

Effects of Phosphoric Acid Pre-Etching on Chemisorption between Enamel and MDP-Containing Universal Adhesives: Chemical and Morphological Characterization, and Evaluation of Its Potential

Fei Han, Ruizhen Liang, and Haifeng Xie*

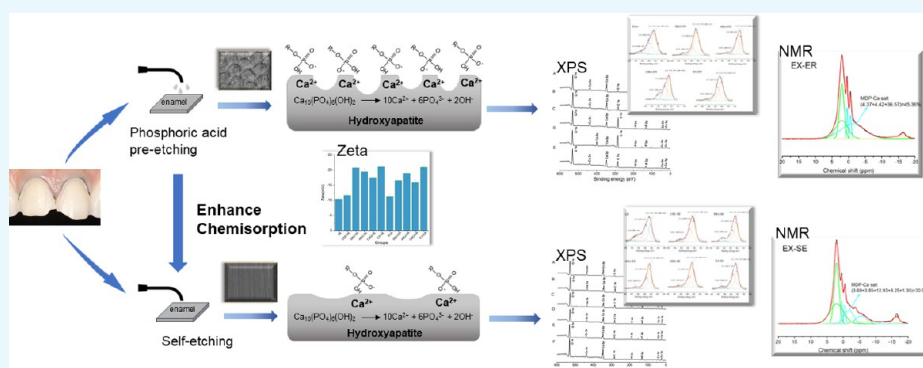
Cite This: *ACS Omega* 2021, 6, 13182–13191

Read Online

ACCESS |

Metrics & More

Article Recommendations



ABSTRACT: This study aimed to provide evidence that phosphoric acid pre-etching is necessary for the chemisorption between enamel and 10-methacryloyloxydecyl dihydrogen phosphate (MDP)-containing universal adhesives. Three MDP-containing universal adhesives: Single Bond Universal (SBU), All Bond Universal (ABU), and Clearfil Universal Bond Quick (CBQ), as well as an experimental MDP-containing adhesive (EX) were investigated. Clearfil SE Bond (CSE) was a control and untreated enamel (UE) was another control. Self-etch (SE) and etch-and-rinse (ER) bonding modes were employed for universal adhesives. The enamel surfaces with different treatments were observed with a scanning electron microscope (SEM). The chemical bonds in the enamel reactants were determined using Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), and nuclear magnetic resonance (NMR). The charge changes with phosphoric acid pre-etching or without were characterized by ζ -potential. FTIR confirmed the chemisorption between enamel and MDP-containing adhesives. XPS and ^{31}P NMR studies detected improved chemical affinity to the phosphoric acid etching + MDP-containing universal adhesives applied with enamel. The ζ -potential of MDP-containing universal adhesives absorbed with enamel, with or without etching became more negative compared with that of UE ($P < 0.05$). Furthermore, single etching created a negative ζ -potential with a higher absolute value ($P = 0.009$). Phosphoric acid pre-etching can increase the negative charge on the enamel surface and facilitate the chemical reactions of MDP and hydroxyapatite (HAP) to produce more calcium salts, thus improving the chemisorption between enamel and MDP-containing universal adhesives.

1. INTRODUCTION

Current enamel bonding systems and strategies have no substantial challenges in achieving acceptable bond strength and durability; however, simplifying operation procedures and reducing technical sensitivity are still attractive goals.^{1,2} Dental universal adhesives containing phosphate ester monomers, e.g., 10-methacryloyloxydecyl dihydrogen phosphate (MDP), have been applied widely in a clinical routine.³ Universal adhesives partially dissolve the smear layer and demineralize hydroxyapatite (HAP) to obtain micromechanical retention in a self-etch (SE) mode via the acidic pH of MDP molecules.^{4,5} The chemical affinity of the molecular structure of MDP (with long and hydrophobic spacer chains) to HAP is another important

contribution to universal adhesives in enamel bonding.⁶ Moreover, MDP molecules chemically interact with HAP^{7–9} in a demineralized enamel within the short clinical pretreatment and combine with HAP through phosphate groups and then self-assemble into an ultrathin film (defined as nanolayering in previous studies) at adhesive–enamel interfaces.^{10,11} Besides,

Received: February 24, 2021

Accepted: April 29, 2021

Published: May 11, 2021



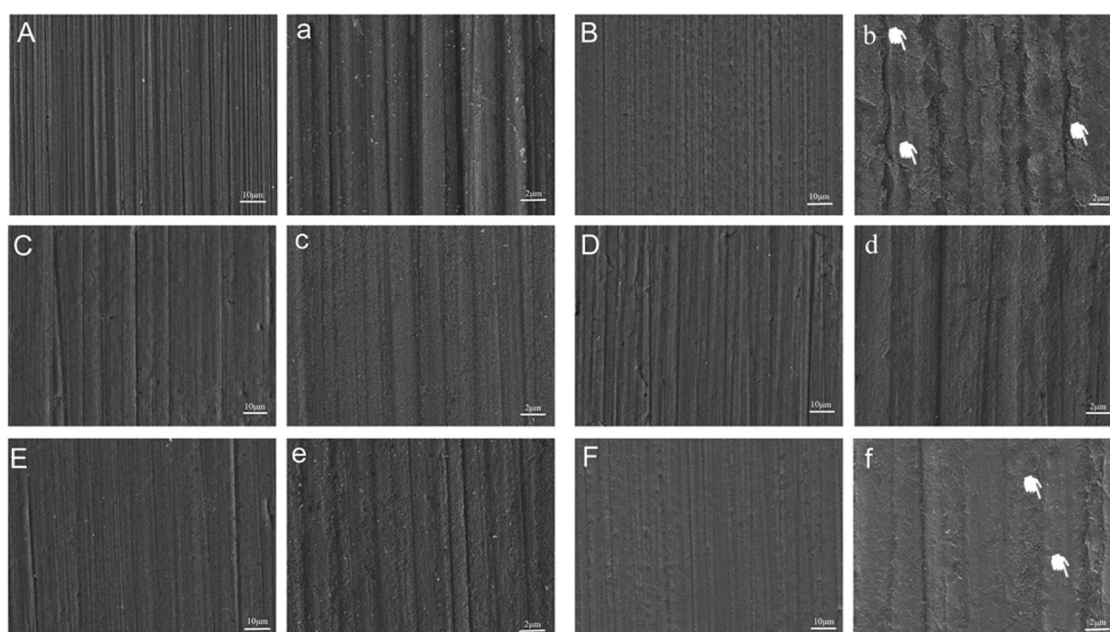


Figure 1. SEM images of (A, a) untreated enamel surfaces conditioned with (B, b) Clearfil SE Bond primer, (C, c) Single Bond Universal, (D, d) All Bond Universal, (E, e) Clearfil Universal Bond Quick, or (F, f) EX. White arrows indicate the exposed enamel-prism contours.

