Comparison of enamel bond fatigue durability between universal adhesives and two-step self-etch adhesives: Effect of phosphoric acid pre-etching

Shunichi SUDA¹, Akimasa TSUJIMOTO¹, Wayne W. BARKMEIER², Kie NOJIRI¹, Yuko NAGURA¹, Toshiki TAKAMIZAWA¹, Mark A. LATTI² and Masashi MIYAZAKI¹

¹ Department of Operative Dentistry, Nihon University School of Dentistry, 1-8-13 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-8310, Japan

² Department of General Dentistry, Creighton University School of Dentistry, 2500 California Plaza, Omaha, NE 68178, USA

Corresponding author, Akimasa TSUJIMOTO; E-mail: tsujimoto.akimasa@nihon-u.ac.jp

The effect of phosphoric acid pre-etching on enamel bond fatigue durability of universal adhesives and two-step self-etch adhesives was investigated. Four universal adhesives and three two-step self-etch adhesives were used. The initial shear bond strengths and shear fatigue strengths to enamel with and without phosphoric acid pre-etching using the adhesives were determined. SEM observations were also conducted. Phosphoric acid pre-etching of enamel was found to increase the bond fatigue durability of universal adhesives, but its effect on two-step self-etch adhesives was material-dependent. In addition, some universal adhesives with phosphoric acid pre-etching showed similar bond fatigue durability to the two-step self-etch adhesives, although the bond fatigue durability of universal adhesives in self-etch mode was lower than that of the two-step self-etch adhesives. Phosphoric acid pre-etching enhances enamel bond fatigue durability of universal adhesives, but the effect of phosphoric acid pre-etching on the bond fatigue durability of two-step self-etch adhesives was material-dependent.

Keywords: Phosphoric acid pre-etching, Enamel bond fatigue durability, Universal adhesive, Two-step self-etch adhesive

INTRODUCTION

The benefits of applying phosphoric acid etching to enamel selectively prior to the application of self-etch adhesives (phosphoric acid pre-etching) to improve the bond strength of the adhesives have been well documented in the literature^{1–4}. Frankenberger *et al.*⁵ published “Selective enamel etching reconsidered: better than etch-and-rinse and self-etch?” and concluded that clinical bonding performance of self-etch adhesives was improved when phosphoric acid was applied on enamel selectively. Therefore phosphoric acid pre-etching has become one of the clinical options when self-etch adhesives are to be used, based on laboratory and clinical studies^{1–5}. Phosphoric acid pre-etching modifies enamel morphology^{6,7}, structure⁸ and interfacial characteristics^{4,9,10} in order to achieve durable bonds.

On the other hand, Van Meerbeek *et al.*¹¹ and Kubo *et al.*¹² have reported that no difference in clinical performance of resin composite restorations was recorded when two-step self-etch adhesive (Clearfil SE Bond, Kuraray Noritake Dental, Tokyo, Japan) was applied in both the presence and absence of phosphoric acid pre-etching. In addition, excellent clinical studies related to the 3, 5, 8 and 13 year follow-up of resin composite restorations in non-carious cervical lesions using two-step self-etch adhesive (Clearfil SE Bond) with and without phosphoric acid pre-etching have been reported by Peumans *et al.*^{13–16}. They suggested that phosphoric acid pre-etching was not critical for the overall clinical performance of the restorations at 3 and 5 year follow-up^{13,14}, and only had some minor positive effects on marginal integrity and absence of marginal discoloration at 8 and 13 year follow-up^{15,16}. Therefore,

questions still remain about the effect of phosphoric acid pre-etching on the bonding performance of two-step self-etch adhesives, and further work is necessary.

Two-step self-etch adhesives have been further developed in recent years, and two two-step self-etch adhesives, OptiBond XTR (Kerr, Orange, CA, USA) and Clearfil SE Bond 2 (Kuraray Noritake Dental), have been introduced to the profession with the claim that they exhibit superior bonding performance to the previous generations of two-step self-etch adhesives, including Clearfil SE Bond. In the literature, Walter *et al.*¹⁷ and Meharry *et al.*¹⁸ reported that Optibond XTR showed superior enamel bond strength compared to Clearfil SE Bond. In addition, Sato *et al.*¹⁹ reported that the bond strength after 24 h and long-term (6 month and 1 year) water storage of Clearfil SE Bond 2, which is offered as an improved version of Clearfil SE Bond with a new photo-initiator, was higher than that of Clearfil SE, and that it showed a higher degree of conversion. Therefore, these adhesives should be compared to the previous generation of two-step self-etch adhesives in order to determine whether phosphoric acid pre-etching is necessary with current adhesives of this type.

Another recent trend in adhesives is universal adhesives, which have gained popularity due to their versatility as they can be used with either total-etch, self-etch, or selective etch techniques²⁰, and can also bond to various substrates, such as glass ceramics, zirconia and metal alloys, in addition to tooth substrates²¹. In a recent systematic review of the bonding performance of universal adhesives in laboratory studies, weak enamel bond strengths have been reported for the adhesives when used without phosphoric acid pre-etching, compared to those

with the pre-etching²²). Therefore many researchers recommended phosphoric acid pre-etching for universal adhesives in order to obtain optimal enamel bonds^{7,10}.

de Goes *et al.*²³) reported that phosphoric acid pre-etching increased bond strength for a universal adhesive (Scotchbond Universal Adhesive) and two-step self-etching adhesive (Clearfil SE Bond), but there was no difference in bond strength depending on the type of adhesive regardless each of the presence or absence of the pre-etching. On the other hand, Makishi *et al.*²⁴) have conducted similar study, but the results were only partially consistent with the study of de Goes *et al.*²³). Makishi *et al.*²⁴) investigated enamel bond strength after 24 h and 1 year water storage of universal adhesives (All-Bond Universal and Scotchbond Universal Adhesive) and two-step self-etch adhesive (Clearfil SE Bond) with phosphoric acid pre-etching, and concluded that the enamel bond strength after 1 year water storage was not influenced by type of adhesives, unlike that after 24 h, when that of two-step self-etch adhesive was higher than those of universal adhesives. The increasing popularity of universal adhesives, driven by their versatility, has made pressing the question of whether the universal adhesive or two-step self-etch adhesive is superior for enamel bonding, regardless of the presence or absence of phosphoric acid pre-etching.

