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Enamel Etching for Universal Adhesives: Examination of Enamel Etching Protocols for Optimization of Bonding Effectiveness

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Clinical Relevance

Conventional phosphoric acid etching with reduced etching times and polyalkenoic acid etching for 15 seconds are potential optimal etching protocols to improve enamel bonding effectiveness with universal adhesives, unlike phosphoric acid ester monomer etching.

SUMMARY

Objective: The purpose of this study was to evaluate whether different enamel etching methods with reduced etching times would improve the bonding effectiveness of universal adhesives.

Methods and Materials: Three enamel etching methods, phosphoric acid ester monomer (PPM) etching, phosphoric acid (PPA) etching, and polyalkenoic acid (PLA) etching, and three universal adhesives, G-Premio Bond (GP), Prime&Bond elect (PE), and Scotchbond Universal Adhesive (SU), were evaluated. Initial bond strengths and fatigue strengths of universal adhesives to ground enamel and ground enamel etched for less than one, five, 10, and 15 seconds using different etching methods were determined. The bonded fatigue specimens were loaded using a sine wave at a frequency

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of 20 Hz for 50,000 cycles or until failure occurred with a staircase method. Atomic force micrograph (AFM) observations along with measurements of surface Ra roughness and modified surface area of enamel with different etching protocols were also conducted.

Results: The bond fatigue durability of universal adhesives to enamel with PPA etching from less than one to 15 seconds and PLA etching for 15 seconds was significantly higher than that to ground enamel. The bond fatigue durability to enamel with PPM etching was not increased compared with ground enamel. The surface Ra roughness and surface area obtained with AFM of enamel increased after PPA and PLA etching, and those values were significantly higher than those of ground enamel. Furthermore, surface Ra roughness and surface area with PPA etching were significantly higher than those with PLA etching. However, surface Ra roughness and surface area of enamel with PPM etching were similar to those of ground enamel regardless of etching time.

Conclusion: PPA etching for less than one to 15 seconds and PLA etching for 15 seconds improve universal adhesive bonding, surface Ra roughness, and surface area of enamel. However, PPM etching is not effective, regardless of etching time, in improving bonds strengths, increasing surface roughness, and increasing surface area.

INTRODUCTION

A recent trend in simplifying and streamlining adhesive systems has been the use of universal adhesives applied in either etch-and-rinse or self-etch modes.[1] Scotchbond Universal Adhesive (3M Oral Care, St Paul, MN, USA) was introduced in 2012 as the first commercial universal adhesive. Other manufacturers later released similar universal adhesives with various distinctive characteristics such as the ability to be used with various substrates,[2] shortened application times,[3] or various levels of tooth substrate wetness.[4] It has been reported that the bond durability of most universal adhesives to enamel in etch-and-rinse and self-etch modes,[5] and dentin in self-etch mode,[6] is lower than that of two-step self-etch adhesives. Nevertheless, the clinical use of universal adhesives is rapidly increasing due to their versatility. Therefore, the optimal conditions for universal adhesive application, including the use of enamel etchants, deserve continued investigation.

A systematic review and meta-analysis of the bond strength (micro-shear and micro-tensile bond strengths) of universal adhesives by Rosa and others[7] reported that, although the dentin bond strength of universal adhesives is not influenced by the bonding strategy used, the enamel bond strength is higher in etch-and-rinse mode than in self-etch mode. These results may indicate that universal adhesives are best used in etch-and-rinse mode. On the other hand, it has been reported in clinical studies of universal adhesives (Scotchbond Universal Adhesive [3M Oral Care] and Tetric N-Bond Universal [Ivoclar Vivadent, Schaan, Liechtenstein]) over three years by Loguercio and others[8,9] that clinical results are not dependent on the bonding strategies used for resin composite restorations in noncarious cervical lesions and that the use of etch-and-rinse mode had only a minor effect for universal adhesives. These clinical studies[8,9] may suggest that the higher laboratory enamel bond strength of universal adhesive in etch-and-rinse mode does not have a strong impact on the clinical results of adhesively bonded resin composite restorations. Based on clinical results, the use of universal adhesives in self-etch mode may be preferable for clinicians because of the simplified process and cost effectiveness, as phosphoric acid etching is not used. The incongruity between the laboratory and clinical studies means that it is still unclear whether best practice with universal adhesives should be etch-and-rinse or self-etch mode. This conflict cannot be easily resolved as both laboratory studies and relatively short-term clinical studies have important limitations.

Many clinicians still prefer to use etch-and-rinse mode for adhesive systems with the standard etching protocol of 30%-40% phosphoric acid (PPA) for 15 seconds.[10] The advantages of the use of PPA etching for enamel include increases in the surface wettability,[11] surface roughness,[12] and surface free-energy,[13] leading to improved bonding, even though the surface hardness of enamel decreases.[14] On the other hand, PPA etching of dentin leads to decreased wettability and increased hydrophobicity of the surface compared with ground dentin due to the aggressive demineralization of the smear layer and the superficial layer of dentin.[15] In addition to these unfavorable characteristics of the adherent for bonding, which are well known, Tay and others[16] reported that the hydrophobicity of demineralized dentin leads to osmosis of water content from deeper dentin, causing weaker bonding due to osmotic blisters and the hydrolysis of the adhesive itself. Furthermore, PPA etching of dentin activates endogenous collagenolytic proteases associated with the degradation of the interface between the adhesive and dentin.[17] In this way, clinicians who prefer to use universal adhesives in etch-and-rinse mode may believe that doing so has disadvantages due to the etchant's influence on enamel and dentin, even if it has many advantages for enamel bonding.

