass, water, photoinitiator (aryl borate catalyst)	A: 551F-2 B: 551F-2	Estelite (A2)	J279

4-MET: 4-methacryloxyethy trimellitate, PPTM: pyrophosphate tetramethacrylate, PEM-F: fluoromethacryloxy cyclophosphazen, UDMA: urethane dimethacrylate, MDP: 10-methacryloxydecyl di-hydrogen phosphate, bis-GMA: 2, 2bis[4-(2-hydrogen-3-methacryloyloxypropoxy)phenyl]propane, HEMA: 2-hydroxyethyl methacrylate, 4-AET: 4-acryloyloxyethyl trimellitic acid, 4-AETA: 4-acryloyloxyethyl trimellitic anhydride, PRG: pre-reacted glass filler, MAC-10: 11-methacryloxy-1,1-undecandicarboxylic acid, MMA: methyl methacrylate

Table 2: *Application Protocols of Single-step Self-etch Systems*

Code	Application Protocol
AB (Single Bottle)	Dispense one drop of liquid into well. Apply to moist dentin for 3 seconds twice. Subject to a mild stream of air for 3 seconds to dry and light cure for 10 seconds.
CT (Single Bottle)	Dispense one drop of liquid into well. Apply to dried dentin for 20 seconds. Subject to a relatively strong stream of air to dry and light cure for 10 seconds.
FB (Two Bottles)	Mix equal amounts of bond agent A and B. Apply to dried dentin for 20 seconds. Briefly air dry and light cure for 10 seconds.
GB (Single Bottle)	Dispense one drop of liquid into well. Apply dried dentin for 10 seconds. Strong air dry and light cure for 10 seconds.
OF (Two Bottles)	Mix equal amounts of bond agents A and B until a pink homogenous liquid mixture is obtained. Apply to dried dentin for 10 seconds with agitation and light cure 10 seconds.

of the embedding media. The labial surfaces were ground on wet 240-grit silicon carbide (SiC) paper to a flat dentin surface. The tooth bonding surfaces were determined to be free of any remnants of enamel upon visual inspection. Each tooth was then mounted in self-curing acrylic resin (Resin Tray II, Shofu Inc, Kyoto, Japan) to expose the flattened area and placed in tap water to reduce the temperature rise from the exothermic polymerization reaction of the acrylic resin. Final finish was accomplished by grinding on wet 600-grit SiC paper. After ultrasonic cleaning with distilled water for one minute to remove the excess debris, these surfaces were washed and dried with oil-free compressed air.

A piece of adhesive tape with a 4-mm diameter hole was firmly attached to define the area for bonding. Half of the specimens were phosphoric acid etched (Etchant, 3M ESPE) for 15 seconds, followed by 10 seconds of rinsing with a three-way syringe and air dried. The adhesive was applied onto the dentin surface according to the manufacturers' instructions (Table 2). The adhesive-applied surfaces were dried with oil-free compressed air and irradiated with the curing unit. A Teflon (Sanplatec Corp, Osaka, Japan) mold 2.0-mm high and 4.0-mm in diameter was used to form and hold the restorative resin onto the dentin surface. The resin composite was condensed into the mold and cured for 30 seconds. The Teflon mold and adhesive tape were removed from the specimen 10 minutes after light irradiation.

Bonded specimens from each group were divided into two treatment groups of 10 specimens each for testing: Group 1) stored in 37°C distilled water for 24 hours after placement, without thermal cycling, and Group 2) stored in 37°C distilled water for 24 hours, followed thermal cycling between 5°C and 55°C for 10,000 cycles.

The specimens in each group were tested in shear mode using a knife-edge testing apparatus in a universal testing machine (Type 4204, Instron Corp, Canton, MA, USA) at a crosshead speed of 1.0 mm/minute. Shear bond strength values in MPa were calculated from the peak load at failure divided by the specimen surface area.