An extremely large number of laboratory enamel bond strength evaluations related to effect of phosphoric acid pre-etching have been conducted over the years using static bond strength testing such as shear macro- and micro-tensile push-out bond strength, and so on^{1-4,6-10,20,21,23,24}). An article search of literature conducted in May 2017 in the databank PubMed using the keywords “bond strength”, “acid etching” and “enamel” yielded 1,102 publications, while searching on just “bond strength” and “enamel” yielded 2,331. However, Heintze and Zimmerli²⁵), and Heintze²⁶) argued that results using static bond strength testing have limited clinical relevance and should not be used to make clinical recommendations. Theoretically, it should be clinically more relevant to test adhesive interfaces dynamically, as in the clinical situation tooth-resin composite bonds are seldom subjected to the acute shear and/or tensile stresses employed in static bond strength tests²⁷). Therefore, evaluations on the effect of phosphoric acid pre-etching using dynamic bond strength testing on current adhesives are desirable.

Over the past decade, a dynamic bond strength testing that assess the bond fatigue strength of adhesive systems have been developed at the Creighton University School of Dentistry (Omaha, NE, USA)²⁸⁻³⁰). A method for dynamic bond strength testing was originally developed by Erickson *et al.*^{31,32}) at Academic Center for Dentistry Amsterdam (Amsterdam, Netherlands) in 2006–2008, and was modified and optimized by Erickson *et al.*³³), Barkmeier *et al.*³⁴), Takamizawa *et al.*³⁵), and Tsujimoto *et al.*³⁶) at the Creighton University School of Dentistry from 2009 to 2017. This dynamic bond fatigue strength testing allows better assessment of an adhesive's response to

the repeated subcritical loading challenges encountered in the oral environment as the cyclic stresses used are more similar to the stresses generated during mastication than the continuous loading to failure applied in traditional static bond strength testing^{31,32}). Thus, it would be worthwhile to evaluate the effects of phosphoric acid pre-etching on the enamel bond fatigue durability of universal adhesives and two-step self-etch adhesives in order to assess the endurance of the adhesives in the face of repetitive subcritical loading.

The purpose of this laboratory study was to investigate all three of these issues by assessing the effects of phosphoric acid pre-etching on the enamel bond fatigue durability of universal adhesives and two-step self-etch adhesives. The null hypothesis was that there would be no differences in the enamel bond fatigue durability between universal adhesives and two-step self-etch adhesives regardless of the presence or absence of phosphoric acid pre-etching.

MATERIALS AND METHODS

Study materials

Six adhesives were used in this study (Table 1): four universal adhesives [1] Adhese Universal (AU, Ivoclar Vivadent, Schaan, Liechtenstein), 2) Clearfil Universal Bond (CU, Kuraray Noritake Dental), 3) G-Premio Bond (GP, GC, Tokyo, Japan), and 4) Scotchbond Universal (SU, 3M ESPE, St. Paul, MN, USA)], and three two-step self-etch adhesives [1) Clearfil SE Bond (CS), 2) Clearfil SE Bond 2 (CS2), and 3) OptiBond XTR (OX)]. Ultra-Etch (Ultradent Products, South Jordan, UT, USA) was used as a 35% phosphoric acid etching agent, and Z100 Restorative (3M ESPE) was used as the resin composite for the bonding procedures.

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 Bosworth Fastray acrylic material (The Harry J. Bosworth Company, Skokie, IL, USA). Flat enamel bonding sites were prepared on the mounted buccal and lingual surfaces by wet grinding using a sequence of silicon carbide (SiC) papers (Struers, Cleveland, OH, USA) up to 4000 grit in a grinder-polisher (Ecomet 4, Buehler, Lake Bluff, IL, USA). This was to minimize the influence of any directionality of the surface grooving created by the abrasives. These surfaces were then washed and dried using a dental three-way syringe at a distance of 5 cm above the surface at air pressure of 3.8 kgf/cm². Thirty specimens were prepared for each of the adhesives for initial shear bond strength testing and forty specimens for shear fatigue strength testing. Half of the specimens for each of the adhesives were pre-etched with Ultra-Etch for 15 s prior to application of the adhesive (Pre-etching group), while other half were not pre-etched (Self-etching group).

Stainless steel metal rings (mold-enclosed method) with an inner diameter of 2.4 mm, an outer diameter

Table 1 Materials used in this stud

Adhesive	Type of Adhesive (Code)	Main components (pH)	Manufacturer
Adhese Universal (164453)	Universal Adhesive (AU)	Bis-GMA, HEMA, MDP, MCAP, Decandiol dimethacrylate, Dimethacrylate, Ethanol, Water, Initiators, Stabilizers, Silicon dioxide (2.5)	Ivoclar Vivadent, Schaan, Lichtenstein
Clearfil Universal Bond (1L0003)	Universal Adhesive (CU)	Bis-GMA, HEMA, MDP, Hydrophilic aliphatic dimethacrylat, Ethanol, Water, Initiators, Silica, Silane coupling agent (2.3)	Kuraray Noritake Dental, Tokyo, Japan
G-Premio Bond (1603091)	Universal Adhesive (GP)	MDP, 4-MET, MEPS, Methacrylate monomer, Acetone, Water, Initiators, Silica (1.5)	GC, Tokyo, Japan
Scotchbond Universal (617265)	Universal Adhesive (SU)	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 (2.7)	3M ESPE, St. Paul, MN, USA
Clearfil SE Bond (Primer: 5L0092; Adhesive: 5K0146)	Two-step Self-etch Adhesive (CS)	Primer: MDP, HEMA, Hydrophilic aliphatic dimethacrylate, Water, Initiators, N,N-diethanol-p-toluidine (2.0) Adhesive: MDP, HEMA, Bis-GMA, Hydrophobic aliphatic dimethacrylate, Initiators, Accelerators, Silica, N, N-diethanol-p-toluidine	Kuraray Noritake
Clearfil SE Bond 2 (Primer: 3F0032; Adhesive: 3M0048)	Two-step Self-etch Adhesive (CS2)	Primer: MDP, HEMA, Hydrophilic aliphatic dimethacrylate, Water, New initiators (1.9) Adhesive: MDP, HEMA, Bis-GMA, Hydrophobic aliphatic dimethacrylate, New initiators, Accelerators, Silica	Kuraray Noritake
OptiBond XTR (Primer: 5093728, Adhesive: 5093730)	Two-step Self-etch Adhesive (OX)	Primer: GPDM, Phosphate monomer, HEMA, Dimethacrylate monomers, Acetone, Ethyl alcohol, Water, Initiators (2.4) Adhesive: Dimethacrylate monomers, Ethyl alcohol, Barium aluminoborosilicate glass, Fumed silica, Sodium hexafluorosilicate, Initiators	Kerr, Orange, CA, USA