MDP most readily deposits Ca salts due to the highest stability of its monomer-Ca salts in the water among the phosphate ester monomers that are commercially available.¹¹ This contributes to the long-term durability of the enamel–resin interface and plays a key role in preventing secondary caries, sealing the edge of restorations, and improving the durability of restorations.^{12,13}

The application of MDP-containing universal adhesives in an SE mode has shown a satisfactory bonding performance in dentin bonding. However, the main mineral content of an enamel is as high as 96%; thus, phosphoric acid etching would be more conducive to change the enamel surface morphology and significantly increase surface energy, which is beneficial for satisfactory enamel wettability.^{14,15} Compared with the SE mode, bonding resin penetrates deeply into the micropores formed by etching. This, in turn, leads to more pronounced resin tags and better micromechanical interlocking after polymerization, with rapid phosphoric acid etching in an etch-and-rinse (ER) mode.¹⁶

Micromechanical retention is still a reliable source of bonding strength in the application of enamel adhesives.⁴ The MDP-containing universal adhesives combined with phosphoric acid pre-etching provided a similar enamel bonding strength as that of the classical three-step ER processing.¹⁷ Therefore, phosphoric acid pre-etching before applying the MDP-containing universal adhesives is still recommended.^{18–20} However, recent studies have found that the hydrolysis of MDP and its coordination with metal oxides is affected by the local pH environment.^{21,22} It is thus necessary to investigate the potential effects of local pH change on the chemisorption of enamel and MDP-containing universal adhesives. This approach can more accurately evaluate the necessity of enamel pre-etching before the application of universal adhesives.

Here, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), nuclear magnetic resonance (NMR), and ζ -potential analyses were used to evaluate the chemical interaction between enamel and MDP-containing universal adhesives with or without phosphoric acid pre-etching. The goal

was to detect the potential effects on the corresponding chemisorption of the implementation of phosphoric acid pre-etching.

2. RESULTS

2.1. Morphological Observation. Representative SEM images of the variously treated enamel surfaces are shown in Figures 1 and 2. Three commercial MDP-containing universal adhesives: Single Bond Universal (SBU), All Bond Universal (ABU), and Clearfil Universal Bond Quick (CBQ), and an experimental MDP-containing adhesive (EX) were evaluated. The classical SE adhesive Clearfil SE Bond (CSE) was a control and the untreated enamel (UE) was the other control. UE (Figure 1A,a) and the enamel conditioned with SBU-SE, ABU-SE, and CBQ-SE showed clear scratches (Figure 1C–E). However, the enamel surface scratches decreased significantly after CSE-SE (Figure 1b) with obvious enamel-prism contours and exposed enamel crystallites. Exposed enamel-prism contours observed in the EX-SE (Figure 1f) group were not as obvious as that of CSE-SE; neither of them formed a distinct honeycomb pattern.

Etching with phosphoric acid for 15 s left a distinct honeycomb pattern on the enamel surface due to the preferential dissolution of enamel-prisms. Spatial repetition of regular patterns created a multitude of deep pits leading to a honeycomb structure (Figure 2A). Etched enamel surfaces conditioned by SBU, ABU, CBQ, and EX presented the exposed enamel crystallites permeated by adhesives. The entire enamel surface was covered by a smear layer when observed under high magnification (Figure 2b–e).

2.2. FTIR Analysis. Figure 3 shows the FTIR spectra (2000–400 cm^{-1}) of the enamel samples with different surface treatments. The C=O stretching vibration (1718 cm^{-1}) of the methacryloxy carbonyl group was detected from CSE-SE, SBU-SE, ABU-SE, CBQ-SE, and EX-SE, as well as SBU-ER, ABU-ER, CBQ-ER, and EX-ER enamel samples but not from UE. After the reaction of the adhesives with enamel and rigorous rinsing with ethanol, the methacryloxy carbonyl peaks were

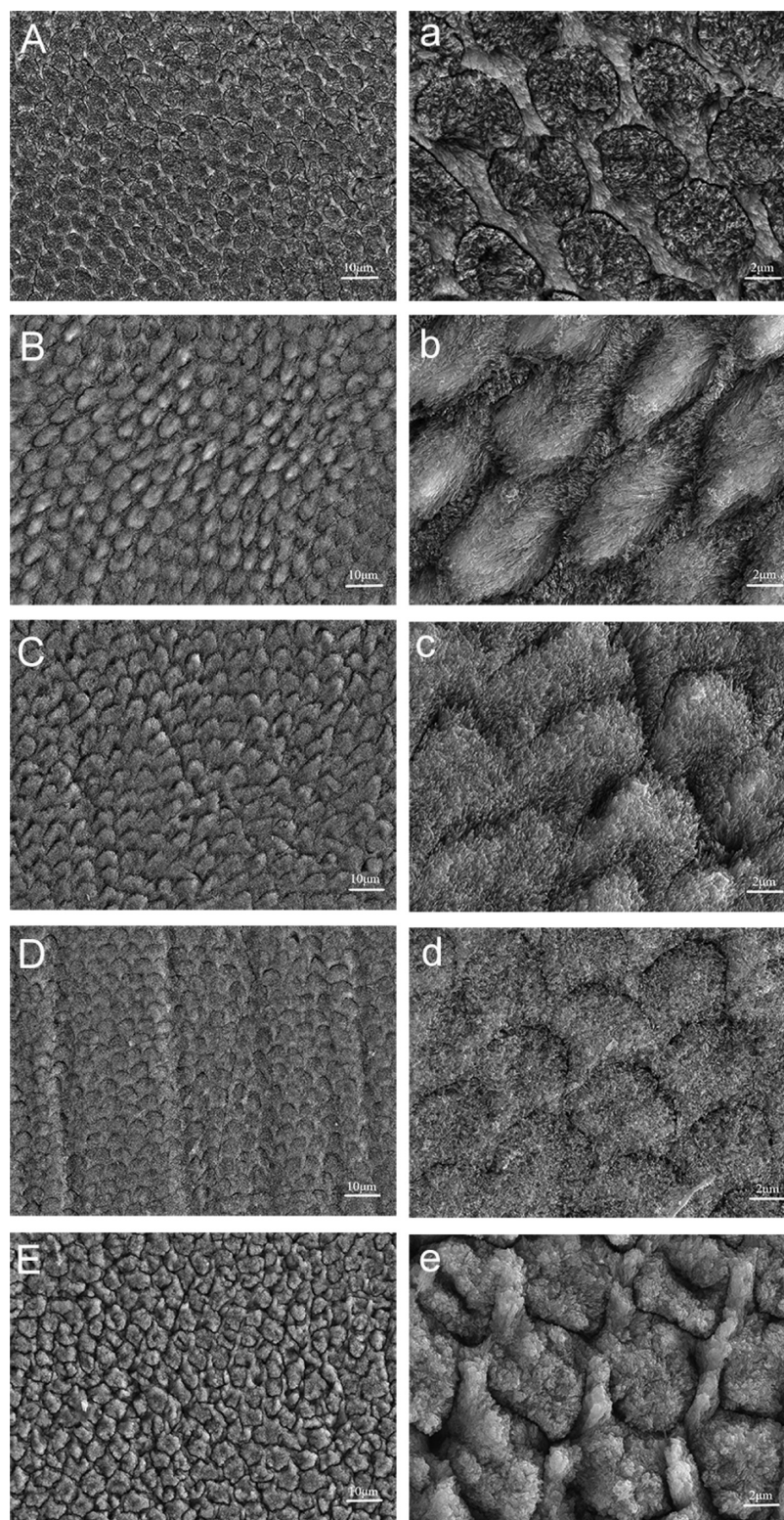


Figure 2. SEM images of enamel surfaces after (A) phosphoric acid etching and (B) conditioned by Single Bond Universal, (C) All Bond Universal, (D) Clearfil Universal Bond Quick, and (E) EX with prior phosphoric acid etching.

retained in the MDP-containing adhesive-treated enamel. The absorption peaks (1036 and 566 cm^{-1}) representing the P–O stretching and bending vibrations were detected in all groups.

