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Effect of Reduced Universal Adhesive Application Time on Enamel Bond Fatigue and Surface Morphology

Y Nagura • A Tsujimoto • NG Fischer • AG Baruth
WW Barkmeier • T Takamizawa • MA Latta • M Miyazaki

Clinical Relevance

Some universal adhesives can achieve stable enamel bonding when applied with reduced application time.

SUMMARY

Objective: The purpose of this study was to evaluate the effect of reduced application times of universal adhesives on enamel bond fatigue and surface morphology of the treated enamel with constant force atomic force microscopy (AFM).

Methods: Four universal adhesives—Adhese Universal (AU), Clearfil Universal Bond Quick (CU), G-Premio Bond (GP), and Scotchbond Universal Adhesive (SU)—were evaluated in a laboratory for their ability to adhesively bond resin composite to enamel. Shear bond strengths were initially determined using 15 specimens per test group for each adhesive. Shear fatigue strengths were then determined using 20 specimens per test group for each the adhesives. The fatigue specimens were loaded using a sine wave at a frequency of 20 Hz for 50,000 cycles or until failure occurred. AFM observations, surface Ra roughness measurements, and geometric surface area evaluations of enamel surface treated with the adhesive agents were also conducted.

Yuko Nagura, graduate student, Department of Operative Dentistry, Nihon University School of Dentistry, Tokyo, Japan

*Akimasa Tsujimoto, assistant professor, Department of Operative Dentistry, Nihon University School of Dentistry, Tokyo, Japan

Nicholas G Fischer, graduate student, MDRCBB, University of Minnesota, Minneapolis, MN, USA

Andrew G Baruth, associate professor, Department of Physics, Creighton University College of Arts and Science, Omaha, NE, USA

Wayne W Barkmeier, special professor and dean emeritus, Department of General Dentistry, Creighton University, Omaha, NE, USA

Toshiki Takamizawa, associate professor, Department of Operative Dentistry, Nihon University School of Dentistry, Tokyo, Japan

Mark A Latta, professor and dean, Department of General Dentistry, Creighton University, Omaha, NE, USA

Masashi Miyazaki, professor and chair, Department of Operative Dentistry, Nihon University School of Dentistry, Tokyo, Japan

*Corresponding author: 1-8-13, Kandasurugadai, Chiyoda-ku, Tokyo, 101-8310, Japan; e-mail: tsujimoto.akimasa@nihon-u.ac.jp

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Results: A strong relationship was found between the initial shear bond strength and shear fatigue strength for enamel surface Ra roughness but not for geometric surface area. The initial shear bond strength and shear fatigue strength of CU and GP were not influenced by different application times, unlike those of AU and SU. While the surface area of enamel treated with the adhesive agents was not significantly influenced by different application times and type of adhesive, surface Ra roughness of the enamel in the AU and SU groups significantly increased with increasing application time, unlike CU and GP.

Conclusions: The results of this study suggest that universal adhesives, used with reduced application times, have adequate Ra surface roughness to provide sufficient resistance to enamel bond fatigue at application times from <1 second to 20 seconds, while the geometric surface area of adhesive-treated enamel did not show any significant changes at these different application times.

INTRODUCTION

A recent trend in adhesives has been the use of universal adhesives in either a self-etch or total-etch mode.[1] Furthermore, they can be used to bond to a variety of substrates, including enamel, dentin, resin composites, glass ceramics, zirconia, and alloys.[2] These flexible adhesive systems permit the use of the simplest adhesive strategies, with the advantage that clinicians can decide which adhesive strategy to use for specific clinical situations.[3] However, Rosa and others,[4] in a systematic review and meta-analysis, concluded that weak enamel bond strengths have been reported for universal adhesives when used in self-etch mode instead of total-etch mode. Clinical studies of resin composite restorations using universal adhesives in noncarious cervical lesions indicate that performance over six months (Mena-Serrano and others[5]) and 18 months (Perdigão and others[6]) does not depend on the bonding strategies employed. Evaluations over two years (Lawson and others[7]) and three years (Loguercio and others[8]) also indicate acceptable clinical performance for both modes, although total-etch mode appears to be superior. Therefore, the use of universal adhesives, along with phosphoric acid pre-etching of enamel, has been recommended by many researchers to achieve optimal bonding.[9-11] This approach increased bonding performance of universal adhesives to enamel in many situations[12,13] and to dentin for universal adhesives with weak acidity (All-Bond Universal: pH 3.2).[14]

A potentially simpler option to improve the enamel bonding of universal adhesives is prolonged application time, which was suggested by Cardenas and others.[15] They concluded that prolonged application times of universal adhesives in the self-etch mode might be a viable approach to enhance enamel bonding of the adhesive. Contrary to these reported findings, newly developed products such as Clearfil Universal Bond Quick (Kuraray Noritake Dental, Tokyo, Japan) and G-Premio Bond (GC, Tokyo, Japan) claim that high bond strength can be achieved even when they are applied with a shortened application time (optional manufacturer's instructions). Although reduced application time is clinically appealing, the procedure might negatively impact the creation of an etching pattern and durable enamel bonding. With the optional instructions, it has become important to examine the influence of different application times on adhesive effectiveness and whether such an approach can be applied to other universal adhesives. To date, there is only one publication, from Saikaew and others,[16] that reports the effect of reduced application times on dentin bond strengths of universal adhesives and no independent research on this approach for enamel bonding.