Bis-GMA: bisphenol-A-glycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate, MDP: 10-methacryloyloxydecyl dihydrogen phosphate, MCAP: methacrylated carboxylic acid polymer, 4-MET: 4-methacryloxyethyl trimellitic acid, MEPS: methacryloyloxyalkyl thiophosphate methylmethacrylate

of 4.8 mm, and a height of 2.6 mm were used to bond a resin composite to the enamel surfaces for both groups. The bonding site surfaces (bottom side) of the metal rings were treated with a releasing agent (3% solution of paraffin in hexane), and the enamel surfaces of the pre-etching and self-etching groups were treated with the adhesives according to the manufacturers' directions (Table 2). A custom fixture was used to position and hold the metal rings over the bonding sites, and the resin composite was placed into the rings using a condensing instrument. This was then light-cured for 40 s using a quartz-tungsten halogen unit (Spectrum 800 Curing Unit, DENTSPLY Caulk, Milford, DE, USA) set at 600 mW/cm² from a standardized distance of 1 mm, and the bonded specimens were then stored in distilled water for 24 h before testing.

Initial shear bond strength testing

Initial shear bond strength tests (24 h water storage at 37°C) were carried out on all groups using the stainless steel ring method described above (mold-enclosed method). A chisel-shaped metal rod was used to apply

the load on the metal rings immediately adjacent to the flat enamel surfaces. The specimens (15 per group) were loaded to failure using an ElectroPuls E1000 machine (Instron Worldwide Headquarters, Norwood, MA, USA) with a crosshead speed of 1 mm/min. Initial shear bond strength values (MPa) were calculated for the peak load at failure divided by the bonded surface area.

Shear fatigue strength testing

A staircase method, previously described by Draughn³⁷⁾ and Dewji *et al.*³⁸⁾, was used to perform the initial shear fatigue strength tests using the ElectroPuls E1000 machine. For the shear fatigue strength tests, twenty specimens were prepared for both the pre-etching and self-etching groups for each adhesive being tested. Subsequently, the specimens were stored in distilled water for 24 h prior to testing. Previous studies reported that bond fatigue strength was not influenced by the frequency rate (5, 10 or 20 Hz with enamel³⁵⁾ and dentin²⁸⁾) or the numbers of cycles (50,000, 100,000 or 1,000,000 cycles with enamel and dentin³⁶⁾), thus the fatigue load was applied using a sine wave at a frequency

Table 2 Application protocol for universal and two-step self-etch adhesives

Adhesive	Adhesive application protocol
AU	Adhesive applied to air-dried tooth surface with rubbing action for 20 s and then medium air pressure applied to surface for 5 s. Adhesive photo-polymerized for 10 s.
CU	Adhesive applied to air-dried tooth surface with rubbing action for 10 s and then medium air pressure applied to surface for 5 s. Adhesive photo-cured 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 photo-cured for 10 s.
SU	Adhesive applied to air-dried tooth surface with rubbing action for 20 s and then medium air pressure applied to surface for 5 s. Adhesive photo-cured for 10 s.
CS	Primer applied to air-dried tooth surface and left it in place for 20 s. Medium air pressure applied to surface for 5 s. Adhesive applied to primed tooth surface and then air-thinned to make uniform bond film. Primer/adhesive photo-cured for 10 s.
CS2	Primer applied to air-dried tooth surface and left it in place for 20 s. Medium air pressure applied to surface for 5 s. Adhesive applied to primed tooth surface and then air-thinned to make uniform bond film. Primer/adhesive photo-cured for 10 s.
OX	Primer applied to air-dried tooth surface with rubbing action for 20 s. Medium air pressure applied to surface for 5 s. Adhesive applied to primed tooth surface with rubbing action for 15 s and then air-thinned for 5 s. Primer/adhesive photo-cured for 10 s.

of 20 Hz for 50,000 cycles or until failure occurred to minimize the resources needed for this study. The initial peak load for shear fatigue strength testing for each of the adhesive systems was set at a level approximately half of the mean initial shear bond strength determined for that adhesive and pre-etching condition. The lower load limit was set near zero (0.4 N). Subsequent loading was adjusted upward or downward approximately 10% from the previous load depending on specimen survival or failure. The test specimens were immersed in room temperature water (23±2°C) during fatigue strength testing to minimize the influence of temperature rise on the bonding specimens. The mean shear fatigue strength (X) and standard deviation (S) were calculated using the formulae listed below which was described by Draughn³⁷⁾:

$$X = X_0 + d \left(\frac{A}{N} - \frac{1}{2} \right)$$

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

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

where X_0 is the lowest stress level considered in the analysis; d is the stress increment employed in the sequential tests; the lowest stress level at which a failure occurs is denoted by $i=0$, the next by $i=1$, etc.; n_i is the number of failures after fatigue strength testing

at each increment.

Failure mode of specimens

The bond failure sites after initial shear bond strength and shear fatigue strength tests were assessed using an optical microscope (MZ16; Leica Microsystems, Heerbrugg, Switzerland) at 20× magnification. The failure types were based on the percentage of substrate area (adhesive, resin composite, or enamel) observed on both the debonded cylinders and the enamel bonding sites. The failure types were classified 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).