2.3. XPS Analysis. The wide-scan XPS spectra of the enamel reactants treated with either of the five adhesives are shown in Figures 4 and 5. The peak intensity (C 1s) of all of the MDP-

containing adhesive-treated enamel samples significantly increased compared with the UE and Etch samples. Meanwhile, the peak intensity (C 1s) of SBU-ER, ABU-ER, CBQ-ER, and EX-ER significantly increased relative to that of SBU-SE, ABU-SE, CBQ-SE, and EX-SE. Narrow-scan spectra of the reacted enamel samples are shown in Figures 6 and 7. The C 1s region of

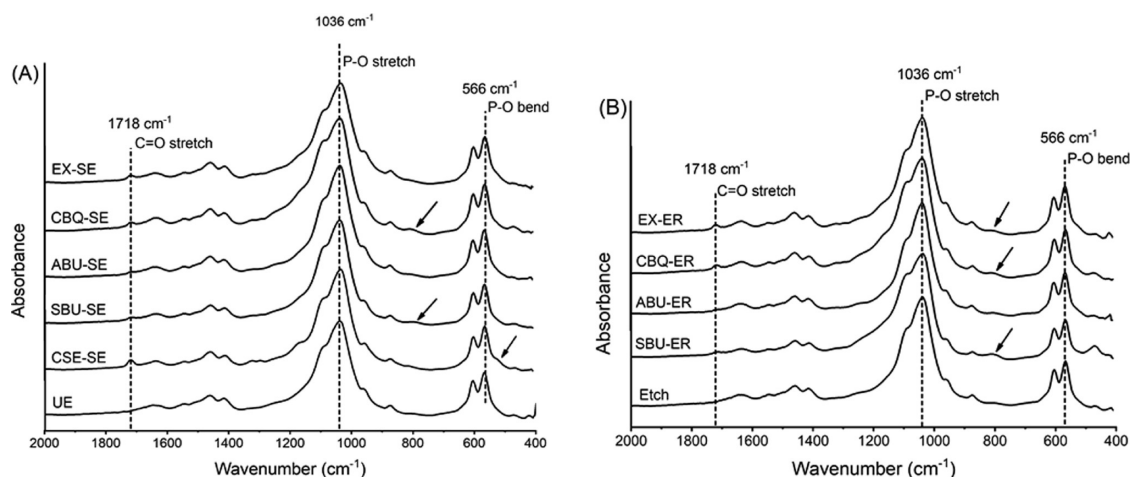


Figure 3. FTIR spectra of the untreated enamel (UE), enamel surfaces conditioned with Clearfil SE Bond primer (CSE-SE), Single Bond Universal (SBU-SE), All Bond Universal (ABU-SE), Clearfil Universal Bond Quick (CBQ-SE), and EX (EX-SE) in a self-etch mode (A); enamel surfaces after phosphoric acid etching (Etch) and conditioned by Single Bond Universal (SBU-ER), All Bond Universal (ABU-ER), Clearfil Universal Bond Quick (CBQ-ER), and EX (EX-ER) with prior phosphoric acid etching (B). Peaks at 1718 cm^{-1} represent the band of C=O stretching vibration of acrylate or methacrylate groups. Arrows indicate the emergence of new phosphate peaks following treatment of enamel with the respective adhesives.

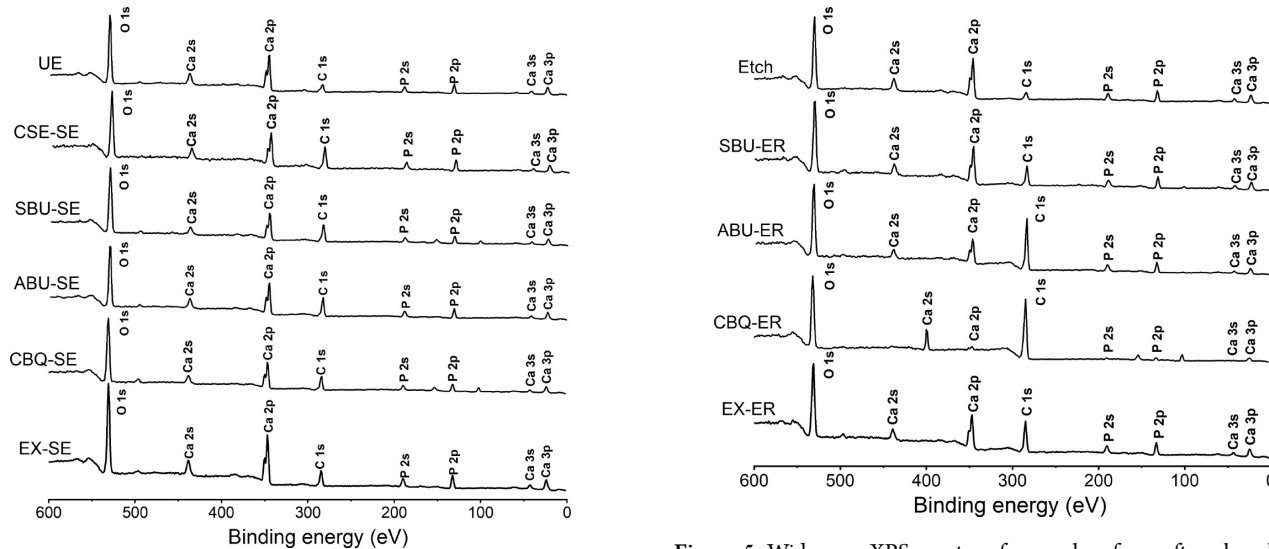


Figure 4. Wide-scan XPS spectra of enamel surfaces of untreated (UE), conditioned with Clearfil SE Bond primer (CSE-SE), Single Bond Universal (SBU-SE), All Bond Universal (ABU-SE), Clearfil Universal Bond Quick (CBQ-SE), and EX (EX-SE). Panels (CSE-SE, SBU-SE, ABU-SE, CBQ-SE, and EX-SE) show the significantly increased C 1s peak intensities versus those of the UE.

UE and Etch samples revealed a backbone (C–C, C–H, and C=C) peak at 284.4 eV , a C–O peak around 285.4 eV , and a C=O peak at 288.1 eV . Furthermore, the C 1s regions for MDP-containing adhesive-treated samples revealed a similar backbone (C–C, C–H, and C=C) peak around 284.4 eV , a C–O peak around 285.4 eV , and a –COO– peak around 288.6 eV .