In the past decade, dynamic adhesive bond strength testing assessing bond fatigue, in terms of shear fatigue strength, has been developed at the Creighton University School of Dentistry (Omaha, NE, USA).[17-21] A method for this bond fatigue strength testing was originally developed by Erickson and others[22,23] at the Academisch Centrum Tandheelkunde Amsterdam (ACTA) using the ACTA fatigue tester in 2006-2008 and was later modified by Erickson and others[17] and Barkmeier and others[18] using a four-station fatigue cycler (Proto-tech, Portland, OR, USA) and then by Latta and Barkmeier[19] using a servohydrolic testing machine (MTS 858 Mini Bionix II, MTS Systems Corp, Eden Prairie, MN, USA). Most recently, fatigue testing methods were further modified by Takamizawa and others[20] and Tsujimoto and others[21] using an all-electric dynamic test instrument (ElectonPuls E1000, Instron, Norwood, MA, USA). The present method allows better assessment of a material's total-life tolerance to the repeated low-magnitude loads encountered in the oral cavity, as the cyclic stresses are thought to be more similar to the stresses generated during oral function than the continuous loading to failure applied with traditional shear bond strength testing.[22,23]

Table 1: *Materials Used in This Study*

Adhesive (Lot Number)	Code (pH)	Main Components	Manufacturer
Adhese Universal (164453)	AU (2.5)	Bis-GMA, HEMA, MDP, MCAP, decandiol dimethacrylate, dimethacrylate, ethanol, water, initiators, stabilizers, silicon dioxide	Ivoclar Vivadent (Schaan, Lichtenstein)
Clearfil Universal Bond Quick (1L0003)	CU (2.3)	Bis-GMA, HEMA, MDP, hydrophilic amide monomer, ethanol, water, initiators, silica, silane coupling agent	Kuraray Noritake Dental (Tokyo, Japan)
G-Premio Bond (1603091)	GP (1.5)	MDP, 4-MET, MEPS, methacrylate monomer, acetone, water, initiators, silica	GC (Tokyo, Japan)
ScotchbondUniversal Adhesive (617265)	SU (2.7)	Bis-GMA, HEMA, MDP, decamethylene dimethacrylate, ethyl methacryalate, propenoic acid, copolymer of acrylic and itaconic acid, dimethylaminobenzoate, methyl ethyl ketone, ethanol, water, silane treated silica, initiators, silane	3M (St Paul, MN, USA)

Abbreviations: Bis-GMA, bisphenol A glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl di-hydrogen phosphate; MCAP, methacrylated carboxylic acid polymer; 4-MET, 4-methacryloyloxyethyl trimellitate; MEPS, methacryloyloxyalkyl thiophosphate methylmethacrylate.

Reported laboratory studies indicate that shear fatigue strength was not influenced by the frequency rate (5, 10, or 20 Hz with enamel in Takamizawa and others[24] and dentin in Scheidel and others[25]) or by the number of cycles (50,000, 100,000, or 1,000,000 cycles with enamel and dentin in Tsujimoto and others[26]). As a result of these studies, the fatigue load for adhesively bonded resin composite to mineralized tooth structures was standardized using a sine wave frequency of 20 Hz for 50,000 cycles or until failure occurs. This is a time-efficient approach for shear fatigue strength testing of rapidly advancing modern dental adhesive systems.

Recently, Tsujimoto and others[27] reported the relationship between shear fatigue strength under standardized conditions and quantitative three-dimensional micrographs obtained with constant force atomic force microscopy (AFM). AFM provides quantitative data about a wide range of surface topography characteristics, including surface roughness and geometric surface area. Additionally, three-dimensional micrographs allow for qualitative comparison of surfaces both in and out of the surface plane. Furthermore, AFM obtains quantitative data at the nanometer scale ($\sim$5 nm lateral and $<$1 nm height resolution) as opposed to the micrometer scale of optical profilometry ($\sim$4.4 μm lateral and $>$100 μm height resolution).[28] In addition, the noninvasive testing requires no sample preparation such as the metallic sputter coatings associated with scanning electron microscopy. Important correlations exist between shear fatigue strength testing and quantitative AFM micrographs,[27] where three-dimensional topographical mapping of adhesive-treated enamel surfaces offers valuable insight into the effects of reduced application times on nanoscale surface roughness and geometric surface area of adhesive-treated enamel.

The purpose of the present study was to evaluate the effect of reduced universal adhesive application times on enamel bond fatigue and nanoscale surface morphology. The null hypotheses tested were that the application time for adhesion to enamel would not influence 1) the shear fatigue strength 2) or the nanoscale surface roughness and geometric surface area of the resultant enamel surface.

METHODS AND MATERIALS

Study Materials

Four universal adhesives were evaluated in this study: 1) Adhese Universal (AU; Ivoclar Vivadent, Schaan, Liechtenstein), 2) Clearfil Universal Bond Quick (CU; Kuraray Noritake Dental, Tokyo, Japan), 3) G-Premio Bond (GP; GC, Tokyo, Japan), and 4) Scotchbond Universal Adhesive (SU; 3M ESPE, St Paul, MN, USA). Z100 Restorative (3M ESPE) was used as the resin composite for the bonding procedures. The adhesive materials are listed in Table 1 with the associated lot numbers and components.