Scanning electron microscopy (SEM) observation of bonding interface

Representative images of the resin-enamel interfaces for three specimens per group were obtained using field-emission SEM (ERA 8800FE, Elionix, Tokyo, Japan). The bonded specimens were stored in distilled water at 37°C for 24 h, embedded in self-cure epoxy resin (Epon 812, Nisshin EM, Tokyo, Japan), and then stored at 37°C for an additional 24 h. They were then sectioned near the center of the bonded resin composite post, and the surfaces of the cut halves were polished with SiC papers up to #4000 grit using a grinder-polisher (Ecomet 4). The surfaces were final polished with a soft

cloth using 1.0 μm -grit diamond paste. SEM specimens of the resin-enamel interfaces were dehydrated by first immersing them in ascending concentrations of aqueous tert-butanol (50% for 20 min, 75% for 20 min, 95% for 20 min, and 100% for 2 h) and then transferring them to a critical-point dryer (Model ID-3, Elionix) for 30 min. To enhance the visibility of the layers, the polished surfaces were etched for 30 s using an argon ion-beam (Type EIS-200ER, Elionix) directed perpendicular to the surface at an accelerating voltage of 1.0 kV and ion current density of 0.4 mA/cm². The surfaces were then coated with a thin film of gold in a vacuum evaporator (Quick Coater Type SC-701, Sanyu Electron, Tokyo, Japan) and observed using field-emission SEM with an operating voltage of 10 kV.

SEM observation of fractured surface after initial shear bond strength and shear fatigue strength tests

Representative fracture surfaces after initial shear bond strength and shear fatigue strength tests were observed in three specimens per group using a field-emission SEM. SEM specimens of the de-bonded specimens were coated with a thin film of gold in a vacuum evaporator and SEM observations were carried out using an operating voltage of 10 kV.

Statistical analysis

Initial shear bond strength data were analyzed using two-way analysis of variance, followed by Tukey's *post*

hoc test, while the shear fatigue strength data were analyzed using a modified *t*-test with a nominal α of 0.05 and a Bonferroni correction (custom program). Fisher's exact test was used to statistically analyze the failure mode. All statistical analyses were conducted using a commercial statistical software package (SPSS Statistics Base, IBM, Armonk, NY, USA).

RESULTS

Initial shear bond strength

The initial shear bond strengths of the universal and two-step self-etch adhesives in the pre-etching and self-etching groups are shown in Table 3. The initial shear bond strengths of both adhesives were significantly higher ($p < 0.05$) in the pre-etching group than in the self-etching group. In the pre-etching group, the initial shear bond strength of SU and GP did not show any significant difference ($p > 0.05$) from that of the two-step self-etch adhesives, while that of AU and CU were significantly lower. In the self-etching group, the initial shear bond strengths of universal adhesives were significantly lower ($p < 0.05$) than those of two-step self-etch adhesives. The initial shear bond strength was material-dependent ($p < 0.05$) for universal adhesives in the pre-etching group. Conversely, the initial shear bond strength of the universal adhesives in the self-etching groups and of the two-step self-etch adhesives in both groups were not influenced ($p > 0.05$) by the type of adhesive. The initial

Table 3 Initial shear bond strength of universal and two-step self-etch adhesives to enamel

Type of adhesive	Adhesive	Pre-etching group	Self-etching group
Universal adhesive	AU	35.9 (3.5) ^{a,A}	26.2 (2.9) ^{a,B}
	CU	37.5 (3.7) ^{a,A}	26.5 (2.8) ^{a,B}
	GP	42.4 (5.5) ^{b,A}	26.1 (2.4) ^{a,B}
	SU	44.3 (4.8) ^{b,A}	27.2 (2.3) ^{a,B}
Two-step self-etch adhesive	CS	45.3 (4.6) ^{b,A}	39.7 (5.4) ^{b,B}
	CS2	46.5 (3.1) ^{b,A}	38.9 (5.9) ^{b,B}
	OX	45.0 (5.0) ^{b,A}	34.3 (5.0) ^{b,B}

Unit: MPa. Values in parenthesis are standard deviations. Same small letter in same individual column indicates no significant difference ($p > 0.05$). Same capital letter within individual rows indicates no significant difference ($p > 0.05$).

Table 4 Shear fatigue strength of universal and two-step self-etch adhesives to enamel

Type of adhesive	Adhesive	Pre-etching group	Self-etching group
Universal adhesive	AU	17.8 (2.5) ^{a,A}	13.2 (2.9) ^{a,B}
	CU	18.5 (2.0) ^{a,A}	13.6 (2.8) ^{a,B}
	GP	21.0 (2.2) ^{b,A}	13.8 (2.4) ^{a,B}
	SU	22.0 (3.1) ^{b,A}	13.9 (3.5) ^{a,B}
Two-step self-etch adhesive	CS	22.3 (3.6) ^{b,A}	20.2 (2.1) ^{b,A}
	CS2	22.4 (3.0) ^{b,A}	19.9 (2.3) ^{b,A}
	OX	22.1 (4.6) ^{b,A}	15.7 (2.6) ^{b,B}

Unit: MPa. Values in parenthesis are standard deviations. Same small letter in same individual column indicates no significant difference ($p > 0.05$). Same capital letter within individual rows indicates no significant difference ($p > 0.05$).

shear bond strengths of SU and GP in the pre-etching group were significantly higher ($p<0.05$) than that of AU and CU.

Shear fatigue strength

The shear fatigue strengths for the universal adhesives and two-step self-etch adhesives in pre-etching and self-etching groups are shown in Table 4. The shear fatigue strengths of the universal adhesives in the pre-etching group were significantly higher than those in the self-etching group. The shear fatigue strengths of CS and CS2 in the pre-etching group did not show any significant difference from those in the self-etching group, although that of OX was higher. The shear fatigue strength was material-dependent for universal adhesives in the pre-etching group and for two-step self-etch adhesives in the self-etching group. Conversely, the shear fatigue strength of the universal adhesive in the self-etching group and of the two-step self-etch adhesives with pre-etching was not influenced by the type of adhesive. The shear fatigue strengths of SU and GP in the pre-etching group were significantly higher than that of AU and CU, while that of CS and CS2 in the self-etching group were significantly higher than that of OX.

Failure mode analysis

The failure mode analyses for initial shear bond strength

and shear fatigue strength testing are shown in Tables 5 and 6, respectively. The failure mode of de-bonded specimens from the initial shear bond strength and shear fatigue strength tests showed more cohesive and mixed failures for both universal adhesives and two-step self-etch adhesives in the pre-etching group than in the self-etching group. However, the Fisher's exact test did not reveal a statistically significant differences ($p>0.05$) in failure mode depending on the type of adhesive, or presence or absence of pre-etching, or testing mode.