Quantitative data for the atomic percentage were obtained from peak areas derived from the O 1s, Ca 2p, P 2p, and C 1s, from which the Ca/P, O/Ca, and C/Ca ratios were calculated (Table 1). The Ca/P ratios were substantially lower for samples treated with MDP-containing adhesives than the UE group, and the Ca/P ratios of the SBU-ER, ABU-ER, CBQ-ER, and EX-ER groups were substantially lower than the SBU-SE, ABU-SE, CBQ-SE, and EX-SE groups, respectively. The C/Ca and O/Ca

Figure 5. Wide-scan XPS spectra of enamel surfaces after phosphoric acid etching (Etch), conditioned by Single Bond Universal (SBU-ER), All Bond Universal (ABU-ER), Clearfil Universal Bond Quick (CBQ-ER), and EX (EX-ER) with prior phosphoric acid etching. The etched enamel surfaces treated with the four MDP-containing adhesives show a significantly increased C 1s peak intensities compared with those of the Etch group.

ratios increased significantly of the MDP-containing adhesive-treated samples compared with those of UE, suggesting that there was adhesive adsorbed on enamel. The C/Ca and O/Ca ratios of ABU-ER, CBQ-ER, and EX-ER groups increased when compared with those of ABU-SE, CBQ-SE, and EX-SE groups, respectively (except SBU-SE and SBU-ER groups).

2.4. NMR Analysis. Figure 8 shows the typical peaks and the curve-fitting analyses of the ^{31}P NMR spectra of the enamel reactants of the EX adhesive, with or without pre-etching. The ^{31}P NMR spectrum of EX-coated enamel powders revealed a strong peak at approximately 2.03 ppm that was assigned to the phosphorus atoms of enamel apatite. Distinct peaks were observed at 0.43 , -0.51 , -1.78 , -3.82 , and -5.44 ppm and at 0.44 , -0.58 , and -1.30 ppm , which were assigned to MDP-Ca salt formation. Peak separation and the subsequent peak area

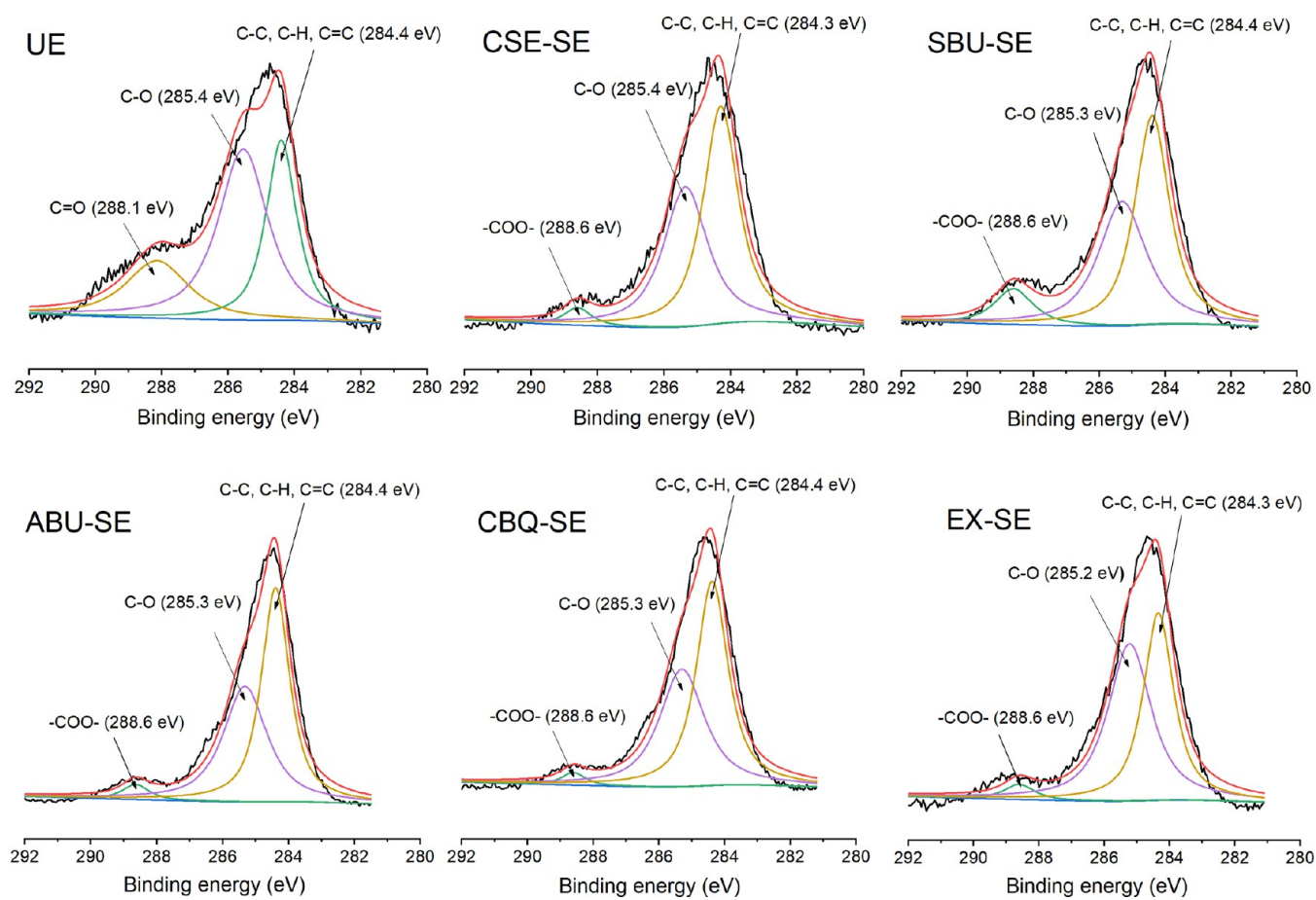


Figure 6. Narrow-scan C 1s XPS spectra. Untreated enamel (UE): the peak at 284.4 eV representing the backbone (C–C, C–H, and C=C) binding, the peak at 285.4 eV representing the C–O binding, and the peak at 288.1 eV representing the C=O binding. Enamels treated with Clearfil SE Bond primer (CSE-SE), Single Bond Universal (SBU-SE), All Bond Universal (ABU-SE), Clearfil Universal Bond Quick (CBQ-SE), and EX (EX-SE) show similar peaks around 284.4 eV (backbone C–C, C–H, and C=C bindings), 285.3 eV (C–O binding), and 288.6 eV (–COO– binding).

calculation revealed that the phosphate atom percentage of MDP-Ca salt was 45.36% with pre-etching and 30.02% without pre-etching.

2.5. ζ -Potential. The ζ -potential of enamel samples with different surface treatments is shown in Table 2. The ζ -potentials of all enamel reactants were negative. The etched sample (–11.867 mV) showed a negative ζ -potential with a higher absolute value compared with that of UE (–10.373 mV) ($P = 0.009$). The ζ -potential of enamel treated with MDP-containing universal adhesives with or without phosphoric acid etching became more negative compared with that of UE ($P < 0.05$). The absolute values decreased after combining the MDP-containing adhesives with phosphoric acid pre-etching compared with the ones without pre-etching (SBU: $P < 0.001$; ABU: $P = 0.443$; CBQ: $P = 0.009$; EX: $P = 0.011$, respectively).