Specimen Preparation

Sectioned buccal and lingual halves of deidentified 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 bonding surfaces were prepared on the mounted buccal and lingual surfaces by wet (water) grinding using a gradually increasing sequence (180, 320, 600, 1200, 2000, and 4000 grit) of silicon carbide papers (Struers, Cleveland, OH, USA) up to 4000 grit in a grinder-polisher (Ecomet 4, Buehler, Lake Bluff, IL, USA). A final 4000-grit surface was used to minimize the influence of any directionality of the surface grooving created by the abrasives. These

Table 2: *Application Protocol for Universal Adhesives*

Adhesive	Adhesive Application Protocol
AU	Adhesive applied to air-dried tooth surface with rubbing motion for 20 s and then medium air pressure applied to surface for 5 s. Adhesive photopolymerized for 10 s.
CU	Adhesive applied to air-dried tooth surface with a rubbing motion and then medium air pressure applied to surface for 5 s. *No waiting time is required.* Adhesive photocured for 10 s.
GP	Adhesive applied to air-dried tooth surface for 10 s and then maximum air pressure applied to surface for 5 s. *Adhesive can provide sufficient bonding strength even when dried immediately after application without waiting time.* Adhesive photocured for 10 s.
SU	Adhesive applied to air-dried tooth surface with rubbing motion for 20 s and then medium air pressure applied to surface for 5 s. Adhesive photocured for 10 s.

Abbreviations: AU, Adhese Universal; CU, Clearfil Universal Bond Quick; GP, G-Premio Bond (GP); SU, Scotchbond Universal Adhesive.

surfaces were then washed with water and dried using a dental three-way syringe at a distance of 5 cm above the surface at an air pressure of 3.8 kgf/cm^2. The specimens were prepared under ambient laboratory conditions of 22°C ± 2°C and 40% ± 20% relative humidity.

Initial Shear Bond Strength Test

Stainless-steel rings (mold-enclosed method) with an inner diameter of 2.4 mm, an outer diameter of 4.8 mm, and a height of 2.6 mm were used to bond the resin composite to the flat ground enamel surfaces. The bonding site surfaces (bottom side) of the stainless-steel rings were treated with a releasing agent (3% solution of paraffin in hexane) to mechanically isolate the bonded enamel/resin composite interface. The flat ground enamel bonding sites were then treated with the universal adhesives in the self-etching mode. The protocols were based on the manufacturers' instructions (Table 2), modified to allow for different application times. Three groups were prepared for each adhesive: 1) ground enamel treated with universal adhesive and immediately air-dried (<1-second group), 2) ground enamel treated with universal adhesive for 10 seconds and air-dried (10-second group), and 3) ground enamel treated with universal adhesive for 20 seconds and then air-dried (20-second group). In the <1-second group, no rubbing motion was used for any adhesive, and the surface was air-dried immediately after application. In the 10-second and 20-second groups, the adhesives for which the manufacturers specify a rubbing motion (AU, CU, and SU) were rubbed during the treatment period; as GP does not have a rubbing motion specified, it was not rubbed during this period. Thus, each adhesive was applied in accordance with the manufacturer's instructions in one condition and with a modified protocol in the others. A purpose-built fixture was used to position and hold the stainless-steel rings over the bonding sites, and the resin composite (Z100 Restorative) was placed into the rings using a packing instrument. The resin composite was photo-cured for 40 seconds at a standardized distance of 1 mm using a quartz-tungsten halogen unit (Spectrum 800 Curing Unit, Dentsply Sirona, York, PA, USA) set at 700 mW/cm^2. The bonded specimens were stored in distilled water for 24 hours (37°C) before initial shear bond strength testing.

Initial shear bond strength tests (24 hours of water storage at 37°C) were carried out on all groups using the stainless-steel ring described above (mold-enclosed method). A chisel-shaped metal rod was used to apply the load on the stainless-steel rings immediately adjacent to the flat enamel surfaces. The specimens (15 per group) 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 for the peak load at failure divided by the bonded surface area.

Shear Fatigue Strength Test

A staircase method, previously described by Draughn[29] and Dewji and others,[30] was used to perform the shear fatigue strength tests (ElectroPuls E1000). Twenty specimens were prepared for each adhesive group, as described above, and stored in distilled water at 37°C for 24 hours before shear fatigue testing. The fatigue load was applied sinusoidally at a frequency of 20 Hz for 50,000 cycles or until failure occurred (Figure 1). The initial peak load was set at approximately half (45-60 N) of the initial mean shear bond strength determined for each adhesive. The lower load limit was at approximately zero (0.4 N). Subsequent loading was adjusted upward or downward approximately 10% from the previous load, depending on specimen survival or failure, respectively. The test specimens were immersed in room-temperature water (23°C ±