After testing, the specimens were examined in an optical microscope (SZH-131, Olympus Ltd, Tokyo, Japan) at 10x magnification to define the location of the bond failure. The type of failure was determined based on the percentage of substrate-free material as follows: adhesive failure, mixed failure (cohesive failure in composite and adhesive resin with partial adhesive failure), cohesive failure in dentin and cohesive failure in composite.[14]

Statistical Analysis

A statistical analysis was done to show how the bond strengths were influenced by thermal cycling. The data for each group were subjected to ANOVA, followed by the Student *t*-test and Tukey HSD test at a level of 0.05 within each adhesive system. The statistical analysis was carried out with the Sigma Stat software system (Ver 3.1, SPSS Inc, Chicago, IL, USA).

FE-SEM

The treated dentin surfaces and restorative/dentin interfaces were observed by FE-SEM. For the etched tooth surface observation, the dentin surfaces were treated, then rinsed with acetone and water to remove the self-etching adhesive. For the ultrastructure observation of the resin/dentin interface by FE-SEM, bonded specimens stored in 37°C distilled water for 24 hours were embedded in self-curing epoxy resin (Epon 812, Nisshin EM, Tokyo, Japan), then stored at 37°C for 12 hours. The embedded specimens were then sectioned to the diameter of the resin composite post and the surfaces of the cut halves were polished with an Ecomet 4/Automet 2 (Buehler Ltd) using SiC papers of 600, 1200 and 4000-grit size, successively. The surface was finally polished on a special soft cloth using diamond paste (Buehler Ltd) with a grit size of 1.0 µm. All the SEM specimens were dehydrated in ascending concentrations of *tert*-butanol (50% for 20 minutes, 75% for 20 minutes, 95% for 20 minutes and 100% for two hours), then transferred to a critical-point dryer for 30 minutes. These surfaces were then subjected to Argon-ion beam etching (Type EIS-200ER, Elionix Ltd, Tokyo, Japan) for 30 seconds, with the ion beam (accelerating voltage 1.0 kV, ion current density 0.4 mA/cm^2) directed perpendicular to the polished surface. The surfaces were coated in a vacuum evaporator, Quick Coater Type SC-701 (Sanyu Denshi Inc, Tokyo, Japan), with a thin film of Au. The specimens were observed in FE-SEM (ERA 8800FE, Elionix Ltd).

RESULTS

Tables 3 and 4 show the mean shear bond strengths to bovine dentin and failure modes after the test. For the specimens stored for 24 hours in water, the mean bond strengths to bovine dentin ranged from 12.8 to 17.1 MPa without prior acid etching and ranged from 6.7 to

Table 3: *Effect of Prior Acid Etching on Bond Strength (Mean (SD) in MPa) to Bovine Dentin After 24 Hours Storage in Distilled Water*

	Bond Strength		Fracture Mode	
	w/o Acid Etching	w Acid Etching	w/o Acid Etching	w Acid Etching
AB	12.8 (3.5)^{b,c,d}	6.7 (1.5)^{a}	9/1/0/0	10/0/0/0
CT	17.1 (1.7)^{e}	13.3 (2.3)^{c,d}	7/2/0/1	2/8/0/0
FB	13.9 (2.7)^{d}	13.3 (2.0)^{c,d}	8/2/0/0	8/2/0/0
GB	13.4 (1.2)^{c,d}	8.3 (1.6)^{a,b}	5/5/0/0	6/4/0/0
OF	13.7 (2.6)^{d}	8.6 (1.3)^{a,b,c}	5/2/0/3	7/3/0/0

SD: standard deviation, N=10
Failure mode: Adhesive failure/Mixed failure/Cohesive failure in dentin/Cohesive failure in composite
The values with same superscript letters indicate no statistical difference (p>0.05)

Table 4: *Effect of Prior Acid Etching on Bond Strength (Mean (SD) in MPa) to Bovine Dentin After 10,000 Thermal Cycles*

	Bond Strength		Fracture Mode	
	w/o Acid Etching	w Acid Etching	w/o Acid Etching	w Acid Etching
AB	13.8 (1.7)^{c}	4.6 (2.9)^{a}	8/2/0/0	10/0/0/0
CT	24.8 (1.4)^{d}	13.9 (2.4)^{c}	3/5/0/2	7/3/0/0
FB	10.7 (3.0)^{b,c}	11.0 (2.2)^{c}	6/4/0/0	7/3/0/0
GB	10.9 (1.9)^{b,c}	5.7 (1.6)^{a}	5/5/0/0	8/2/0/0
OF	14.1 (1.5)^{c}	6.1 (2.0)^{a}	2/6/1/1	10/0/0/0