3. DISCUSSION

The micromorphological observations showed that phosphoric acid pre-etching can obviously obtain a better visually concave morphology. There is no doubt that the three-dimensional braided coarsening texture with a uniform arrangement is more beneficial for the penetration of bonding resin and could lead to the following micromechanical interlocking.²³ The 35% phosphoric acid etching leads to the dissolution of HAp of the superficial enamel layer because the pH value is around 0.2;¹⁶ however, a similar enamel structure could not be achieved using

the other four MDP-containing adhesives (SBU, ABU, CBQ, and EX) in the SE mode or using CSE in the SE mode. This is because the demineralization depth of the enamel surface is closely related to the pH value.¹⁰ Nevertheless, the pH values of EX and CSE were close to each other and also the lowest among all of the adhesives employed in this study (pH \approx 1.98 and pH = 1.9, respectively).

Enamel treated with CSE in the SE mode showed obvious enamel-prism contours and exposed enamel crystallites. Enamel treated with EX in the SE mode also showed enamel-prism contours, but these were not as obvious as the former, and neither of them formed typical honeycomb etching patterns. Previous studies have shown that the etching pattern caused by a higher pH value is shallower, which results in less adhesive penetration into the enamel surface.^{16,17} There is less bonding resin penetration inside the enamel-prism (microresin tags) and between the enamel-prisms (macroscopic resin tags), which may reduce enamel bonding strength and durability.^{10,16}

Both micromechanical retention and chemical bonding contribute to the bonding performance of MDP-containing universal adhesives to enamel; the latter stems from the chemical affinity of MDP to HAp.^{24–29} Among the functional monomers contained in universal adhesives, MDP has been found to adhere to HAp and dental tissue most readily and intensively. MDP has long and hydrophobic spacer chains that separate well the polymerizable methacrylate from the phosphate functionality,

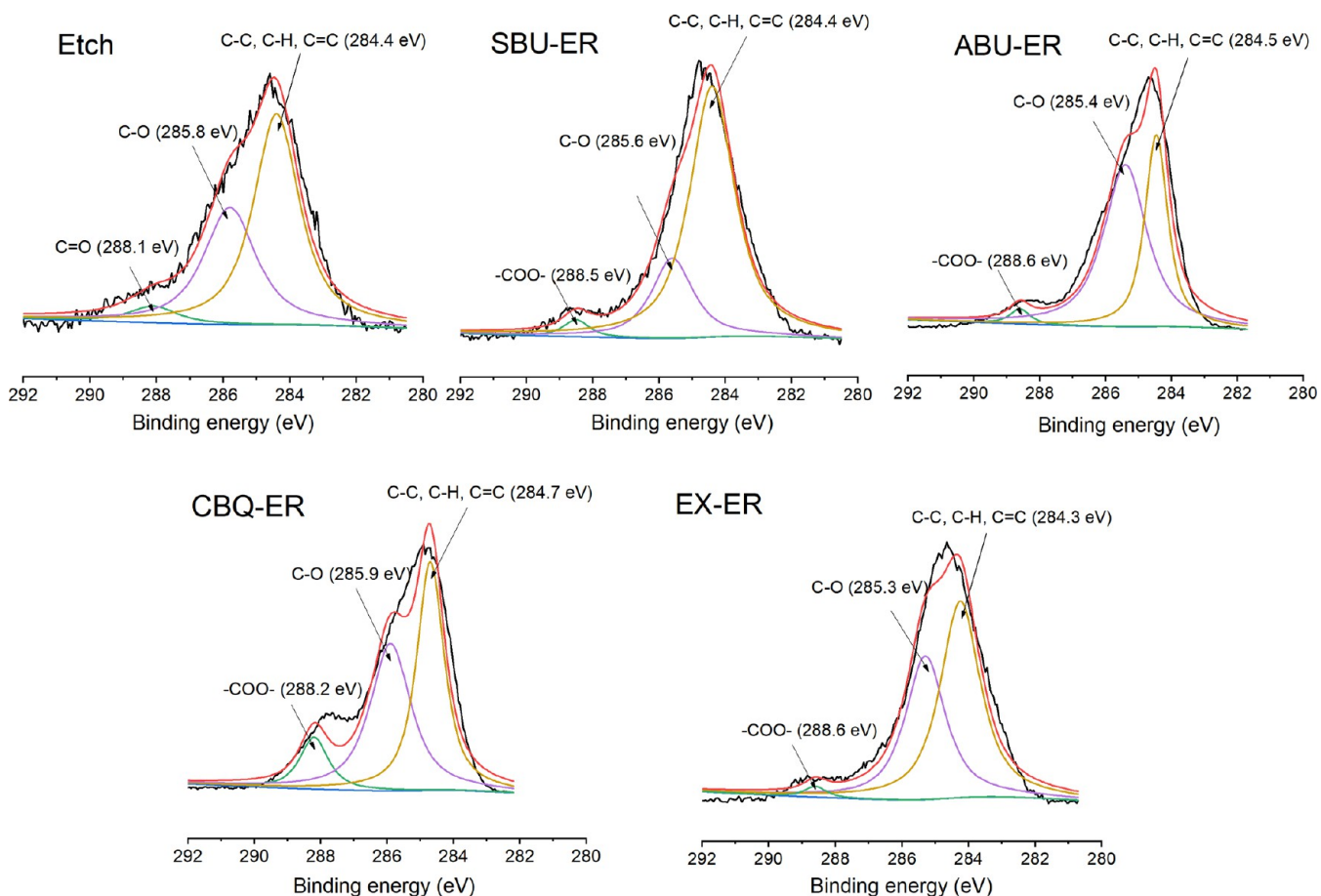


Figure 7. Narrow-scan C 1s XPS spectra of the C 1s region of the etched enamel surface (Etch) revealing: the peak at 284.4 eV representing backbone (C–C, C–H, and C=C) bindings, the peak at 285.8 eV representing C–O binding, and the peak at 288.1 eV representing the C=O binding. Enamels treated with Single Bond Universal (SBU-ER), All Bond Universal (ABU-ER), Clearfil Universal Bond Quick (CBQ-ER), and EX (EX-ER) with prior phosphoric acid etching show similar peaks around 284.5 eV (backbone C–C, C–H, and C=C bindings), 285.4 eV (C–O binding), and 288.6 eV (–COO– binding).

Table 1. Atomic Percentage Ratio of Enamel Treated with Different Types of MDP-Containing Adhesives Derived from XPS Analysis

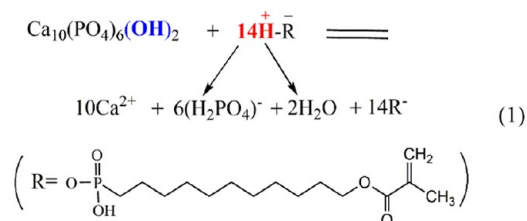
sample group	atomic % ratio		
	Ca/P	C/Ca	O/Ca
UE	1.36	0.95	2.68
Etch	1.31	0.84	2.62
CSE-SE	1.07	2.72	3.18
SBU-SE	1.28	2.69	3.76
ABU-SE	1.19	2.18	2.86
CBQ-SE	1.18	2.02	3.85
EX-SE	1.26	1.22	2.70
SBU-ER	1.10	2.15	3.14
ABU-ER	0.77	8.70	5.11
CBQ-ER	0.53	64.83	29.38
EX-ER	1.03	3.38	3.25

which appears to be essential for durable bonding to enamel and dentin.³⁰ The bond between MDP and HAP should be stable because the MDP-Ca salt is nearly insoluble in water.^{7,9–11} The C=O stretching vibration (1718 cm⁻¹) of the methacryloxy carbonyl group was detected from all of the enamel samples treated with MDP-containing adhesives, but not from the untreated enamel, emphasizing the chemical affinity of MDP to HAP.^{12,31} However, the C=O stretching vibration peaks