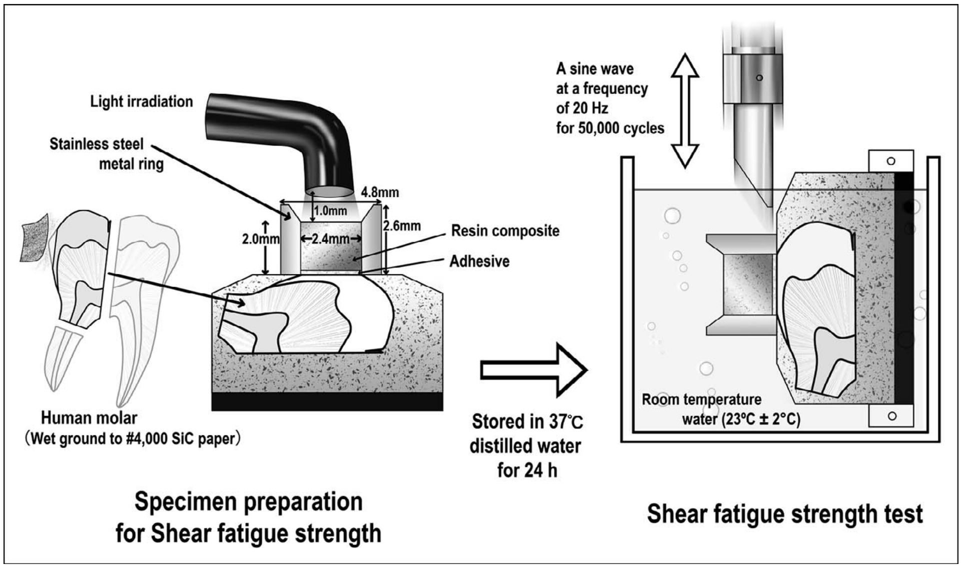

Figure 1. *Schematic illustration of the experimental setup for shear fatigue strength testing under standardized conditions.*

2°C) during testing to minimize the influence of temperature increases on the bonded specimens. The mean shear fatigue strength (X) and standard deviation (S) were calculated using the formulas listed below and as described by Draughn:[29]

$$\mathrm{X} = \mathrm{Xo} + \mathrm{d}\left(\frac{A}{N} - \frac{1}{2}\right)$$

$$\mathrm{S} = 1.62\mathrm{d}\left(\frac{NB - A^2}{N^2} + 0.029\right)$$

$$\mathrm{N} = \sum n_i,\ \mathrm{A} = \sum i n_i, \mathrm{B} = \sum i^2 n_i$$

where Xo is the lowest stress level considered in the analysis, d is the stress increment employed in the sequential tests, $i = 0$ is the lowest stress level at which a failure occurs, $i = 1$ is the next etc, and n is the number of failures after bond fatigue strength testing at each increment.

Failure Mode Analysis

The bond failure sites after initial shear bond strength and shear fatigue strength tests were assessed using an optical stereomicroscope (MZ16, Leica Microsystems, Heerbrugg, Switzerland) at 20× magnification. The failure types were classified by a calibrated investigator as 1) adhesive failure at the interface, 2) cohesive failure in resin composite, 3) cohesive failure in enamel, and 4) mixed failure (partially adhesive and cohesive failure).

AFM Evaluation

Six representative specimens were selected per group for AFM evaluation. Each specimen was imaged in three separate locations near the center of the specimen. Prior to measurements, specimens were washed with three alternating rinses using acetone and water to remove the adhesive and then five seconds of 80-psi compressed, dried air was used in a sweeping motion to remove any surface debris. Scanning probe AFM measurements were performed (5420 SPM/AFM Microscope, Agilent Technologies, Santa Clara, CA, USA) in an acoustical and mechanical isolation chamber under ambient laboratory conditions (22°C ± 2°C and 40% ± 20% 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 [k] of 0.2 N/m) (BudgetSensors, Sofia, Bulgaria) at 512 lines per image at a rate of 4.0 lines per second. In this mode, the AFM is in constant feedback with the cantilever to maintain a constant deflection by increasing or decreasing the AFM specimen separation with piezoelectric motors (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 roughness was quantified in terms of Ra (μm), the arithmetic average of the absolute values of the profile height deviations from the mean, recorded within an equivalent imaging area. Similarly, the geometric surface area measures the modified surface area (above the anticipated 900 μm^2 for a flat surface) due to height variations across the

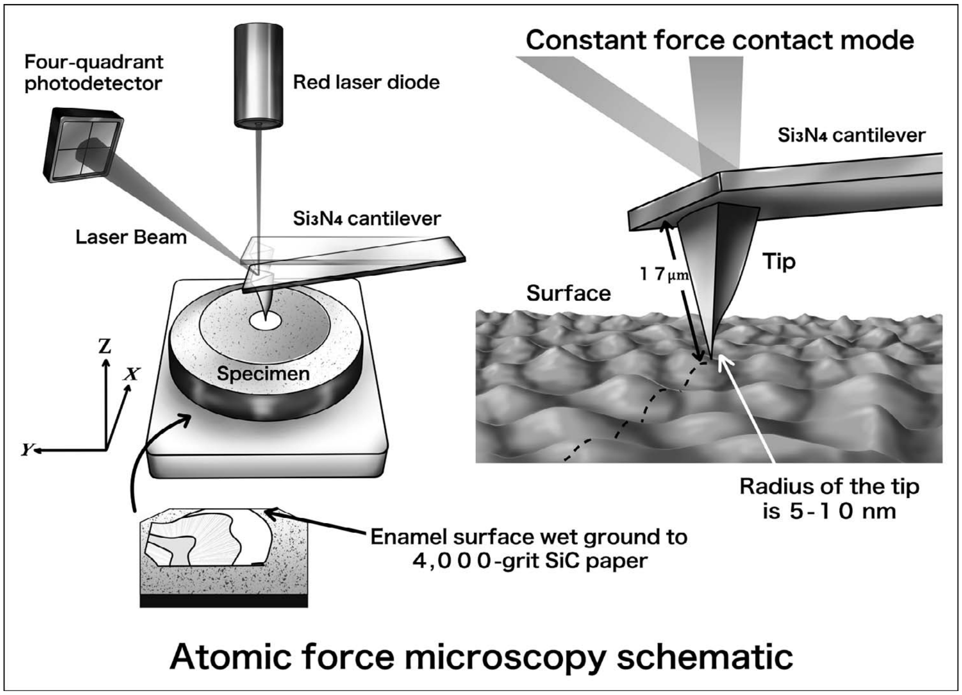

Figure 2. *Schematic illustration of constant force atomic force microscopy, where a flexible cantilever with an atomically sharp tip is systematically rastered across the surface of a sample. A laser beam focused on the back of the reflective cantilever captures three-dimensional topography changes that are reflected in changes to the laser beam position on a four-quadrant photodetector.*

surface. Qualitatively, intra- and intergroup differences were assessed by viewing the AFM micrographs.