SD: standard deviation, N=10
Failure mode: Adhesive failure/Mixed failure/Cohesive failure in dentin/Cohesive failure in composite
The values with same superscript letters indicate no statistical difference (p>0.05)

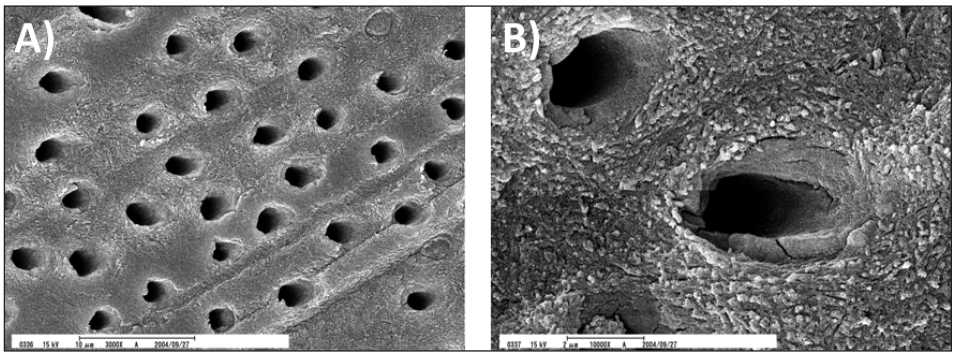

Figure 1: *SEM observation of a dentin surface conditioned with Clearfil tri-S Bond, which was not light-cured, followed by rinsing with acetone and water (Figure 1A, original magnification 3,000x). The micrograph shows removal of the smear layer and the peritubular dentin appears to be slightly etched (Figure 1B, original magnification 10,000x).*

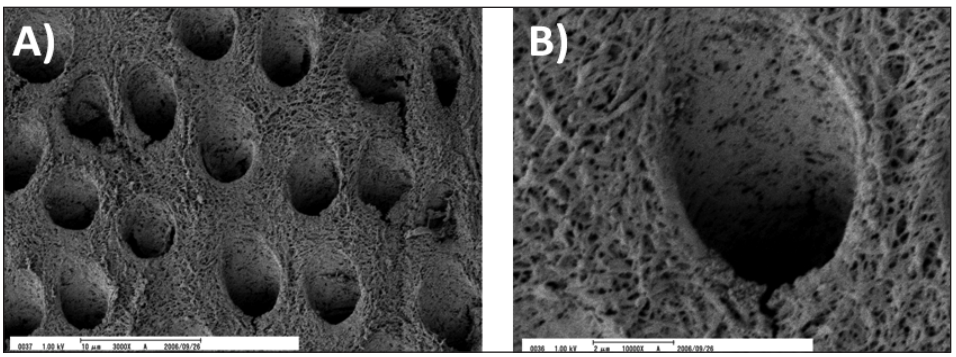

Figure 2: *SEM observation of a dentin surface conditioned with prior acid etching followed by TS, which was not light-cured, followed by rinsing with acetone and water (Figure 2A, original magnification 3,000x). Complete removal of the smear layer and plugs, as well as complete dissolution of peritubular dentin is shown. The intertubular dentin appears more aggressively etched and the collagen fibers appear to be much more distinguishable (Figure 2B, original magnification 10,000x).*