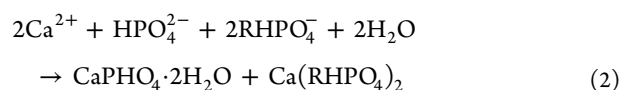
detected for SBU and ABU were less intense than those detected for CSE in the current study, which might be due to the difference in the MDP concentration in the adhesives or the chemical interference by other components.²⁵

The residual HAP microcrystals in the enamel structure have been found to be tightly wrapped by MDP and formed a copolymer.^{24,32,33} Decalcification of HAP and its interaction with MDP can be described as follows^{5,10,32}

(1) MDP reacts as follows:



(2) When MDP-Ca salts with low solubility are formed:



The acidity of the functional monomer influences reaction 1,³⁴ which is also dependent on reaction time or temperature. If

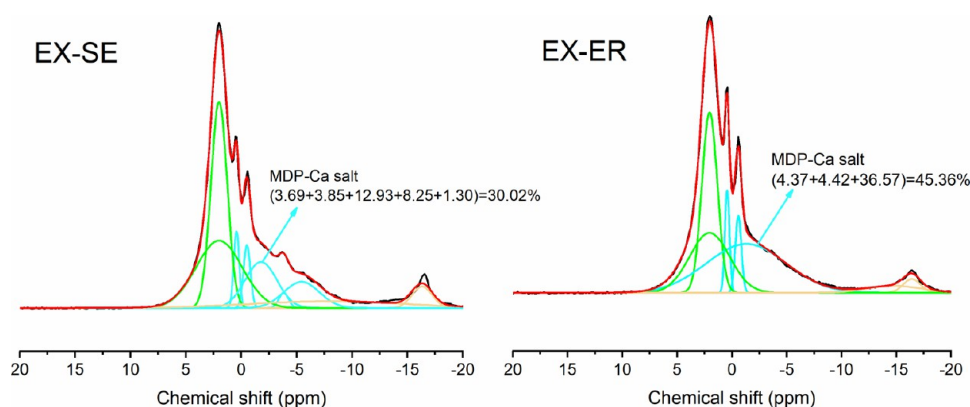


Figure 8. Typical ^{31}P NMR spectra and corresponding curve-fitting analyses of the enamel reactants after EX conditioning with (EX-ER) or without pre-etching (EX-SE). Black and red lines represent the observed and reconstructed ^{31}P NMR spectra of the enamel reactants, whereas the green and blue lines denote the simulated peaks used to fit the spectra of enamel apatite and MDP-Ca salts.

Table 2. Mean and Standard-Deviation ζ -Potential (mV) of Enamel Powders after Different Experimental Treatments

sample group	ζ -potential (mV)	
	mean \pm SD	confidence interval (95%)
UE	-10.373 ± 0.657^f	-12.006 to -8.740
Etch	-11.867 ± 0.252^e	-12.850 to -9.617
CSE-SE	-11.633 ± 0.404^e	-12.637 to -10.629
SBU-SE	-20.700 ± 0.300^{ab}	-21.445 to -19.955
ABU-SE	-19.400 ± 1.044^b	-21.994 to -16.806
CBQ-SE	-17.467 ± 0.322^c	-18.265 to -16.668
EX-SE	-21.600 ± 0.500^a	-22.842 to -20.358
SBU-ER	-16.567 ± 0.208^{cd}	-17.084 to -16.050
ABU-ER	-18.967 ± 1.069^b	-21.623 to -16.310
CBQ-ER	-15.867 ± 0.862^d	-18.008 to -13.725
EX-ER	-20.067 ± 0.971^b	-22.479 to -17.654

^{a–f}Values with different superscript lowercase letters are significantly different ($P < 0.05$).

monomer-Ca salts with a low solubility are produced, then reaction 2 will proceed until a thermodynamic equilibrium is reached.¹⁰ Furthermore, this reaction shift toward stable Ca-salt deposition may further promote chemical reactions 2 and 1, thus leading to further HAP decalcification.¹⁰

XPS was used to determine the effect of the pH change on the chemical bonding of MDP and HAP because the enamel surface atomic content such as Ca and P may change after pre-etching with phosphoric acid with $\text{pH} < 0.5$. We found that the peak intensity (C 1s) of the enamel surface treated with adhesives increased significantly with or without etching, which was consistent with the results of previous studies.^{29,35} Meanwhile, the peak intensity (C 1s) of the enamel surface with etching significantly increased relative to that without pre-etching. However, 2-hydroxyethylmethacrylate (HEMA) and bisphenol A glycidyl dimethacrylate (Bis-GMA) of four commercial MDP-containing adhesives should have been completely washed away after being ultrasonically rinsed with acetone and thoroughly dried prior to XPS analysis.

The wide-scan XPS spectra indicated that the peak intensity (C 1s) of the etched enamel surface increased significantly with MDP-containing universal adhesive conditioning, suggesting a chemical bonding layer on the enamel surface after SE adhesive conditioning. The pre-etching makes the original chemisorption more intense. It is also suggested that phosphoric acid pre-etching has a positive enhancement effect on the chemisorption

of MDP-containing universal adhesives on enamel. This could be because the etching process exposed more enamel-prism crystallites, resulting in deeper and more intercrystal bonding resin penetration. The local pH change caused by pre-etching might enhance the HAP demineralization caused by MDP, resulting in the formation of MDP-Ca salt and the deposition on the enamel surface, which can resist ultrasonic rinsing based on the low solubility of the MDP-Ca salt.^{9,36}

The Ca/P ratios were lower for samples with MDP-containing adhesives compared with those of the untreated enamel because of the increase in the P percentage due to the monomer adsorption.¹² The Ca/P ratios of the enamel surface pre-etched with phosphoric acid were lower than those of the unetched ones, indicating that monomer adsorption on enamel with pre-etching was higher than that in the SE mode. The C/Ca and O/Ca ratios increased for all MDP-containing adhesives compared with those of untreated samples because of the adsorption of MDP on enamel. The C/Ca and O/Ca ratios with pre-etching were higher than those of unetched ones.¹²

The XPS results were also validated by the ^{31}P NMR results. The percentage of MDP-Ca salt in the etched enamel reactants (45.36%) was higher than that in the SE group (30.02%), which might have been caused by the pre-etching-induced increase in the roughness of the enamel surfaces⁵ and surface wettability. This might have led to a more abundant bonding of the resin with the enamel.

Phosphoric acid pre-etching can release cationic calcium, as well as induce the formation of HPO_4^{2-} ions,³⁷ resulting in an increased negative charge on the enamel surface. HPO_4^{2-} is alkaline and is a necessary substance for reaction 2. When MDP interacts chemically with HAP, calcium ions are released into the adhesive solution. This process facilitated reaction 2 to produce more calcium salts and enhanced the chemical affinity of MDP and HAP, which was consistent with the ^{31}P NMR results.