Statistical Analysis

The initial shear bond strength, Ra surface roughness, and geometric surface area data were analyzed using two-way analysis of variance (factors: adhesive and treatment time) followed by the Tukey *post hoc* honest significant difference test (α=0.05). A linear regression analysis between initial shear bond strength and shear fatigue strength on the Ra surface roughness and geometric surface area was also conducted. The Fisher exact test was used to analyze the failure mode after initial shear bond strength and shear fatigue strength testing. These statistical analyses were conducted using a commercial software package (SPSS Statistics Base for Windows, IBM, Armonk, NY, USA). The shear fatigue strength data were analyzed using a modified t-test with a nominal α of 0.05 (custom program).

Table 3: *Effect of Reduced Application Times on Initial Shear Bond Strength of Universal Adhesives (in MPa)*[a]

Adhesive	0 s	10 s	20 s
AU	20.4 (3.0) aA	21.8 (3.0) aA	26.2 (2.9) aB
CU	25.6 (3.1) bA	26.1 (3.2) bA	26.5 (2.8) aA
GP	25.5 (3.2) bA	25.9 (3.6) bA	26.1 (2.4) aA
SU	20.9 (3.7) aA	21.2 (4.0) aA	27.2 (2.5) aB

[a] *Values in parentheses are standard deviations. Same lowercase letter in same column indicates no significant difference (p>0.05). Same capital letter within individual rows indicates no significant difference (p>0.05). Abbreviations: AU, Adhese Universal; CU, Clearfil Universal Bond Quick; GP, G-Premio Bond (GP); SU, Scotchbond Universal Adhesive.*

RESULTS

Regression Analysis

A strong positive correlation (R=0.9712, R^2=0.9433, p<0.0001) was found between the initial bond strength and shear fatigue strength. In addition, a positive correlation was evident in surface roughness (Ra) for both the initial shear bond strength (R=0.8415, R^2=0.7082, p=0.0006) and shear fatigue strength (R=0.8507, R^2=0.7237, p=0.0005). These correlations were statistically significant at α=0.05. Significant positive correlations were not observed for geometric surface area for either initial shear bond strength (R=0.0730, R^2=0.0053, p=0.8215) or shear fatigue strength (R=0.0738, R^2=0.0054, p=0.8197).

Initial Shear Bond Strength

The results for the effect of different application times on the initial shear bond strength of the universal adhesives to enamel are shown in Table 3. The initial shear bond strengths of AU and SU were influenced by the application time of the adhesive agent, as the initial shear bond strengths of the <1-second and 10-second groups for AU and SU were significantly lower than those of 20-second group. On

Table 4: *Effect of Reduced Application Times on Shear Fatigue Strength of Universal Adhesives (in MPa)*[a]

Adhesive	0 s	10 s	20 s
AU	9.7 (1.6) aA	10.3 (1.8) aA	13.2 (2.9) aB
CU	12.6 (2.0) bA	13.2 (1.6) bA	13.6 (2.8) aA
GP	12.5 (2.1) bA	13.7 (2.0) bA	13.8 (2.4) aA
SU	10.2 (2.4) aA	11.0 (1.8) aA	13.9 (3.5) aB

[a] *Values in parentheses are standard deviations. Same lowercase letter in same column indicates no significant difference (p>0.05). Same capital letter within individual rows indicates no significant difference (p>0.05).*
Abbreviations: AU, Adhese Universal; CU, Clearfil Universal Bond Quick; GP, G-Premio Bond (GP); SU, Scotchbond Universal Adhesive.

the other hand, there was no significant difference ($p>0.05$) in the initial shear bond strength of CU and GP over the three application times. The initial shear bond strengths for the <1-second and 10-second groups of AU and SU were lower than those of all other groups.

Shear Fatigue Strength

The results for the effect of different application times on the shear fatigue strength of the universal adhesives to enamel are shown in Table 4. The shear fatigue strengths of AU and SU were influenced by the application time of the adhesive, as the shear fatigue strengths of the <1-second and 10-second groups for AU and SU were significantly lower than those of the 20-second group. There was no significant difference ($p>0.05$) in the shear fatigue strength of CU and GP over the different application times. The shear fatigue strengths for the <1-second and 10-second groups of AU and SU were lower than those of all other groups.

Failure Mode Analysis

The failure modes for initial shear bond strength and shear fatigue strength testing are shown in Table 5. Adhesive failure was the dominant mode of failure for both the shear bond strength and the shear fatigue strength specimens. The Fisher exact test revealed no statistically significant differences ($p>0.05$) in failure mode, depending on the type of adhesive or different application times. The shear fatigue strength failure mode was adhesive for all adhesives and all application times.