Recent clinical studies[8,9] suggest that the use of universal adhesives in etch-and-rinse mode does not have a strong and immediate effect on clinical results of resin composite restorations. However, a recent review of laboratory results[7] does suggest a strong effect on the enamel bond strength and no effect on dentin bonding of universal adhesives in etch-and-rinse mode. Taken together, these studies suggest that it may be possible to develop optimal protocols of universal adhesives in etch-and-rinse mode that preserve benefits for enamel bonding while minimizing negative influences on other tooth substrates. That is, a lower level of demineralization of enamel and dentin may be sufficient to achieve the clinical benefits while minimizing damage to both types of tooth substrate. A previous study[17] suggested that aggressive demineralization may create a potential vulnerability for acidic attacks and secondary caries formation, although the need for some degree of etching is undeniable. One possibility is to use a weaker acid, and another is to reduce the etching time below 15 seconds. Thus, evaluations of optimal protocols, such as the use of alternative etching methods and reduced etching times with universal adhesives, may be desirable.

Several alternative etching methods are currently available. Polyalkenoic acid (PLA), which is a family of complex acids including polyacrylic, polyitaconic, and polymaleic acids, is used in etching for cavity cleansing and conditioning in glass ionomer cement restorations.[18] In these restorative procedures, PLA etching promotes the formation of irregularities on the surface of the substrate, forming an intermediate layer that facilitates ion exchange between the glass ionomer matrix and the calcium in the partially demineralized smear layer.[19] In addition, PLA etching forms insoluble salts with calcium due to its high molecular weight that serve as receptors for primary chemical bonds between glass ionomer cement and the carboxyl groups of polyalkenoic acid.[20] Alternatively, another method for etching has been a newly developed phosphoric acid ester monomer (PPM) etching. Whereas PPA has three hydroxyl groups, a PPM has at least one of these groups replaced with an ester; thus, the monomer can simultaneously demineralize and also bond to substrates. PLA and PPM etching can remove the smear layer and modify enamel and dentin surfaces with less demineralization than PPA etching, minimizing the unfavorable effects for bonding to tooth substrates. Because of these characteristics, PLA and PPM are potentially attractive for application with universal adhesives in etch-and-rinse mode. Accordingly, a comparison between different etching methods and conventional PPA etching for universal adhesives in terms of enamel bond durability may be valuable.

The purpose of this laboratory study was to investigate the central hypothesis of whether different etching methods with reduced etching times would improve the enamel bonding effectiveness of universal adhesives. The two null hypotheses tested were as follows: 1) there would be no differences in bond durability of universal adhesives to enamel among different etching protocols; and 2) different etching protocols would not influence enamel surface morphology.

METHODS AND MATERIALS

Study Materials

Three universal adhesives, 1) G-Premio Bond (GP, GC, Tokyo, Japan); 2) Prime&Bond elect (PE, Dentsply Sirona, Milford, DE, USA); and 3) Scotchbond Universal Adhesive (SU, 3M Oral Care), and three etchants, 1) a PLA etchant (Enamel Conditioner, Shofu, Kyoto, Japan); 2) a PPM etchant (Multi Etchant, Yamakin, Tokyo, Japan); and 3) a PPA etchant (Ultra-Etch, Ultradent Product, South Jordan, UT, USA), were evaluated. Z100 Restorative (3M Oral Care) was used as the resin composite for the bonding procedures. The study materials are listed in Table 1 with associated components.

Specimen Preparation

Sectioned buccal and lingual halves of de-identified extracted human molar teeth with the apical portions removed were mounted in 25-mm brass rings using an acrylic resin (Bosworth Fastray, Keystone Industries, Myerstown, PA, USA). Flat enamel surfaces were prepared on the mounted buccal or lingual surfaces by wet grinding using a gradually increasing grit sequence (180, 320, 600, 1200, 2000 and 4000 grit) of silicon carbide papers (Struers, Cleveland, OH, USA) in a grinder-polisher (Ecomet 4, Buehler, Lake Bluff, IL, USA). These

Table 1: *Materials Used in This study*

Materials	Type of Material (Code)	Components	Manufacturer
G-Premio Bond	Universal adhesive (GP)	MDP, 4-MET, MEPS, methacrylate monomer, acetone, water, silica, initiator	GC
Prime&Bond elect	Universal adhesive (PE)	Dipentaerythritol pentaacrylate monophosphate, polymerizeable dimethacrylate resin, polymerizeable trimethacrylate resin, diketon, organic phosphine oxide, stabilizers, cetylamine hydrofluoride, acetone, water	DENTSPLY Caulk
Scotchbond Universal Adhesive	Universal adhesive (SU)	Bis-GMA, HEMA, decamethylene dimethacrylate, ethyl methacrylate, propenoic acid, methyl-reaction products with decanediol and phosphorous oxide, copolymer of acrylic and itaconic acid, dimethylaminobenzoate, methyl ethyl ketone, ethanol, water, silane treated silica, initiator	3M Oral Care
Enamel Conditioner	Etchant (PLA)	Polyalkenoic acids, thickener, pigment	Shofu
Multi Etchant	Etchant (PPM)	M-TEG-P, thickener, pigment	Yamakin
Ultra-Etch	Etchant (PPA)	35% Phosphoric acid, glycol, cobalt aluminate blue spinel	Ultradent Products
Z100 Restorative	Resin composite	Bis-GMA, TEGDMA, silane treated ceramic, benzotriazolyl methylphenol	3M Oral Care

Abbreviations: Bis-GMA, bisphenol A glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate, MDP, methacryloyloxydecyl dihydrogen phosphate; MEPS, methacryloyloxyalkyl thiophosphate methylmethacrylate; M-TEG-P, 11-methacryloyloxy-4-ethyleneglycol dihydrogen phosphate; TEGDMA: triethylene glycol dimethacrylate; 4-MET, 4-methacryloyloxyethyl trimellitate.

surfaces were then washed with an air-water spray and air-dried using a dental three-way syringe at a distance of 5 cm above the surface and an air pressure of 2.5 kgf/cm^2.