SEM observation of bonding interface

Representative SEM images of the resin-enamel interface of pre-etching and self-etching groups are shown in Figs. 1 and 2. The thickness of the adhesive layer of universal adhesives in the pre-etching group was approximately 10 μm , while that of two-step self-etch adhesives differed depending on the adhesive tested (approximately 40 μm for CS, 20 μm for CS2, and 10 μm for OX). The resin-enamel interface of the tested adhesives showed excellent adaptation to enamel regardless of the presence or absence of phosphoric acid pre-etching.

SEM observations of de-bonded specimens after shear fatigue strength tests

Representative SEM images of de-bonded specimens

Table 5 Failure mode analysis of de-bonded specimens after initial shear bond strength tests

Type of adhesive	Adhesive	Pre-etching group	Self-etching group
Universal adhesive	AU	[86/0/7/7] ^{a,A}	[100/0/0/0] ^{a,A}
	CU	[86/0/14/0] ^{a,A}	[100/0/0/0] ^{a,A}
	GP	[86/0/7/7] ^{a,A}	[100/0/0/0] ^{a,A}
	SU	[67/0/13/20] ^{a,A}	[100/0/0/0] ^{a,A}
Two-step self-etch adhesive	CS	[53/7/0/40] ^{a,A}	[87/00/13/0] ^{a,A}
	CS2	[80/0/0/20] ^{a,A}	[86/0/7/7] ^{a,A}
	OX	[67/0/13/20] ^{a,A}	[100/0/0/0] ^{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 ($p>0.05$).

Table 6 Failure mode analysis of de-bonded specimens after shear fatigue strength tests

Type of adhesive	Adhesive	Pre-etching group	Self-etching group
Universal adhesive	AU	[90/0/5/5] ^{a,A}	[100/0/0/0] ^{a,A}
	CU	[80/0/10/10] ^{a,A}	[100/0/0/0] ^{a,A}
	GP	[80/0/10/10] ^{a,A}	[90/0/10/0] ^{a,A}
	SU	[60/10/20/10] ^{a,A}	[100/0/0/0] ^{a,A}
Two-step self-etch adhesive	CS	[60/10/10/20] ^{a,A}	[90/0/5/5] ^{a,A}
	CS2	[80/0/0/20] ^{a,A}	[85/5/5/5] ^{a,A}
	OX	[70/0/10/20] ^{a,A}	[100/0/0/0] ^{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 ($p>0.05$).

Fig. 1 Representative field-emission SEM images of the resin-enamel interface of pre-etching group at magnification of 2,500 $\times$ (a) and of the enamel-adhesive interface at 10,000 $\times$ (b). Arrows indicate the thickness of the adhesive layer. The universal adhesives in the pre-etching group had an adhesive layer with a thickness of approximately 10 μ m, while that of the two-step self-etch adhesive differed depending on the type of adhesive. The thickness of two-step self-etch adhesives in the pre-etching group was approximately 40 μ m for CS, 20 μ m for CS2, and 10 μ m for OX. The resin-enamel interface of the tested adhesives at higher magnification showed excellent adaptation to pre-etched enamel. A: adhesive; R: resin composite; E: enamel; AB-P: All Bond Universal in pre-etching group, AU-P: Adhese Universal in pre-etching group; CS-P: Clearfil SE Bond in pre-etching group; CS2-P: Clearfil SE Bond 2 in pre-etching group; CU-P: Clearfil Universal Bond in pre-etching group; OX-P: OptiBond XTR in pre-etching group; GP-P: G-Premio Bond in pre-etching group; and SU-P: Scotchbond Universal in pre-etching group.

Fig. 2 Representative field-emission SEM images of the resin-enamel interface in the self-etching group at a magnification of 2,500 $\times$ (a) and of the enamel-adhesive interface at 10,000 $\times$ (b). Arrows indicate the thickness of the adhesive layer. The universal adhesives in the self-etching group had an adhesive layer with a thickness of in the approximately 10 μ m while that of the two-step self-etch adhesives was different depending on the type of adhesive. The thickness of two-step self-etch adhesives in the self-etching group was approximately 40 μ m for CS, 20 μ m for CS2, 10 μ m for OX. The resin-enamel interface of the tested adhesives at higher magnification showed excellent adaptation to enamel. A: adhesive; R: resin composite; E: enamel. AB-N: All Bond Universal in self-etching group; AU-S: Adhese Universal in self-etching group; CS-S: Clearfil SE Bond in self-etching group; CS2-S: Clearfil SE Bond 2 in self-etching group; CU-S: Clearfil Universal Bond in self-etching group; OX-S: OptiBond XTR in self-etching group; GP-S: G-Premio Bond in self-etching group; and SU-N: Scotchbond Universal in self-etching group.

Fig. 3 Representative field-emission SEM images of debonded specimens after shear fatigue strength tests at (a) 40× magnification and (b) 2,500× magnification.

The debonded specimens predominantly exhibited adhesive failure at lower magnification, and debonding from the interface between enamel and adhesive could be clearly seen in the specimens at higher magnification. SEM observations revealed more cracks and cleavages in the fracture surfaces of universal adhesives after shear fatigue strength tests than in two-step self-etch adhesives. In addition, SEM observation did not show a clear difference in the morphological appearance regardless of presence or absence of phosphoric acid pre-etching. AB: All Bond Universal in pre-etching group; AU: Adhese Universal; CS: Clearfil SE Bond; CS2: Clearfil SE Bond 2; CU: Clearfil Universal Bond; OX: OptiBond XTR, GP: G-Premio Bond; and SU: Scotchbond Universal.

after shear fatigue strength tests are shown in Fig. 3. The specimens predominantly exhibited adhesive failure, and de-bonding from the interface between enamel and the adhesive was clearly observed. Following shear fatigue strength tests, SEM observations revealed more cracks and cleavages in the fracture surfaces of universal adhesives after shear fatigue strength tests than in the two-step self-etch adhesives. In addition, no clear difference in morphological appearance could be observed, regardless of the presence or absence of phosphoric acid pre-etching.