Three-Dimensional Topographic Observations

Qualitative changes in three-dimensional topography are evident in representative AFM topographic images (Figure 3). For AU, different appearances were observed for different application times. Although a smear layer and grinding debris were observed in

Table 5: *Failure Mode Analysis of Debonded Specimens After Initial Shear Bond Strength and Shear Fatigue Strength Testing*[a]

Adhesive	Condition	Initial Shear Bond Strength	Shear Fatigue Strength
AU	0 s	[100/0/0/0] aA	[100/0/0/0] aA
	10 s	[100/0/0/0] aA	[100/0/0/0] aA
	20 s	[86/0/7/7] aA	[100/0/0/0] aA
CU	0 s	[100/0/0/0] aA	[100/0/0/0] aA
	10 s	[93/0/7/0] aA	[100/0/0/0] aA
	20 s	[86/0/14/0] aA	[100/0/0/0] aA
GP	0 s	[93/0/7/0] aA	[100/0/0/0] aA
	10 s	[100/0/0/0] aA	[100/0/0/0] aA
	20 s	[86/0/7/7] aA	[100/0/0/0] aA
SU	0 s	[100/0/0/0] aA	[100/0/0/0] aA
	10 s	[100/0/0/0] aA	[100/0/0/0] aA
	20 s	[67/0/13/20] aA	[100/0/0/0] aA

[a] *Percentage of failure mode [adhesive failure/cohesive failure in composite/cohesive failure in enamel/mixed failure]. Same 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).*
Abbreviations: AU, Adhese Universal; CU, Clearfil Universal Bond Quick; GP, G-Premio Bond (GP); SU, Scotchbond Universal Adhesive.

topographic images of the <1-second group, these were less prominent in the 10-second group and had disappeared in the 20-second group. For CU, there were no obvious morphological differences for increased application times. Periodic grooves and an absence of smear layer and grinding debris were observed. For GP, a demineralized enamel surface with clear enamel prisms was seen regardless of the application time. There were no clear morphological differences in enamel prisms or microirregularities of hydroxyapatite crystals with increased application times. For SU, different morphologies were observed with increased application time. Topographic images of the <1-second and 10-second groups revealed periodic grooves on the ground surfaces without smear layer and grinding debris, while enamel prisms or microirregularities of hydroxyapatite crystals were observed only in the 20-second group.

Ra Surface Roughness and Geometric Surface Area Measurements

The surface roughness (Ra) results are shown in Table 6. For AU and SU, values significantly increased ($p<0.05$) with increasing application times. In CU and GP, there were no differences ($p>0.05$) with increased application time. Although time independent, the values for CU and GP were significantly greater at short application times when compared to AU and SU, with GP being the largest.