One control group per adhesive was prepared by rinsing ground enamel with an air-water spray for 10 seconds and air drying without the application of etching agents. Four etching time groups were prepared for each etching method and adhesive: 1) ground enamel with etching agent applied and then immediately rinsed with an air-water spray for 10 seconds and air dried (less than one-second group); 2) ground enamel with etching agent for five seconds and then rinsed with an air-water spray for 10 seconds and air dried (five-second group); 3) ground enamel with etching for 10 seconds and then rinsed with an air-water spray for 10 seconds and air dried (10-second group); and 4) ground enamel with etching agent for 15 seconds and then rinsed with an air-water spray for 10 seconds and air dried (15-second group). The specimens were prepared under ambient laboratory conditions of 23 ± 2°C and 50 ± 10% relative humidity.

Initial Bond Strength Tests

Stainless steel molds with an inner diameter of 2.38 mm, an outer diameter of 4.8 mm, and a height of 2.6 mm were used to bond a resin composite to enamel surfaces in all groups. The mold-enclosed method was used to minimize the impact of repeated application of force to the resin in the sample and to make the applied force as close to a pure shear force as possible. The bonding side surfaces of the metal molds were prepared with a releasing agent (3% solution of paraffin in hexane) to chemically isolate the bonded enamel/resin composite interface. The enamel surfaces of the different etching protocols were then treated with the universal adhesives according to the manufacturers' instructions (Table 2). A fixture was used to position and hold the molds over the bonding surfaces as the resin composite was placed into the mold using a condenser to an approximate height of 2.5 mm. The resin composite was photo-cured for 40 seconds at a standardized distance of 1 mm using a quartz-tungsten halogen (QTH) curing unit (Spectrum 800 Curing Unit, Dentsply Sirona) set at 800 mW/cm^2. The bonded

Table 2: *Application Protocol for Tested Adhesives*

Adhesive	Application Protocol
GP	Adhesive applied to air-dried enamel/dentin surface for 10 seconds. Strong stream of air applied over the liquid adhesive for five seconds or until adhesive no longer moved and the solvent had completely evaporated. Adhesive light cured for 10 seconds.
PE	Adhesive applied to air-dried enamel/dentin surface with rubbing for 20 seconds. Gentle stream of air applied over the liquid for at least five seconds. Adhesive light cured for 10 seconds.
SU	Adhesive applied to air-dried enamel/dentin surface with rubbing action for 20 seconds, and then medium air pressure applied to surface for five seconds. Adhesive light cured for 10 seconds.

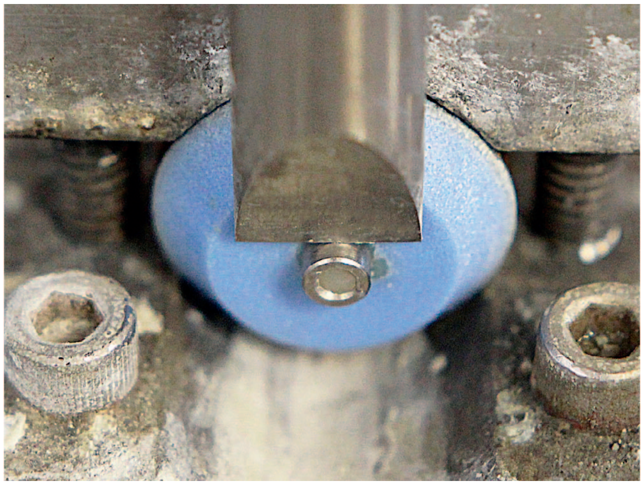

Figure 1. *Figure illustrating the "Mold-Enclosed" bonding jigs.*

specimens were stored in 37°C distilled water for 24 hours before initial bond strength testing.

Shear bond strength tests were carried out on all groups with the stainless steel mold described above (mold-enclosed method). A chisel-shaped metal rod was used to apply the load on the stainless steel molds immediately adjacent to the flat ground enamel surfaces (Figure 1). The specimens (n=15) were loaded to failure using an all-electric dynamic test instrument (ElectroPuls E1000, Instron, Norwood, MA, USA) with a crosshead speed of 1 mm/min. Initial shear bond strengths (MPa) were calculated from the peak load at failure divided by the bonded surface area.

Bond Fatigue Strength Test

A staircase method was used to perform the bond fatigue strength tests using the all-electric dynamic test instrument. Twenty specimens (n=20) were prepared for all groups for each of the adhesives being tested. Subsequently, the specimens were stored in 37°C distilled water for 24 hours prior to testing. Tsujimoto and others[21,22] reported that the bond fatigue strength, using the mold enclosed system used in this study, was not influenced by the frequency rate (2 or 20 Hz) or the numbers of cycles (50,000, 100,000, or 1,000,000 cycles); thus, the fatigue load was applied using a sine wave at a frequency of 20 Hz for 50,000 cycles or until failure occurred. The initial peak load for bond fatigue strength testing for each of the adhesives was set at a level approximately half of the initial shear bond strength determined for each group and the lower load limit was set at 0.4 N. Subsequent specimen loading was adjusted upward or downward approximately 10% from the previous load depending on specimen survival or failure. This procedure was repeated for the 20 specimens in each test group. The test specimens were immersed in room temperature water (23±2°C) during bond fatigue strength testing to minimize the influence of any temperature changes on the bonded assemblies during testing. The bond fatigue strength and standard deviation were calculated using the formula described by Draughn.[23]

Failure Mode Analysis

Bond failure sites were assessed after the initial bond strength and fatigue strength tests by a single experienced individual using an optical microscope (MZ16, Leica Microsystems, Heerbrugg, Switzerland) at 20× magnification. The failure modes were assessed on the percentage of substrate area (adhesive, resin composite, or enamel) observed on both the debonded resin composite cylinders and the enamel bonding sites. The failure modes were classified as follows: 1) adhesive failure at the interface; 2) cohesive failure in resin composite; 3) cohesive failure in enamel; or 4) mixed failure.