DISCUSSION

The results of the present study show that the initial shear bond strengths and shear fatigue strengths of universal adhesives in the pre-etching group were significantly higher than those in the self-etching groups. The percentage increase in initial shear bond strength in the pre-etching group was approximately 37% for AU, 42% for CU, 63% for GP, and 63% for SU. Shear fatigue strength with pre-etching were increased approximately 35% for AU, 36% for CU, 52% for GP, and 58% for SU. Recently reported studies^{39,40} have reported that the enamel bond durability of universal adhesives is greater with phosphoric acid pre-etching than without pre-etching. These findings agree with the present study, although the level of increase in bond fatigue durability of universal adhesives associated with phosphoric acid pre-etching of enamel was material-dependent. There is sufficient evidence available to support using phosphoric acid pre-etching of enamel as part of the routine bonding regimen for universal adhesives.

The two-step self-etch adhesives in the pre-etching group showed significantly higher initial shear bond strength than those in the self-etching group. The percentage increase in initial shear bond strength was approximately 14% for CS, 20% for CS2, and 31% for OX, and the increase level associated with phosphoric acid pre-etching was lower than that observed in universal adhesives. On the other hand, unlike OX (41% greater), there were no significant differences in shear fatigue strength of CS (10% greater) and CS2 (13% greater) between the pre-etching and self-etching groups, that is, the effect of phosphoric acid pre-etching on enamel bond fatigue durability of two-step self-etch adhesives was material-dependent. Although many manufacturers have introduced various self-etch adhesives over the past two decades, CS has proved to be one of the most reliable ones⁴¹. Laboratory studies have demonstrated stable bond durability to tooth substrates^{42,43} and clinical studies have also reported promising results^{11–16,44}. Moreover, the manufacturers indicated that, in addition to a composition similar to that of CS, CS2 included a new integrated photo-initiator chemistry that may provide more free radicals and lead to higher monomer conversion rates and resulting in stronger bonds than CS. Furthermore, SEM observations of the enamel-resin interface revealed that the thickness of the adhesive

layer of CS and CS2 was greater than the other adhesive tested regardless of the presence or absence of phosphoric acid pre-etching. Previous studies have reported that greater thickness of the adhesive layer may reduce the stress created by mechanical loading, and with potentially concurrent improvement in bond durability^{45,46}. In addition, another previous study reported that a relatively thick adhesive layer may serve as an artificial elastic layer at the interface and act as a shock absorber against mechanical loading, leading to higher bond strengths⁴⁷. These features demonstrate that CS and CS2 are fundamentally resilient against fatigue, which may explain why their fatigue durability was not significantly lower in the self-etch mode when compared to phosphoric acid pre-etching. These findings are clinically relevant. In practice, it is often difficult to precisely etch only the enamel surface due to the cavity design, tooth position, or existence of adjacent teeth, especially in high stress bearing areas. This may result in a high probability of inadvertently etching the contiguous dentin surface, which may result in decreased dentin bond durability due to incomplete penetration of the acidic functional monomers into the demineralized dentin⁴⁸. This has been described as a disadvantage of phosphoric acid pre-etching of dentin. However, the present study found that the bond fatigue strength of CS and CS2 to enamel is not significantly influenced by the presence or absence of phosphoric acid pre-etching, consistent with the results of the clinical studies reported by Van Meerbeek *et al.*¹¹, Kubo *et al.*¹² and Peumans *et al.*^{13–16}. Thus, use of these adhesives may be the best option in these situations.

The initial shear bond strength and shear fatigue strength of SU and GP in the pre-etching group was similar to that of two-step self-etch adhesives, and significantly higher than that of AU and CU, results that may shed light on the conflict between the results of de Goes *et al.*²³ and Makishi *et al.*²⁴. Tsujimoto *et al.*^{4,9,10} reported that phosphoric acid pre-etching increased surface free-energy and polarized the enamel surface, leading to enhanced chemical bonding reactions between adhesive and enamel. 10-methacryloyloxydecyl dihydrogen phosphate (MDP) is a key acidic functional monomer for bonding of universal adhesives. In addition to MDP, SU also contains a specific polyalkenoic acid copolymer (Vitrebond copolymer, 3M ESPE)⁴⁹. Vitrebond copolymer bonds chemically and spontaneously to hydroxyapatite, and a previous study demonstrated a higher bond strength for adhesives with Vitrebond copolymer than those without it³⁵. It is likely that the presence of polyalkenoic acid copolymer favors additional bonding of universal adhesives to pre-etched enamel. Conversely, GP contains 4-methacryloxyethyl trimellitic acid (4-MET) as a functional monomer in addition to MDP. It has been reported that 4-MET forms a strong chemical bonds with calcium-containing substrates⁵⁰. Therefore, the chemical bonding of specific components in SU and GP may be enhanced by the higher chemical bonding sensitivity of enamel created by phosphoric acid pre-etching, yielding the higher bond

fatigue durability in the pre-etching group than AU and CU. In addition, the pH of GP is lower than that of other tested universal adhesives (Table 1), and this may contribute to the higher enamel bond fatigue durability of GP due to the additional etching effect.

The initial shear bond strengths of universal adhesives in the self-etching groups were significantly lower than that of two-step self-etch adhesives, and the shear fatigue strength was overall lower than that of two-step self-etch adhesives (CS and CS2). Universal adhesives contain a hydrophilic and hydrophobic monomer mixture, and the residual water in this mixture may degrade the mechanical properties of the adhesive layer⁵¹. SEM observations of the fracture surfaces after shear fatigue strength tests revealed more cracks and cleavages in the adhesive layer of universal adhesives compared to that of two-step self-etch adhesives, regardless of the presence or absence of phosphoric acid pre-etching. The weaker mechanical properties of universal adhesives, especially in self-etch mode might lead to lower bond fatigue durability compared to some two-step self-etch adhesives (CS and CS2) that have relatively thicker adhesive layers, as previously described.

Therefore, based on the results of this study, the null hypothesis that there would be no difference in the enamel bond fatigue durability of universal adhesives and two-step self-etch adhesives regardless of the presence or absence of phosphoric acid pre-etching, was rejected.

CONCLUSION

The results of this study suggest that phosphoric acid pre-etching of enamel increases the bond fatigue durability of universal adhesives, but its effect on two-step self-etch adhesives is material-dependent. In addition, some universal adhesives with phosphoric acid pre-etching exhibit similar bond fatigue durability when compared to two-step self-etch adhesives, although the bond fatigue durability of universal adhesives in self-etch mode is lower than that of two-step self-etch adhesives.