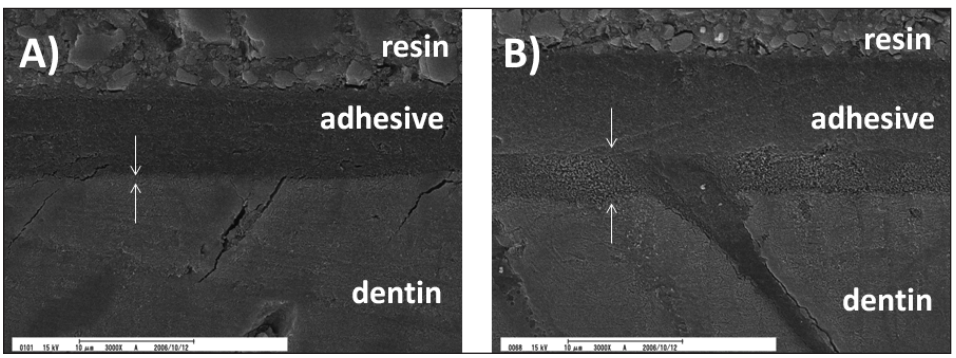

Figure 3: *SEM observation of dentin-resin interface bonded with CT (Figure 3A, original magnification 3,000x). The hybrid-like layer appeared to be very thin (0.2~0.3 μm) and difficult to discern at some locations. When the dentin was prior acid etching followed by TS, a hybrid-like layer could be clearly observed (4~6 μm) and appeared to be more granular than those of specimens bonded without prior acid etching.*

13.3 MPa for the specimens with prior acid etching. When the specimens were subjected to thermal cycling, the mean bond strengths ranged from 10.7 to 24.8 MPa for specimens without prior acid etching and 4.6 to 13.9 MPa for specimens with prior acid etching. The changes in dentin bond strengths were different among the adhesive systems tested. Based on the statistical analysis, the effect of prior acid etching on dentin bond strengths did not depend on specimen storage conditions. There was not a statistically significant interaction between prior acid etching and storage conditions. For the materials tested, significant decreases in dentin bond strengths were found with prior acid etching, except for FB, regardless of storage conditions. There was a trend toward differences in failure mode between the with- and without prior acid etching groups. The predominant mode of failure was mixed failure for the specimens without prior acid etching, and it changed to adhesive failure for the specimens with prior acid etching, regardless of storage conditions.

SEM observations of the dentin surface after acidic solution application are shown in Figures 1 and 2. From the SEM pictures of the treated dentin surfaces, the dentin etching pattern with exposed collagen fibrils was more marked in specimens with prior acid etching. For specimens without prior acid etching, the smear layer was, in general, removed, but the smear plugs remained in some tubule orifices. For specimens with prior acid etching, the smear layer was totally removed and denatured collagen fibrils were observed.

SEM observations of the resin-dentin interface are shown in Figure 3. The tooth resin interface in both groups revealed excellent adaptation, with the formation of a transitional layer between the adhesive resin and the tooth structure. For prior acid etching specimens, a so-called hybrid layer was clearly observed. The thickness of this layer was about 0.5 μm for specimens without prior acid etching and 3~5 μm for specimens with prior acid etching.

DISCUSSION

The success of dentin bonding has been believed to be dependent on the infiltration of resin monomers into acid etched dentin followed by polymerization *in situ*.[16-17] The hydrophilic monomers may form a complex structure, with exposed collagen fibrils and partially demineralized dentin containing residual hydroxyapatite. When the adherend dentin surfaces were treated with phosphoric acid, significant decreases in bond strength were observed for the single-step self-etch adhesives except for FB, which showed no significant difference. Thus, the hypothesis that prior acid etching would increase dentin bond strengths was rejected for all the adhesive systems tested. Lower bond strengths obtained for the group prior to acid etching might be explained by incomplete infiltration of the demineralized collagen network by resin monomers and subsequent poor adaptation of the adhesive to the underlying dentin.[18-19] The collapse of unsupported collagen after phosphoric acid treatment and exposure to air has been shown to inhibit resin monomer penetration to the entire depth of decalcified dentin.[20] Since the single-step self-etch adhesives contain water and low molecular weight hydrophilic monomers, collapsed collagen fibrils after acid etching might partially re-expand. While the hydrophilic components may penetrate, the resin component may have been hampered in penetrating the exposed collagen network, leading to a decrease in bond strength.