Atomic Force Microscopy Evaluation

Six enamel specimens were prepared for each of the previously described groups for atomic force microscopy (AFM) evaluation. Each specimen was imaged in three different locations near the center of the specimen. Prior to measurement, the specimens were blown with dried air in a sweeping motion for approximately five seconds at 0.55 MPa to remove any dust particles and surface debris. AFM evaluations were performed using a scanning probe microscope/AFM (5420 SPM/AFM Microscope, Agilent Technologies, Santa Clara, CA, USA) in an acoustical and mechanical isolation chamber under ambient laboratory conditions (23±2°C and 50±10% relative humidity). Micrographs were obtained in constant force contact mode with a silicon nitride (Si_3N_4) cantilever (tip radius of ≤10 nm and spring constant of 0.2 N/m; BudgetSensors, Sofia, Bulgaria) at 512 lines per image at a rate of three to four lines per second. In this mode, the AFM is in constant feedback with the cantilever to maintain a constant deflection by modulating the AFM/specimen separation with piezoelectric motors. A schematic diagram of the AFM is shown in Figure 2.

Micrographs (30×30 μm) were analyzed, quantitatively and qualitatively, with image analysis software (Gwyddion, Central European Institute of Technology, Brno, Czech Republic). Enamel surface

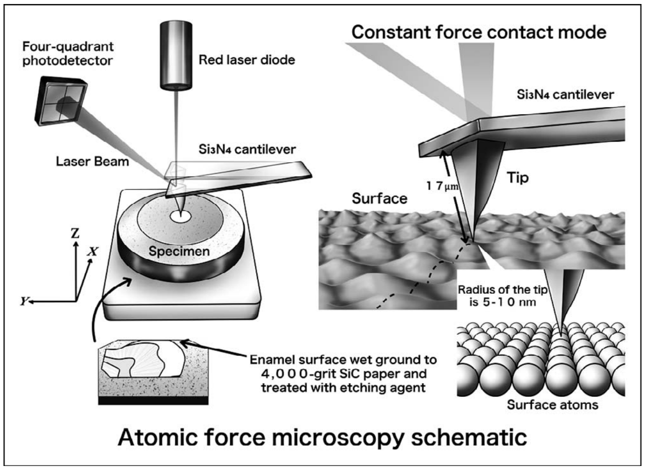

Figure 2. *Schematic illustration of AFM, where a flexible cantilever with an atomically sharp tip is systematically swept across the surface of a sample. A laser beam positioned at the back of the aluminum coated, reflective cantilever captures three-dimensional topography changes, which are reflected in changes to the laser beam position on a four-quadrant photodetector monitored by a specialized computer system.*

roughness was quantified in terms of Ra (nm), the arithmetic average of the absolute values of the profile height deviations from the mean line, recorded within an equivalent imaging area. Among several parameters for measurement of surface roughness, the average Ra is most commonly reported.[24-26] Similarly, the geometric surface area (μm^2) was obtained, which measures the modified surface area (above the anticipated 900 μm^2 for a flat surface) due to height variations across the surface. Experienced investigators assessed qualitative intra- and intergroup differences.

Statistical Analysis

The initial bond strength, surface Ra roughness, and surface area data obtained with AFM were analyzed using a three-way analysis of variance (ANOVA; factors: 1) etching method, 2) etching time and 3) adhesive) followed by Tukey's *post hoc* honest significant differences test with a significance level of $\alpha = 0.05$. Fisher's exact test was used to statistically analyze the failure mode after initial bond strength and fatigue strength testing with a significance level of $\alpha = 0.05$. These statistical analyses were conducted using a commercial statistical software package (SPSS Statistics, International Business Machines, Armonk, NY, USA). The bond fatigue strength data were analyzed using a modified *t*-test with Bonferroni correction and significance level of $\alpha = 0.05$ (custom program).

RESULTS

Initial Bond Strength

The results for the effects of etching protocols on the initial bond strength of the universal adhesives to enamel are shown in Table 3. The three-way ANOVA revealed that the main factors 1) etching method (F=23.231, p<0.001), 2) etching time (F=18.112, p<0.001), and 3) adhesive (F=8.112, p=0.012) significantly affected these values, and etching method and etching time were the most influential. In addition, an interaction between etching method and etching time (F=3.112, p=0.032) was observed, but there was no significant interaction between the other factors.

The initial bond strengths of the universal adhesives increased immediately after PPA etching and were significantly higher (p<0.05) than those of the control, but the values were not influenced by etching times (p>0.05). The initial bond strength of the universal adhesives with PLA etching gradually increased with the increase of etching times, and the values with PLA etching for 15 seconds were similar to those with PPA etching at all etching times.