ACKNOWLEDGMENTS

The authors would like to thank Mr. Jason M. Moody for his technical contribution to this study. This work was supported, in part, by Grant-in-Aid for Scientific Research (C) (Grant numbers: 17K11716 to M. M., 16K11565 to T.T.) and by Grant-in-Aid for Young Scientists (B) (Grant number: 17K17141 to A.T.) from the Japan Society for the Promotion of Science.

REFERENCES

- 1) Torii Y, Itou K, Nishitani Y, Ishikawa K, Suzuki K. Effect of phosphoric acid etching prior to self-etching primer application on adhesion of resin composite to enamel and dentin. *Am J Dent* 2002; 15: 305-308
- 2) Erickson RL, Barkmeier WW, Kimmes NS. Bond strength of self-etch adhesives to pre-etched enamel. *Dent Mater* 2009;

- 25: 1187-1194.
- 3) Taschner M, Nato F, Mazzoni A, Frankenberger R, Krämer N, Di Lenarda R, Petschelt A, Breschi L. Role of preliminary etching for one-step self-etch adhesives. *Eur J Oral Sci* 2010; 118: 517-524.
- 4) Tsujimoto A, Barkmeier WW, Takamizawa T, Latta MA, Miyazaki M. The effect of phosphoric acid pre-etching times on bonding performance and surface free energy with single-step self-etch adhesives. *Oper Dent* 2016; 41: 441-449.
- 5) Frankenberger R, Lohbauer U, Roggendorf MJ, Naumann M, Taschner M. Selective enamel etching reconsidered: better than etch-and-rinse and self-etch? *J Adhes Dent* 2008; 10: 339-344.
- 6) Erickson RL, Barkmeier WW, Latta MA. The role of etching in bonding to enamel: a comparison of self-etching and etch-and-rinse adhesive systems. *Dent Mater* 2009; 25: 1459-1467.
- 7) Tsujimoto A, Fischer NG, Barkmeier WW, Baruth A, Takamizawa T, Latta MA, Miyazaki M. Effect of shortened phosphoric acid pre-etching times on bond fatigue durability of universal adhesives and surface morphology of enamel. *J Adhes Dent* 2017; 13: 267-273.
- 8) Barkmeier WW, Erickson RL, Kimmes NS, Latta MA, Wilwerding TM. Effect of enamel etching time on roughness and bond strength. *Oper Dent* 2009; 34: 217-222.
- 9) Tsujimoto A, Iwasa M, Shimamura Y, Murayama R, Takamizawa T, Miyazaki M. Enamel bonding of single-step self-etch adhesives: influence of surface energy characteristics. *J Dent* 2010; 38: 123-130.
- 10) Tsujimoto A, Barkmeier WW, Takamizawa T, Watanabe H, Johnson WW, Latta MA, Miyazaki M. Influence of duration of phosphoric acid pre-etching on bond durability of universal adhesives and surface free-energy characteristics of enamel. *Eur J Oral Sci* 2016; 124: 377-386.
- 11) Van Meerbeek B, Kanumilli P, De Munck J, Van Landuyt K, Lambrechts P, Peumans M. A randomized controlled study evaluating the effectiveness of a two-step self-etch adhesive with and without selective phosphoric-acid etching of enamel. *Dent Mater* 2005; 21: 375-383.
- 12) Kubo S, Kawasaki A, Hayashi Y. Factors associated with the longevity of resin composite restorations. *Dent Mater J* 2011; 30: 374-383.
- 13) Peumans M, Munck J, Van Landuyt K, Lambrechts P, Van Meerbeek B. Three-year clinical effectiveness of a two-step self-etch adhesive in cervical lesions. *Eur J Oral Sci* 2005; 113: 512-518.
- 14) Peumans M, De Munck J, Van Landuyt K, Lambrechts P, Van Meerbeek B. Five-year clinical effectiveness of a two-step self-etching adhesive. *J Adhes Dent* 2007; 9: 7-10.
- 15) Peumans M, De Munck J, Van Landuyt KL, Poitevin A, Lambrechts P, Van Meerbeek B. Eight-year clinical evaluation of a 2-step self-etch adhesive with and without selective enamel etching. *Dent Mater* 2010; 26: 1176-1184.
- 16) Peumans M, De Munck J, Van Landuyt K, Van Meerbeek B. Thirteen-year randomized controlled clinical trial of a two-step self-etch adhesive in non-carious cervical lesions. *Dent Mater* 2015; 31: 308-314.
- 17) Walter R, Swift EJ Jr, Boushell LW, Braswell K. Enamel and dentin bond strengths of a new self-etch adhesive system. *J Esthet Restor Dent* 2011; 23: 390-396.
- 18) Meharry MR, Moazzami SM, Li Y. Comparison of enamel and dentin shear bond strengths of current dental bonding adhesives from three bond generations. *Oper Dent* 2013; 38: E237-E245.
- 19) Sato K, Hosaka K, Takahashi M, Ikeda M, Tian F, Komada W, Nakajima M, Foxton R, Nishitani Y, Pashley DH, Tagami J. Dentin bonding durability of two-step self-etch adhesives with improved degree of conversion of adhesive resins. *J Adhes Dent* 2017; 19: 31-37.