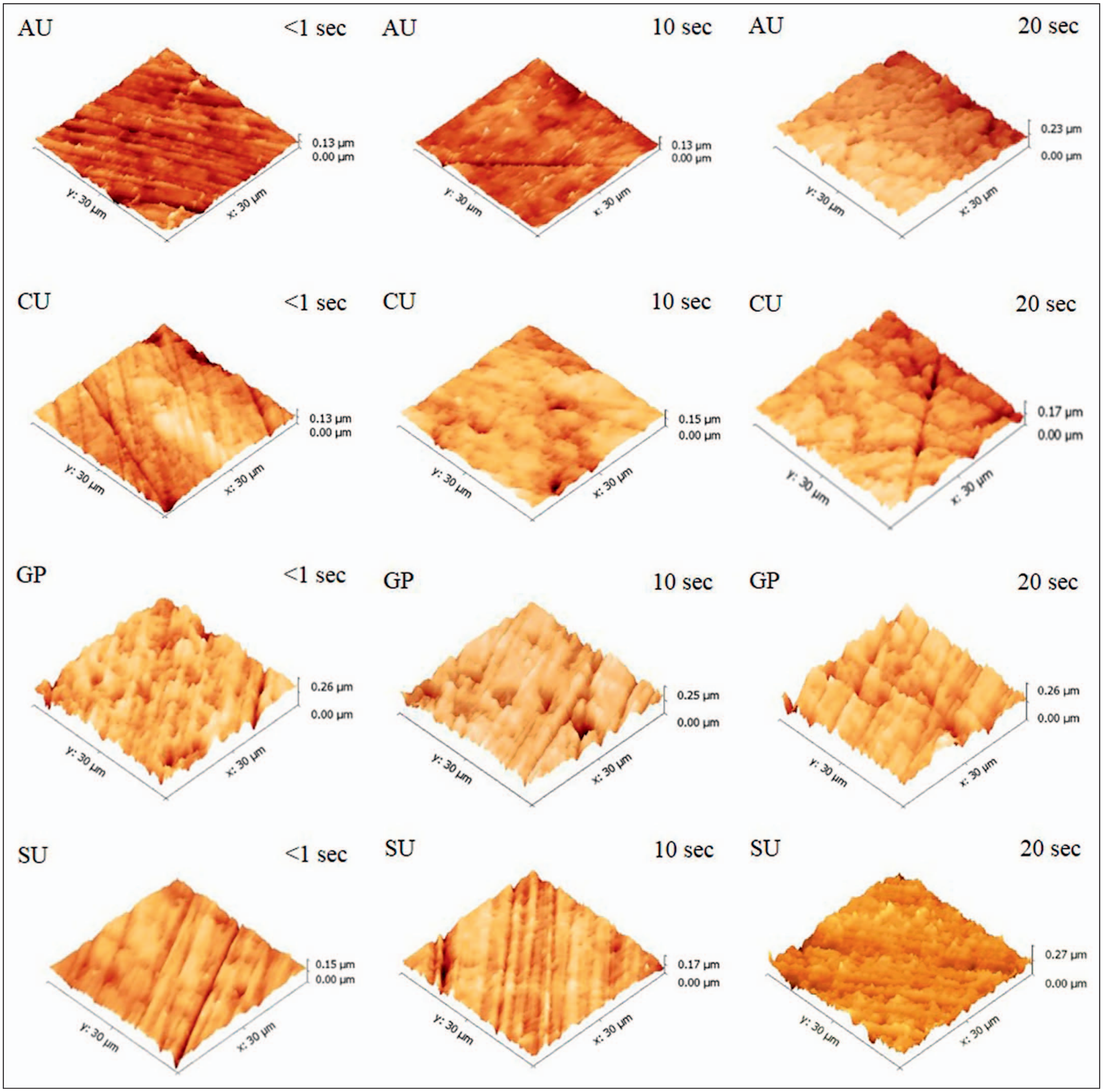

Figure 3. *False color three-dimensional topographic images (30×30 μm) of universal adhesive–treated enamel surfaces obtained by constant force atomic force microscopy. Micrograph heights were normalized for accurate visual inspection, where the vertical scale bar shows the maximum feature height in the given micrograph. AU, Adhese Universal; SU, Scotchbond Universal Adhesive; CU, Clearfil Universal Bond Quick; GP, G-Premio Bond (GP).*

These significant differences in Ra were not reflected in the geometric surface area values, as seen in Table 7. A perfectly flat surface would have a surface area of 900 μm^2. Therefore, the measured values (ranging from 903.4 to 906.0 μm^2 on average) varied only slightly from a flat surface and were not influenced by increased application time.

DISCUSSION

The initial shear bond strength and shear fatigue strength of CU and GP were not influenced by different application times, unlike those of AU and SU. Thus, the claim by the manufacturers that these adhesives can be used even with reduced application

Table 6: *Effect of Reduced Application Times on Surface Ra Roughness of Enamel Surfaces (in nm)*[a]

Adhesive	0 s	10 s	20 s
AU	10.2 (1.1) aA	12.9 (2.6) aA	18.3 (3.4) aB
CU	16.5 (3.9) bA	16.8 (1.1) bA	15.3 (2.8) bA
GP	21.1 (2.2) cA	21.1 (3.4) cA	19.6 (3.6) aA
SU	13.0 (1.8) aA	13.6 (3.2) aA	21.2 (4.3) aB

[a] *Values in parentheses are standard deviations. Same lowercase letter in same column indicates no significant difference ($p>0.05$). Same capital letter within individual rows indicates no significant difference ($p>0.05$). Abbreviations: AU, Adhese Universal; CU, Clearfil Universal Bond Quick; GP, G-Premio Bond (GP); SU, Scotchbond Universal Adhesive.*

Table 7: *Effect of Reduced Application Times on Geometric Surface Area of Enamel Surfaces (in μm^2)*[a]

Adhesive	<10 s	10 s	20 s
AU	905.0 (2.3) aA	904.9 (3.0) aA	905.0 (2.3) aA
CU	903.9 (2.2) aA	903.4 (1.7) aA	903.4 (2.8) aA
GP	906.0 (2.5) aA	905.8 (2.1) aA	904.9 (3.3) aA
SU	904.6 (3.3) aA	904.2 (3.4) aA	904.7 (1.9) aA

[a] *Values in parentheses are standard deviations. Same lowercase letter in same column indicates no significant difference ($p>0.05$). Same superscript letter within individual rows indicates no significant difference ($p>0.05$). Abbreviations: AU, Adhese Universal; CU, Clearfil Universal Bond Quick; GP, G-Premio Bond (GP); SU, Scotchbond Universal Adhesive.*

times appears to be accurate from a bond fatigue perspective. Per the results of the shear bond strength and shear fatigue strength tests, the first null hypothesis that the adhesive application time to enamel would not influence the shear bond strength and shear fatigue strength was rejected. However, there were no significant differences in failure mode after the shear bond strength or shear fatigue strength tests, and adhesive failures were the most frequently observed type of failure, regardless of the application time or adhesive agent. This is not surprising, as the bonding procedures were performed without phosphoric acid pre-etching of the enamel surface and an absence of etching is known to increase the frequency of adhesive failures.[31] In addition, the testing was performed using a mold-enclosed method, which is also known to result in an increase in adhesive failures.[32]

Strong positive correlations were found between both shear bond strength and shear fatigue strength and the surface roughness. For GP, the Ra was not influenced by the different application times and was significantly higher than those of other tested adhesives, except that of SU-treated enamel for 20 seconds. Topographic images of GP-treated enamel, regardless of the application time, showed a demineralized enamel surface with enamel prisms or microirregularities of hydroxyapatite crystals. The pH of GP (1.5) was lower than the other tested adhesives (2.3-2.7), which may contribute to the enamel bond fatigue strength of GP and Ra of GP-treated enamel even with reduced application times. In addition, GP contains 4-methacryloxyethyl trimellitic acid (4-MET) and 10-methacryoyloxydecyl dihydrogen thiophosphate (10-MDTP) as functional monomers in addition to 10-methacryoyloxydecyl dihydrogen phosphate (10-MDP). The former two monomers are acidic, and it has been reported that 4-MET forms strong chemical bonds with calcium-containing substrates such as enamel[33] (10-MDTP reportedly forms chemical bonds to metal substrates,[34] a property that was probably not important in this study). The other tested universal adhesives (SU, CU, and AU) contained only a single acidic functional monomer (10-MDP), which suggests that the presence of multiple acidic functional monomer types is beneficial for enamel bonding. However, as manufacturers do not release specific information on the concentration of acidic functional monomers, it is impossible to rule out the possibility that GP simply has a higher concentration of acidic functional monomers and that the variety of types is not important in this context.