Here, phosphoric acid etching obviously increased the negative charge of enamel. With a higher negative charge, the surface of the demineralized enamel could attract more calcium ions (positive charge).²⁸ When MDP partially dissolves HAP, the released calcium ions were adsorbed on the demineralized enamel surface; meanwhile, the calcium ions chemically combined with the phosphate groups in MDP, and were then deposited on the enamel surface immediately. Based on this theory, and combined with the current XPS and NMR results, we conclude that phosphoric acid pre-etching can change the enamel surface morphology and increase the surface charge.

Table 3. Five Different Types of MDP-Containing Adhesives Used in This Study^a

brand name	manufacturer	lot number	composition	pH
Clearfil SE Bond (CSE)	Kuraray Noritake; Tokyo, Japan	primer: 1P0349 adhesive: 1U0618	primer: MDP, HEMA, hydrophilic dimethacrylate, photoinitiator (CQ, DEPT, others), and water adhesive: MDP, Bis-GMA, HEMA, hydrophilic dimethacrylate, and microfiller	1.9 ²⁵
Single Bond Universal (SBU)	3M ESPE; St. Paul, MN, USA	91212B	MDP, Bis-GMA, HEMA, methacrylate resins, silane methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, and initiators	2.7 ²⁵
All Bond Universal (ABU)	Bisco; Schaumburg, IL, USA	1900007480	MDP, Bis-GMA, HEMA, ethanol, water, and initiators	3.1 ⁶
Clearfil Universal Bond Quick (CBQ)	Kuraray Noritake Dental Inc.	9L0048	MDP, Bis-GMA, HEMA, hydrophilic amide monomer, colloidal silica, ethanol, camphorquinone, initiators, accelerators, water, and sodium fluoride	2.3 ⁵
our designed adhesive (EX)			MDP, UDMA, TEGDMA, EDMAB, CQ, acetone, and water	1.98

^aAbbreviations: MDP, 10-methacryloyloxydecyl dihydrogen phosphate; bis-GMA, bisphenol A glycidyl dimethacrylate; CQ, camphorquinone; DEPT, *N,N*-diethanol-*p*-toluidine; EDMAB, 4-dimethylamino-benzoic acid ethyl ester; HEMA, 2-hydroxyethylmethacrylate; UDMA, urethane dimethacrylate; and TEGDMA, triethylene glycol dimethacrylate.

Table 4. Application Protocols According to the Manufacturer's Instructions

sample group	self-etch approach (SE)	etch-and-rinse approach (ER)
CSE	apply primer for 20 s; gently air blow; apply bonding agent; light cure for 10 s	
SBU	apply with rubbing action for 20 s, medium pressure air thin for 5 s	etch with 35% phosphoric acid for 15 s, rinse for 15 s and immediately air dry; then, apply as the SE approach
ABU	apply two separate coats with rubbing action for 10–15 s per coat without light-curing in-between, air thin for 10 s	etch with 35% phosphoric acid for 15 s, rinse for 15 s, and immediately air dry; then apply as SE approach
CBQ	apply with rubbing action for 10 s, medium pressure air thin for 5 s	etch with 35% phosphoric acid for 15 s, rinse for 15 s, and immediately air dry; then, apply as SE approach
EX	apply with rubbing action for 20 s, medium pressure air thin for 5 s	etch with 35% phosphoric acid for 15 s, rinse for 15 s, and immediately air dry; then, apply as SE approach

There is a synergistic effect on the chemical bonding between enamel and MDP-containing universal adhesives, which enhances the bonding durability between enamel and MDP-containing universal adhesives. Therefore, the hypothesis that phosphoric acid pre-etching had no effect on the chemical bonding of enamel and MDP-containing universal adhesives can be rejected.

4. CONCLUSIONS

Phosphoric acid pre-etching can increase the negative charge on the enamel surface, thereby facilitating chemical reactions to produce more calcium salts. Phosphoric acid pre-etching impacts the formation of the MDP-Ca salt and enhances the chemisorption between enamel and MDP-containing universal adhesives. Therefore, selective enamel etching is recommended to improve the bonding strength of universal adhesives.

5. MATERIALS AND METHODS

5.1. Preparation of Enamel Specimens and Experimental Design. A total of 64 freshly extracted carious-free human third molars were collected with the approval of the Ethical Committee of the Nanjing Medical University, China, file number: (2019) 277. The molars were stored in a Hanks balanced salt solution at 4 °C and used within 2 weeks after extraction. Dental calculus, pigments, and soft tissue attachments were removed from the enamel surface and samples were prepared. A total of 11 3 mm thick enamel slices were sectioned using a low-speed diamond saw (Isomet, Buehler; Lake Bluff, IL) under water irrigation. To standardize the smear layer, the enamel surfaces were wet polished with a 600-grit SiC paper for 1 min. Three commercial MDP-containing universal adhesives: Single Bond Universal (SBU), All Bond Universal (ABU), and Clearfil Universal Bond Quick (CBQ), and an experimental

MDP-containing adhesive (EX)⁷ were evaluated. The classical SE adhesive Clearfil SE Bond (CSE) was a control and the untreated enamel (UE) was the other control. After calibration using pH 4.00 and pH 7.00 standard solutions, the pH value of EX was measured using an electronic pH meter (FE28, Mettler Toledo, Zurich, Switzerland). The average pH value of EX was determined from five measurements.

Applications of the universal adhesives on the enamel surface were performed according to the corresponding manufacturer in either an SE or an ER mode by etching with a phosphoric acid gel (35%, Bisco, Schaumburg, IL) for 15 s. The composition of the adhesives and their application are shown in Tables 3 and 4.

5.2. Morphological Observation. The enamel samples conditioned with phosphoric acid and adhesives were rinsed ultrasonically with absolute acetone for 1 min to remove the residual monomers and then gradually dehydrated in increasing ethanol concentrations.

All enamel samples and an untreated enamel sample were sputter-coated with gold and examined using an SEM (TESCAN, MAIA3, Kohoutovice, Czech Republic) to observe the enamel surface morphology in a secondary electron mode in a vacuum environment with an accelerating voltage of 20 kV; the working distance was 5 mm.

5.3. Fourier Transform Infrared Spectroscopy (FTIR). Based on a previous study,³⁸ the enamel samples were ground into a powder using a diamond bur under water-cooling conditions. The powder-containing water was allowed to settle for 24 h, and then the sedimented enamel powder was collected. The powder was divided equally into 11 groups. Then, 0.2 g of the enamel powder was suspended in adhesives (1.0 g). The pre-etching groups were treated with a 1% phosphoric acid solution (Sigma Chemical Co., St. Louis, MO) for 10 min to simulate the etching procedure, and then the enamel samples were washed

three times with deionized water and dried. The etched enamel samples were suspended in the adhesives.³⁹ After the reaction, the enamel reactants were washed three times with absolute ethanol and air-dried at room temperature in an ambient atmosphere.

The enamel reactants obtained by the reaction of enamel samples with the adhesives in different modes were also analyzed by FTIR (Nicolet 6700, Thermo Scientific) in a transmission mode from 2000 to 400 cm⁻¹ with an attenuated total reflection setup at 4 cm⁻¹ resolution and 32 scans.