- 20) Hanabusa M, Mine A, Kuboki T, Momoi Y, Van Ende A, Van Meerbeek B, De Munck J. Bonding effectiveness of a new 'multi-mode' adhesive to enamel and dentine. *J Dent* 2012; 40: 475-484.
- 21) Tsujimoto A, Barkmeier WW, Takamizawa T, Wilverding TM, Latta MA, Miyazaki M. Interfacial characteristics and bond durability of universal adhesive to various substrates. *Oper Dent* 2017; 42: E59-E70.
- 22) Rosa WL, Piva E, Silva AF. Bond strength of universal adhesives: a systematic review and meta-analysis. *J Dent* 2015; 43: 765-776.
- 23) de Goes MF, Shinohara MS, Freitas MS. Performance of a new one-step multi-mode adhesive on etched vs non-etched enamel on bond strength and interfacial morphology. *J Adhes Dent* 2014; 16: 243-250.
- 24) Makishi P, André CB, Ayres A, Martins AL, Giannini M. Effect of storage time on bond strength and nanoleakage expression of universal adhesives bonded to dentin and etched enamel. *Oper Dent* 2016; 41: 305-317.
- 25) Heintze SD, Zimmerli B. Relevance of in vitro tests of adhesive and composite dental materials. A review in 3 parts. Part 3: in vitro tests of adhesive systems. *Schweiz Monatsschr Zahnmed* 2011; 121: 1024-1040.
- 26) Heintze SD. Clinical relevance of tests on bond strength, microleakage and marginal adaption. *Dent Mater* 2013; 29: 59-84.
- 27) Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, De Munck J. Relationship between bond-strength tests and clinical outcomes. *Dent Mater* 2010; 26: e100-e121.
- 28) Scheidel DD, Takamizawa T, Barkmeier WW, Erickson RL, Tsujimoto A, Miyazaki M. Effect of frequency on the fatigue strength of dentin bonds. *J Oral Sci* 2016; 58: 539-546.
- 29) Tsujimoto A, Barkmeier WW, Takamizawa T, Watanabe H, Johnson WW, Latta MA, Miyazaki M. Comparison between universal adhesives and two-step self-etch adhesives in terms of dentin bond fatigue durability in self-etch mode. *Eur J Oral Sci* 2017; 125: 215-222.
- 30) Tsujimoto A, Barkmeier WW, Takamizawa T, Latta MA, Miyazaki M. Effect of oxygen inhibition in universal adhesives on bond durability and interfacial characteristics. *Am J Dent* 2017; 30: 71-76.
- 31) Erickson RL, De Gee AJ, Feilzer AJ. Fatigue testing of enamel bonds with self-etch and total-etch adhesive systems. *Dent Mater* 2006; 22: 981-987.
- 32) Erickson RL, De Gee AJ, Feilzer AJ. Effect of pre-etching enamel on fatigue of self-etch adhesive bonds. *Dent Mater* 2008; 24: 117-123.
- 33) Erickson RL, Barkmeier WW, Kimmes NS. Fatigue of enamel bonds with self-etch adhesives. *Dent Mater* 2009; 25: 716-720.
- 34) Barkmeier WW, Erickson RL, Latta MA. Fatigue limits of enamel bonds with moist and dry techniques. *Dent Mater* 2009; 25: 1527-1531.
- 35) Takamizawa T, Scheidel DD, Barkmeier WW, Erickson RL, Tsujimoto A, Latta MA, Miyazaki M. Influence of frequency on shear fatigue strength of resin composite to enamel bonds using self-etch adhesives. *J Mech Behav Biomed Mater* 2016; 62: 291-298.
- 36) Tsujimoto A, Barkmeier WW, Erickson RL, Takamizawa T, Latta MA, Miyazaki M. Influence of number of cycles on shear fatigue strength of resin composite bonded to enamel and dentin using adhesives in self-etch mode. *Dent Mater J* 2018; 37: 113-121.
- 37) Draughn RA. Compressive fatigue limits of composite restorative materials. *J Dent Res* 1979; 58: 1093-1096.
- 38) Dewji HR, Drummond JL, Fadavi S, Punwani I. Bond strength of Bis-GMA and glass ionomer pit and fissure sealants using cyclic fatigue. *Eur J Oral Sci* 1998; 106: 594-599.
- 39) McLean DE, Meyers EJ, Guillery VL, Vandewalle KS. Enamel bond strength of new universal adhesive bonding agents. *Oper Dent* 2015; 40: 410-417.
- 40) Vermelho PM, Reis AF, Ambrosano GMB, Giannini M. Adhesion of multimode adhesives to enamel and dentin after one year of water storage. *Clin Oral Investig* 2017; 21: 1707-1715.
- 41) Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt K. State of the art of self-etch adhesives. *Dent Mater* 2011; 27:17-28.
- 42) Nikaido T, Kunzelmann KH, Ogata M, Harada N, Yamaguchi S, Cox CF, Hickel R, Tagami J. The in vitro dentin bond strengths of two adhesive systems in class I cavities of human molars. *J Adhes Dent* 2002; 4: 31-39.
- 43) Mine A, De Munck J, Cardoso MV, Van Landuyt KL, Poitevin A, Kuboki T, Yoshida Y, Suzuki K, Lambrechts P, Van Meerbeek B. Bonding effectiveness of two contemporary self-etch adhesives to enamel and dentin. *J Dent* 2009; 37: 872-883.
- 44) Burrow MF, Tyas MJ. Clinical evaluation of three adhesive systems for the restoration of non-carious cervical lesions. *Oper Dent* 2007; 32: 11-15.
- 45) Hashimoto M, Sano H, Yoshida E, Hori M, Kaga M, Oguchi H, Pashley DH. Effects of multiple adhesive coatings on dentin bonding. *Oper Dent* 2004; 29: 416-423.
- 46) Ito S, Tay FR, Hashimoto M, Yoshiyama M, Saito T, Brackett WW, Waller JL, Pashley DH. Effects of multiple coatings of two all-in-one adhesives on dentin bonding. *J Adhes Dent* 2005; 7: 133-141.
- 47) Coelho PG, Calamia C, Harsono M, Thompson VP, Silva NR. Laboratory and FEA evaluation of dentin-to-composite bonding as a function adhesive layer thickness. *Dent Mater* 2008; 24: 1297-1303.
- 48) Yoshihara K, Yoshida Y, Hayakawa S, Nagaoka N, Torii Y, Osaka A, Suzuki K, Minagi S, Van Meerbeek B, Van Landuyt KL. Self-etch monomer-calcium salt deposition on dentin. *J Dent Res* 2011; 90: 602-606.
- 49) Mitra SB, Lee CY, Bui HT, Tantbirojn D, Rusin RP. Long-term adhesion and mechanism of bonding of a paste-liquid resin-modified glass-ionomer. *Dent Mater* 2009; 25: 459-466.
- 50) Nagakane K, Yoshida Y, Hirata I, Fukuda R, Nakayama Y, Shirai K, Ogawa T, Suzuki K, Van Meerbeek B, Okazaki M. Analysis of chemical interaction of 4-MET with hydroxyapatite using XPS. *Dent Mater J* 2006; 25: 645-649.
- 51) Chen C, Niu LN, Xie H, Zhang ZY, Zhou LQ, Jiao K, Chen JH, Pashley DH, Tay FR. Bonding of universal adhesives to dentine —Old wine in new bottles? *J Dent* 2015; 43: 525-536.