The hybrid layer thickness of the single-step self-etch adhesive was very thin after argon-ion etching of the resin-dentin interface. Though different hybrid layer thicknesses were observed, no correlation with dentin bond strength was found.[2] Hybrid layer thickness and the presence of resin tags may not be the only mechanisms influencing dentin bond strengths. Other factors that might play an important role are the cohesive strengths of the adhesives and resin composites. The intrinsic strength of the bonding agent and the degree of porosity of dentin substrate are believed to be important factors influencing bond strength. The role of collagen fibrils in dentin bonding has not been proven, and some reports have revealed that collagen fibrils offer no direct, quantitative contribution to interfacial bond strength.[21] It has been demonstrated that, in addition to collapse, part of the demineralized dentin collagen is in a denatured, unstable state, making it sensitive to hydrolysis and enzymatic degradation.[22] Also, a dense web of exposed collagen creates a low surface energy that results in an increase in the contact angle of adhesive resin.

After thermal cycling, a significantly higher bond strength was observed for CT when the dentin surfaces were not treated with phosphoric acid. On the other hand, no significant difference was found for the prior acid-etching group. The complex thermal cycling process offers many possibilities for the entrapment of flaws inside the dentin-resin interface.[23] The thermal cycling test induces stress between the tooth substrate and restorative material due to differences in the coefficient of thermal expansion. During the thermal cycling test, hot water may also accelerate hydrolysis of the resin and extract poorly polymerized resin oligomers,[24] leading to a decrease in mechanical properties of the polymers. Most single-step adhesives contain hydroxyethyl methacrylate (HEMA), which can polymerize in the presence of water to form microporous hydrogel.[25] Differential water movement across the cured adhesive layer may occur in the presence of increased concentrations of dissolved inorganic ions, uncured and water-soluble hydrophilic resin monomers or dissolved collagen/proteoglycan components within the oxygen inhibited layer of the cured adhesive.[26] This water sorption will plasticize the polymer and reduce the mechanical properties. The decreased mechanical properties of resin composite might contribute to a decreased bond strength with any adhesive system. The change in mechanical properties after thermal cycling could result in bond failure trends or tendencies due to weakened adhesive resins, which exist between the dentin and resin.

From a previous study performed to compare the chemical bonding efficacy of functional monomers (MDP; 10-methacryloxydecyl dihydrogen phosphate, 4-MET; 4-methacryloxyethyl trimellitic acid, and phenyl-P; 2-methacryloxyethyl phenyl hydrogen phosphate), MDP has been reported to have a high chemical bonding potential to hydroxyapatite within a clinically reasonable application time.[27] Furthermore, the calcium salt of MDP was highly insoluble and, consequently, was able to resist ultrasonic cleaning. According to the adhesion-decalcification concept,[28] the less soluble the calcium salt of the acidic molecule, the more intense and stable the molecular adhesion to a hydroxyapatite-based substrate. And, the functional monomer 4-acryloxyethyltrimellitic acid (4-AET) has been shown to interact with Ca^{2+} from apatite crystallites within the partially demineralized hybrid layer to form an insoluble calcium salt (4-AETCa) that may aid in bonding this resin system to dentin.[29] Like polyalkenoic acids that can bond chemically to hydroxyapatite[30] or collagen,[31] 4-AET has also been shown to bond chemically to both dentin apatite and collagen. A chemical interaction between hydroxyapatite and functional monomers in the adhesive leads to higher bond strengths than the adhesives that rely only on micromechanical retention to dentin substrate.

In addition to a functional monomer, the adhesive FS contains a pre-reacted glass-ionomer filler that provides adhesion and a source of fluoride release.[32] These fillers are formed by the complete reaction of FASG

glass with polyalkenoic acids in the presence of water to form a wet siliceous hydrogel. Upon freeze-drying, the desiccated "xerogel" is further milled and silane-treated to form F-PRG fillers of a specific size range. A study has examined the ultrastructure and elemental composition of resin-dentin interfaces that were treated with this type of adhesive, and the presence of a surface interaction layer on top of a partially demineralized zone along the resin-dentin interface was observed. It was concluded that, either a glass-ionomer type reaction or precipitation of insoluble carboxylate salts around remnant apatite crystallites, might occur when the adhesive interacts with dentin.

CONCLUSIONS

The results of this study suggest that there is no benefit to using phosphoric acid prior to the application of single-step self-etch adhesives in terms of increasing dentin bond strengths. A further understanding of the factors that contribute to the durability of the adhesives and their bonding characteristics is needed.

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