The initial bond strengths of universal adhesives with PPM etching did not increase and were similar (p>0.05) to those of the control regardless of pre-etching time. Furthermore, the initial bond

Table 3: *Effects of Etching Protocols on the Initial Shear Bond Strength (MPa) of the Universal Adhesives to Enamel*[a]

Etching Time (s)	G-Premio Bond			Prime&Bond elect			Scotchbond Universal		
	PPA	PLA	PPM	PPA	PLA	PPM	PPA	PLA	PPM
Control		25.4 (3.3)^{a}			26.9 (4.2)^{a}			27.1 (3.8)^{a}	
<1	32.5 (4.3)^{b,A}	27.5 (3.4)^{a,B}	19.9 (3.8)^{b,C}	37.1 (4.3)^{b,A}	29.3 (4.7)^{a,B}	24.2 (4.5)^{a,C}	42.2 (5.3)^{b,A}	35.4 (4.2)^{b,B}	24.9 (3.8)^{a,C}
5	33.1 (4.1)^{b,A}	27.7 (4.6)^{a,B}	20.5 (3.7)^{b,C}	38.2 (3.9)^{b,A}	28.3 (5.7)^{a,B}	24.0 (5.5)^{a,B}	42.1 (4.3)^{b,A}	36.5 (4.9)^{b,B}	24.2 (5.1)^{a,C}
10	32.9 (3.8)^{b,A}	28.0 (3.4)^{a,B}	20.7 (3.5)^{b,C}	39.2 (3.2)^{b,A}	31.2 (5.0)^{a,b,B}	22.6 (4.5)^{a,C}	43.2 (4.4)^{b,A}	38.0 (5.4)^{b,B}	25.1 (4.4)^{a,C}
15	33.7 (4.1)^{b,A}	32.3 (4.3)^{b,A}	21.3 (4.0)^{b,B}	39.0 (4.3)^{b,A}	37.1 (5.3)^{b,A}	22.3 (3.5)^{a,B}	43.7 (4.3)^{b,A}	41.3 (4.7)^{c,A}	24.1 (4.7)^{a,B}

[a] *Values in parentheses are standard deviations. Same superscript lowercase letter in same column indicates no significant difference (*p>0.05*). Same superscript capital letter within individual rows indicates no significant difference (*p>0.05*).*

Table 4: *Effects of Etching Protocols on the Bond Fatigue Strength (MPa) of the Universal Adhesives to Enamel*[a]

Etching Time (s)	G-Premio Bond			Prime&Bond elect			Scotchbond Universal		
	PPA	PLA	PPM	PPA	PLA	PPM	PPA	PLA	PPM
Control		12.1 (1.3)^{a}			13.2 (1.2)^{a}			13.7 (1.4)^{a}	
<1	15.5 (1.3)^{b,A}	13.0 (1.2)^{a,B}	9.7 (1.0)^{b,C}	18.1 (1.3)^{b,A}	13.3 (1.5)^{a,B}	11.5 (1.2)^{a,B}	20.1 (1.3)^{b,A}	16.7 (1.2)^{b,B}	12.1 (1.2)^{a,C}
5	16.2 (1.1)^{b,A}	13.1 (1.2)^{a,B}	9.9 (1.4)^{b,C}	18.2 (1.4)^{b,A}	13.5 (1.1)^{a,B}	11.6 (1.5)^{a,B}	20.4 (1.3)^{b,A}	17.7 (1.2)^{b,B}	12.3 (1.1)^{a,C}
10	16.1 (1.2)^{b,A}	13.3 (1.3)^{a,B}	10.1 (1.5)^{b,C}	19.2 (1.2)^{b,A}	14.9 (1.0)^{a,b,B}	11.3 (1.3)^{a,C}	21.0 (1.4)^{b,A}	18.3 (1.4)^{b,B}	12.4 (1.4)^{a,C}
15	16.5 (1.1)^{b,A}	16.4 (1.2)^{b,A}	10.8 (1.0)^{b,B}	19.0 (1.0)^{b,A}	18.1 (1.3)^{b,A}	11.3 (1.5)^{a,B}	21.7 (1.3)^{b,A}	20.2 (1.7)^{c,A}	12.3 (1.3)^{a,B}

[a] *Values in parentheses are standard deviations. Same superscript lowercase letter in same column indicates no significant difference ($p>0.05$). Same superscript capital letter within individual rows indicates no significant difference ($p>0.05$).*

strengths of universal adhesives in the control group were not influenced ($p>0.05$) by the type of adhesive.

Bond Fatigue Strength

The results for the effects of etching protocols on the bond fatigue strength of the universal adhesives to enamel are shown in Table 4. The bond fatigue strengths of the universal adhesives increased significantly, immediately after PPA etching, and were significantly higher ($p<0.05$) than those of the control group, regardless of etching time.

The bond fatigue strengths of GP and PB with PLA pre-etching for 15 seconds were significantly higher ($p<0.05$) than those of the control and other etching time groups. On the other hand, the bond fatigue strengths of SU with PLA etching increased significantly with increased etching times and were significantly higher ($p<0.05$) than those of the control group. There were no statistically significant differences in bond fatigue strengths of universal adhesives between those with PLA etching for 15 seconds and those with PPA etching, regardless of adhesive.

However, the bond fatigue strengths of the universal adhesives with PPM etching did not increase and were similar ($p>0.05$) to those of the control group, regardless of etching time. The bond fatigue strength of universal adhesives in the control was not influenced by the type of adhesive.

Failure Mode Analysis

The failure mode analyses for initial bond strength and fatigue strength testing are shown in Tables 5 and 6, respectively. Failure mode was overwhelmingly adhesive failure. Fisher's exact tests did not reveal statistically significant differences ($p>0.05$) in failure mode depending on the type of adhesive, the etchant, or the etching time. All adhesives with PPM etching showed exclusively adhesive failure at all etching times. Similarly, only a single case of nonadhesive failure was observed for etching times below 10 seconds, which was also the only case of nonadhesive failure for GP. Other cases showed a small number of cohesive failure in enamel, and two cases of mixed failure were observed overall, but these other failure modes were not significant; in no case were fewer than 85% of failures classified as adhesive failure. This is normal for studies of enamel bonding using the mold-enclosed method.