For CU, the Ra of treated enamel was not influenced by increasing the application time (similar to GP), but the Ra was significantly lower than all groups of GP and the 20-second group of AU and SU. Topographic images (Figure 3) of CU-treated enamel showed weaker demineralization compared to all groups of GP-treated enamel and the 20-second group of AU- and SU-treated enamel (periodic grooves on the enamel surfaces from grinding are visible) and revealed no application time-dependent morphological differences. CU includes a new hydrophilic amide monomer that purportedly is a key technological factor in the development of rapid bonds. This new hydrophilic amide monomer rapidly permeates tooth substrates due to its higher hydrophilicity, which eliminates chairside waiting time. It also shows a higher degree of polymerization than 2-hydroxyethyl methacrylate, reducing water absorption, which is important to bond durability. CU also includes a new integrated photoinitiator chemistry, also used in Clearfil SE Bond 2, that may provide more free radicals and lead to higher monomer conversion rates.[35] These factors may explain the higher bond fatigue durability of CU with reduced application times, even with lower Ra values and weaker demineralization, compared to all groups of GP and the 20-second group of SU. CU and GP could therefore be interpreted as demonstrating fundamentally different successful approaches (more acidic functional monomers vs rapid penetration and higher monomer polymerization rate, respectively) to the development of universal adhesives. It is an open question as to whether their combined integration might lead to further improvements.

Naively, an increase in shear bond strength and shear fatigue strength could be associated with an increase in geometric surface area. However, the geometric surface area of universal adhesive–treated enamel was not influenced by different application times for any of the adhesives tested. In addition, there was no correlation between the shear bond strength or shear fatigue strength and surface area of enamel surfaces treated with the universal adhesives. Therefore, the geometric surface area alone of universal adhesive–treated enamel does not seem to be an important factor for bond fatigue strength. Based on the results of Ra values and surface areas, the second null hypothesis that the application time to enamel would not influence the surface roughness was partially rejected.

The overall study results demonstrated that bond fatigue strength of some universal adhesives and surface morphologies were not influenced by different application times, and thus some universal adhesives may be suitable for use with a reduced application time. Generally, clinicians would desire to reduce the application time, which would encourage the use of those kinds of universal adhesives. However, there is some reason to be concerned about the direct clinical applicability of these results. Mine and others[36] reported that the surface-preparation method of enamel significantly affected the nature of the smear layer and the interaction with a mild (pH 2.7) one-step self-etch adhesive (Clearfil S^3 Bond, Kuraray Noritake Dental). In the bond strength tests used in the present study, the enamel bonding surface was prepared by grinding to 4000-grit SiC paper to minimize the influence of surface scratches and irregularities on bond fatigue strength testing. Tani and Finger[37] reported that the thickness of the smear layer decreases with increasing SiC grit numbers. The thickness of the smear layer on the bonding surface used in this study may have been so thin that CU and GP could effectively interact with the enamel substrate. In addition, it has been reported that characteristics of the smear layer vary according to preparation variables[38] and that smear layers prepared with a diamond bur are more compact than those prepared by SiC paper when examined under scanning electron microscopy[39] and transmission electron microscopy.[40] Therefore, it is possible that the denser smear layer created by a coarse diamond bur might hinder functional monomer penetration, and hence the results of this study may not be directly applicable to clinical situations using a diamond bur. Furthermore, a one-step self-etch adhesive (Clearfil S^3 Bond) has been shown to be affected by the specific type of diamond bur used, with fine-grit diamond burs producing the highest microtensile bond strength as compared to medium and coarse grit.[40] Therefore, differences in smear layer preparation and subsequent bonding performance may exist when diamond burs are utilized in laboratory studies to mimic clinical practice.

A previous study[16] that reported the effect of reduced application times of universal adhesives to dentin concluded that bonding to diamond bur–cut dentin always resulted in significantly lower bond strength regardless of the universal adhesive or application time. In addition, lower dentin bond strengths were found with reduced application times of adhesives to bur-cut dentin. Further research is needed on the effect of reduced application time on the bonding performance of universal adhesives with bur-cut enamel.

While recommendations have been made for abrasive papers to mimic bur-cut smear layers for self-etch adhesives on dentin,[38] these remain unclear for universal adhesives on enamel. Establishing a methodology for reproducing a clinical bur-cut smear layer with abrasive papers is important, as cutting with hand pieces is affected by a number of factors, including surface orientation, material removal rate, and application force.[41] This may make reproducibility more difficult, but this could be ameliorated by the use of abrasive papers as long as they faithfully reproduce the smear layer.

CONCLUSIONS

The results of this study suggest that certain universal adhesives can be used with reduced application times. These adhesives appear to have adequate enamel bond fatigue strength and surface roughness (Ra) values at application times ranging from <1 second to 20 seconds, while the geometric surface area did not show any significant changes with different application times.

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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 Biomedical Institutional Review Board at Creighton University, Omaha, NE, USA. The approval code for this study is 760765-1.

Conflict of Interest

The authors of this article 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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