AFM Observations

Representative three-dimensional topographic images of enamel surfaces obtained by AFM are shown in Figures 3A through 3M. In the control (Figure 3a), periodic grooves made by polishing were observed, and a smear layer was not clearly observed on the ground surfaces. There were clear morphologic differences between enamel with PPA and PLA etching and the control. Topographic

Table 5: *Effect of Etching Protocols on Surface Roughness (nm) and Surface Area (μm^2) of Enamel Surfaces*[a]

Etching Time (s)	Surface Roughness			Surface Area		
	PPA	PLA	PPM	PPA	PLA	PPM
Control		10.9 (2.4)^{a}			901.5 (1.2)^{a}	
<1	139.5 (28.3)^{b,A}	23.0 (6.2)^{b,B}	8.4 (1.9)^{a,C}	1150.8 (65.4)^{b,A}	912.4 (4.7)^{b,B}	902.1 (1.6)^{a,C}
5	154.3 (19.8)^{b,A}	23.9 (6.9)^{b,B}	9.0 (2.7)^{a,C}	1178.5 (74.6)^{b,A}	911.5 (4.2)^{b,B}	902.2 (2.8)^{a,C}
10	173.9 (25.5)^{b,c,A}	23.4 (6.3)^{b,B}	9.2 (1.5)^{a,C}	1202.2 (38.5)^{b,c,A}	910.9 (2.5)^{b,B}	900.9 (0.4)^{a,C}
15	194.3 (28.2)^{c,A}	24.8 (8.0)^{b,B}	9.0 (3.8)^{a,C}	1223.4 (42.1)^{c,A}	912.2 (3.3)^{b,B}	903.0 (0.1)^{a,C}

[a] *Values in parentheses are standard deviations. Same superscript lowercase letter in same column indicates no significant difference ($p>0.05$). Same superscript capital letter within individual rows indicates no significant difference ($p>0.05$).*

Table 6: *Failure Mode Analysis of Debonded Specimens After Shear Fatigue Strength Tests*[a]

Etching Time (s)	G-Premio Bond			Prime&Bond Elect			Scotchbond Universal		
	PPA	**PLA**	**PPM**	**PPA**	**PLA**	**PPM**	**PPA**	**PLA**	**PPM**
Control		[100/0/0/0][a]			[100/0/0/0][a]			[100/0/0/0][a]	
<1	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[100/0/0/0][a,A]
5	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[100/0/0/0][a,A]
10	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[86/0/14/0][a,A]	[93/0/7/0][a,A]	[100/0/0/0][a,A]	[86/7/7/0][a,A]	[100/0/00][a,A]	[100/0/0/0][a,A]
15	[93/0/7/0][a,A]	[100/0/0/0][a,A]	[100/0/0/0][a,A]	[86/0/14/0][a,A]	[93/0/7/0][a,A]	[100/0/0/0][a,A]	[86/7/7/0][a,A]	[93/0/7/0][a,A]	[100/0/0/0][a,A]

[a] *Percentage of failure mode [adhesive failure/cohesive failure in resin/cohesive failure in enamel/mixed failure]. Same small letter in same column indicates no significant difference ($p > 0.05$). Same capital letter within individual rows indicates no significant difference ($p > 0.05$).*

images of enamel surfaces with PPA (Figures 3b through 3e) and PLA (Figures 3f through 3i) etching showed micro-irregularities that were different between PPA and PLA etching, and the degree of demineralization for enamel with PLA etching was weaker than those with PPA etching, regardless of etching time. The degree of demineralization appeared to increase with an increase of etching time for both PPA and PLA etching. On the other hand, topographic images of enamel surfaces with PPM etching (Figures 3j through 3m) did not show any morphologic differences compared with those of the control, independent of etching time.

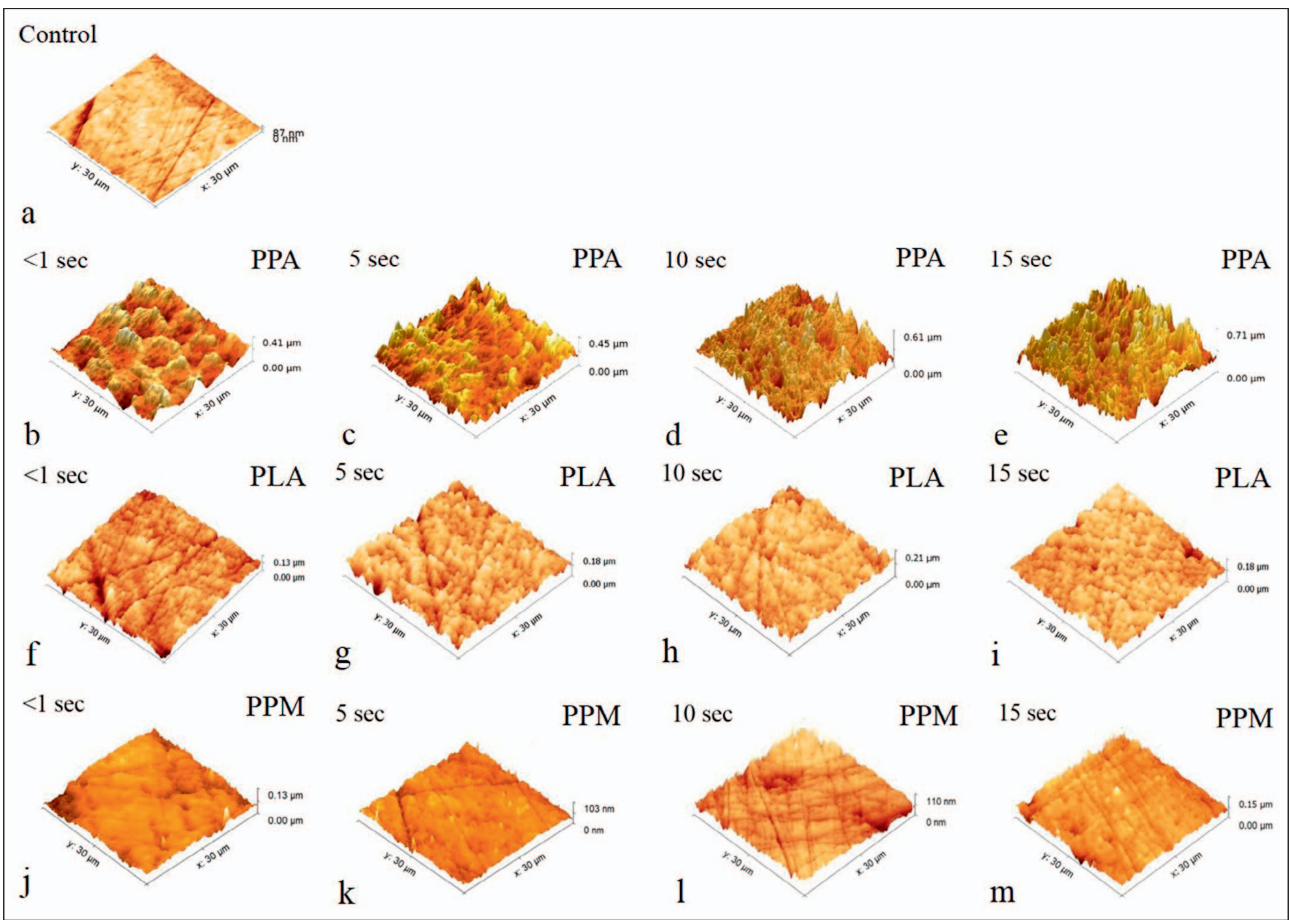

Figure 3. *False color three-dimensional topographic images (30×30 μm) of enamel surfaces obtained by constant force atomic force microscopy. Micrograph heights were normalized for accurate visual inspection, where the vertical scale bar shows the maximum feature height in the given micrograph. (a): Control; (b-e): PAA etched <1 to 15 sec; (f-i): PLA etched <1 to 15 sec; and (j-m): PPM etched <1 to 15 sec.*

Table 7: *Effect of Etching Protocols on Surface Roughness and Surface of Enamel Surfaces*[a]

Etching Time (s)	Surface Roughness (nm)			Surface Area (μm²)		
	PPA	PLA	PPM	PPA	PLA	PPM
Control		10.9 (2.4)[a]			901.5 (1.2)[a]	
<1	139.5 (28.3)[b,A]	23.0 (6.2)[b,B]	8.4 (1.9)[a,C]	1150.8 (65.4)[b,A]	912.4 (4.7)[b,B]	902.1 (1.6)[a,C]
5	154.3 (19.8)[b,A]	23.9 (6.9)[b,B]	9.0 (2.7)[a,C]	1178.5 (74.6)[b,A]	911.5 (4.2)[b,B]	902.2 (2.8)[a,C]
10	173.9 (25.5)[b,c,A]	23.4 (6.3)[b,B]	9.2 (1.5)[a,C]	1202.2 (38.5)[b,c,A]	910.9 (2.5)[b,B]	900.9 (0.4)[a,C]
15	194.3 (28.2)[c,A]	24.8 (8.0)[b,B]	9.0 (3.8)[a,C]	1223.4 (42.1)[c,A]	912.2 (3.3)[b,B]	903.0 (0.1)[a,C]

[a] *Values in parentheses are standard deviations. Same superscript lowercase letter in same column indicates no significant difference ($p>0.05$). Same superscript capital letter within individual rows indicates no significant difference ($p>0.05$).*

Surface Roughness and Geometric Surface Area Measurements

The surface roughness (Ra, nm) and surface area (μm^2) obtained with AFM are shown in Table 7. Significantly higher ($p<0.05$) surface roughness and surface area of enamel with the PPA and PLA etching were observed compared with those in the control, but the values of surface roughness and surface area of enamel with PPA etching were significantly higher ($p<0.05$) than those with PLA etching. The surface area of the enamel with PPA etching increased significantly ($p<0.05$) with increased etching time. Surface roughness of the enamel with PPA etching appeared to increase with increased etching time, but this was not significant ($p>0.05$). Although the surface roughness and surface area of enamel with PLA etching were also increased immediately after etching, those values were not influenced by the etching time. On the other hand, the surface roughness and surface area of enamel with PPM etching were not increased compared with the control, and there was no statistically significant difference ($p>0.05$) in the values of surface roughness and surface area between PPM etching and the control.

DISCUSSION

One aspect of this study confirms the effect of reduced PPA etching times on enamel bonding with universal adhesives. PPA etching for 15 seconds is generally recommended by manufacturers when universal adhesives are used in etch-and-rinse mode, based on earlier research results by Barkmeier and others[12,27,28] and Uno and Finger.[29] Those studies reported that PPA etching times in excess of 15 seconds did not increase bond strength to enamel even though the surface roughness of enamel continued to increase. However, the universal adhesives investigated herein are different from the adhesive systems used in those studies. In addition, a recently published article by Tsujimoto and others[30] on enamel adhesion with single-step self-etch adhesives and Stape and others[31] on dentin with universal adhesives revealed that PPA etching with a reduced etching time of three seconds is a potential protocol to improve the bonding of adhesives. Thus, the question of optimal PPA etching time for universal adhesives was revisited.

In the present study, the bond fatigue durability of universal adhesives to enamel with PPA etching was significantly higher than that to ground enamel, but the value was not statistically increased depending on the etching time. In the results for surface characteristics obtained from AFM, surface roughness (67.8-85.3 nm) and surface area (921.9-943.9 μm^2) were increased immediately after PPA etching compared with those of ground enamel (surface Ra roughness: 10.1 nm; surface area: 901.5 μm^2) and micro-irregularities of the surfaces were seen regardless of etching times. Previous studies[24-26] reported that the surface roughness of enamel with PPM etching obtained by AFM showed 160-321 nm; thus, the surface Ra roughness observed in the present study was relatively low compared with earlier studies. However, the surface Ra roughness of the baseline of the previous studies[24-26] was 30-50 nm, whereas that of the baseline of the present study was 10.1 nm. Moreover, slight differences in methodologies used to measure Ra roughness, including image size and resolution, strongly impact subsequent values. In the present study, an enamel surface ground with 4000-grit silicon carbide papers was used as baseline to minimize the influence of any directionality or inhomogeneities of the surface grooving or scratches created by the abrasives on bond fatigue strength testing. Therefore, a lower baseline surface Ra roughness of enamel was found in the present study, but the resultant increase in Ra was similar in magnitude to the earlier studies. In addition, Tsujimoto and others[30] reported that PPA etching with reduced etching times of under 15 seconds can improve the bond durability of simplified adhesives and the interfacial characteristics of enamel to an adequate level. These findings are consistent with the results of the present study. Thus, the results of this study clearly indicate that PPA etching is still the gold standard for improving enamel bonding, even with universal adhesives, and that PPA etching with reduced etching time for universal adhesives, used in etch-and-rinse mode, may be a potential protocol to improve bonding effectiveness of universal adhesives for use in the clinic. Although, both the study by Stape and others[31] and the present study revealed the effectiveness of PPA etching with reduced etching time for universal adhesives, it is necessary to garner additional data on bonding to unground enamel before making clinical recommendations.

The fact that PPA etching with reduced etching times may be effective for enamel bonding with universal adhesives suggests that different, weaker types of acids or acidic agents may also be effective, particularly if the etching times are extended. This is worth consideration because strong acids may inflict considerable damage on tooth substrates. Therefore, the effect of different etching methods (both PLA and PPM etching) on enamel bonding with universal adhesives, including the effect of reduced etching times, was evaluated.

PLA etching has been widely used over a long period for restoration with glass ionomer cement, and this combination has been extensively investigated.[18,19,32] However, there appear to have been no investigations of whether PLA etching can be used with resin composites, much less any investigation of whether they can be used with universal adhesives. The PLA etchant used in this study is mainly composed of polyacrylic acid and has a pH of approximately 1.5. The pH of PLA is higher than that of PPA etching agent ($pH<1.0$), which may have accounted for the less aggressive demineralization of enamel. The present study showed that the bond fatigue durability of all universal adhesives to enamel with PLA etching for 15 seconds was significantly higher than that to ground enamel and was similar to enamel with PPA etching. In addition, although PLA etching did change the Ra values and surface area of enamel immediately after etching, as with PPA etching, the values measured were significantly lower than those for enamel with PPA etching. Therefore, even with less demineralization of enamel, PLA etching was able to effectively improve the enamel bond fatigue durability of all universal adhesives if applied for 15 seconds. Consequently, PLA etching for 15 seconds may be a potential protocol to improve the enamel bonding of universal adhesives with less damage to enamel than with PPA etching.

On the other hand, the PPM etchant used in this study is mainly composed of 11-methacryloyloxy-4-ethyleneglycol dihydrogen phosphate (11-M-TEG-P). These 11-M-TEG-P monomers can remove the smear layer, demineralize tooth substrates, and chemically bond to hydroxyapatite. For universal adhesives, 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) is a key technological factor for chemical bonding with tooth substrates regardless of the bonding strategies used.[34] The idea behind using PPM etching is to enhance the chemical bonding with universal adhesives by supplementing the chemical bonding capacity of MDP to tooth substrates by applying a PPM in two separate steps. In the present study, the bond fatigue durability of PE and SU was not improved by PPM etching, and the bond fatigue durability of GP to enamel with PPM etching was even decreased, regardless of etching time. One plausible rationale for this observation may be that enamel with PPM etching had already reacted with the 11-M-TEG-P in the PPM etchant, and thus the 10-MDP in the universal adhesive could not react with the hydroxyl groups of enamel, leading to a lack of improvement, or even a deterioration, in the bond fatigue durability of the adhesives. Future work is warranted to further elucidate this issue. In addition, measurements of surface Ra roughness and surface area obtained with AFM did not increase with PPM etching, and the morphology of enamel with PPM etching was similar to that of ground enamel. This apparent lack of demineralization may also be related to the enamel bond fatigue durability not being increased by PPM etching. Therefore, the use of PPM etching with universal adhesives for any etching times was not an effective protocol for improving enamel bond fatigue durability in this study.

From the overall results of this study, the null hypotheses that 1) there would be no differences in bond durability of universal adhesives to enamel among different etching protocols, and 2) different etching protocols would not influence enamel surface morphology were both rejected. This study suggests that it may be possible to use universal adhesives with etching protocols for PPA etching with reduced etching times or PLA etching for 15 seconds, but it is imperative that additional data be generated for unground enamel before making any clinical recommendations. However, under the experimental conditions of this study, PPM etching was not effective in improving enamel bonding with universal adhesives.

CONCLUSION

The results of this study suggest that 1) enamel bond durability with universal adhesives is different depending on the etching protocol and 2) different etching protocols influence enamel surface morphology. Overall, the results show that the enamel bonding of universal adhesives was improved with etching protocols of phosphoric acid etching for reduced etching times from less than one to 15 seconds or with polyacrylic acid etching for 15 seconds. However, phosphoric acid ester monomer etching was not effective in improving bonding with universal adhesives regardless of etching time.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Creighton University School of Dentistry. The approval code for this study is 760765-1.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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