5.4. X-Ray Photoelectron Spectroscopy (XPS) Analysis.

The adhesive-applied enamel samples with and without phosphoric acid pre-etching were also examined by XPS (Escalab250xi, Thermo Fisher Scientific, U.K.) using monochromatic Al K α radiation (1486.6 eV photo energy, energy step size 0.05 eV). Moreover, narrow-scan spectra of the C 1s region were obtained and peak-fitted using XPS Peak 4.1 software, with a Lorentz–Gauss ratio (L/G ratio) fixed at 80% and a Shirley function to subtract the background. The best-fitting parameters, peak positions, amplitudes, and peak half-width were calculated based on the least-squares procedure. The atomic percentage ratios were obtained from peak areas.

5.5. Nuclear Magnetic Resonance (NMR) Analysis.

The ³¹P NMR spectra of the enamel reactants of EX with and without phosphoric acid pre-etching were measured using an NMR spectrometer (AVANCE III HD 400M, Bruker, Karlsruhe, Germany). Diammonium phosphate was used as an external reference. The curve-fitting analyses of the ³¹P NMR spectra were performed using OriginPro 8.0 Data Analysis and Graphing Software (OriginLab Co., Northampton, MA). The intensity of each simulated peak used for the curve-fitting analyses of the enamel reactants was determined. We performed the NMR analysis twice to ensure repeatability.

5.6. ζ -Potential Characterization. The adhesive-applied enamel samples (10 mg) in different modes were suspended in 1 mL of deionized water; the suspensions were then dispersed ultrasonically to obtain a uniform suspension before measurement. The ζ -potential of the enamel reactants with or without phosphoric acid etching was measured using a Zetasizer Nano ZS90 (Malvern Instruments, Ltd., U.K.) at room temperature with each group measured three times. After tests of normal distribution and homogeneity of variance, the effects of different surface treatments on the enamel reactant charge were statistically analyzed using one-way ANOVA and post-hoc (LSD) testing.

AUTHOR INFORMATION

Corresponding Author

Haifeng Xie – Jiangsu Key Laboratory of Oral Diseases, Department of Prosthodontics, Affiliated Hospital of Stomatology, Nanjing Medical University, Nanjing 210029, China; orcid.org/0000-0003-2008-3846; Phone: +8625 69593081; Email: xhf-1980@126.com

Authors

Fei Han – Jiangsu Key Laboratory of Oral Diseases, Department of Prosthodontics, Affiliated Hospital of Stomatology, Nanjing Medical University, Nanjing 210029, China

Ruizhen Liang – Jiangsu Key Laboratory of Oral Diseases, Department of Endodontics, Affiliated Hospital of Stomatology, Nanjing Medical University, Nanjing 210029, China

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acsomega.1c01016>

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank Taoran Ma (Kuraray Noritake Dental, Japan) for providing the Clearfil Universal Bond Quick adhesive and thank Yan Fang (NIGPAS, China) for providing technical support in SEM observation. This work was supported by the National Key Research and Development Program of China [Grant Number 2016YFA0201704], the Medical Research Projects of the Health Department of Jiangsu Province [Grant Number M2020066], and the Priority Academic Program Development of Jiangsu Higher Education Institutions [Grant Number 2018-87].

REFERENCES

- (1) Dressano, D.; Salvador, M. V.; Oliveira, M. T.; Marchi, G. M.; Fronza, B. M.; Hadis, M.; Palin, W. M.; Lima, A. F. Chemistry of novel and contemporary resin-based dental adhesives. *J. Mech. Behav. Biomed. Mater.* **2020**, *110*, No. 103875.
- (2) Fujiwara, S.; Takamizawa, T.; Barkmeier, W. W.; Tsujimoto, A.; Imai, A.; Watanabe, H.; Erickson, R. L.; Latta, M. A.; Nakatsuka, T.; Miyazaki, M. Effect of double-layer application on bond quality of adhesive systems. *J. Mech. Behav. Biomed. Mater.* **2018**, *77*, 501–509.
- (3) Nikaido, T.; Takagaki, T.; Sato, T.; Burrow, M. F.; Tagami, J. The concept of super enamel formation - Relationship between chemical interaction and enamel acid-base resistant zone at the self-etch adhesive/enamel interface. *Dent. Mater. J.* **2020**, *39*, 534–538.
- (4) Cruz, J.; Sousa, B.; Coito, C.; Lopes, M.; Vargas, M.; Cavalheiro, A. Microtensile bond strength to dentin and enamel of self-etch vs. etch-and-rinse modes of universal adhesives. *Am. J. Dent.* **2019**, *32*, 174–182.
- (5) Han, F.; Dai, S.; Yang, J.; Shen, J.; Liao, M.; Xie, H.; Chen, C. Glycerol phosphate dimethacrylate: An alternative functional phosphate ester monomer to 10-methacryloyloxydecyl dihydrogen phosphate for enamel bonding. *ACS Omega* **2020**, *5*, 24826–24837.
- (6) de Oliveira da Rosa, W. L.; Piva, E.; da Silva, A. F. Bond strength of universal adhesives: a systematic review and meta-analysis. *J. Dent.* **2015**, *43*, 765–776.
- (7) Fujita Nakajima, K.; Nikaido, T.; Arita, A.; Hirayama, S.; Nishiyama, N. Demineralization capacity of commercial 10-methacryloyloxydecyl dihydrogen phosphate-based all-in-one adhesive. *Dent. Mater.* **2018**, *34*, 1555–1565.
- (8) Hass, V.; Abuna, G.; Pinheiro Feitosa, V.; Martini, E. C.; Sinhoret, M. A.; Furtado Carvalho, R.; Coelho Bandéca, M.; Sauro, S.; Loguercio, A. D. Self-etching enamel bonding using acidic functional monomers with different-length carbon chains and hydrophilicity. *J. Adhes. Dent.* **2017**, *19*, 497–505.
- (9) Yoshida, Y.; Nagakane, K.; Fukuda, R.; Nakayama, Y.; Okazaki, M.; Shintani, H.; Inoue, S.; Tagawa, Y.; Suzuki, K.; De Munck, J.; Van Meerbeek, B. Comparative study on adhesive performance of functional monomers. *J. Dent. Res.* **2004**, *83*, 454–458.
- (10) Yoshihara, K.; Hayakawa, S.; Nagaoka, N.; Okihara, T.; Yoshida, Y.; Van Meerbeek, B. Etching efficacy of self-etching functional monomers. *J. Dent. Res.* **2018**, *97*, 1010–1016.
- (11) Yoshihara, K.; Yoshida, Y.; Hayakawa, S.; Nagaoka, N.; Irie, M.; Ogawa, T.; Van Landuyt, K. L.; Osaka, A.; Suzuki, K.; Minagi, S.; Van Meerbeek, B. Nanolayering of phosphoric acid ester monomer on enamel and dentin. *Acta Biomater.* **2011**, *7*, 3187–3195.
- (12) Tian, F. C.; Wang, X. Y.; Huang, Q.; Niu, L. N.; Mitchell, J.; Zhang, Z. Y.; Pranatik, C.; Zhang, L.; Chen, J.-H.; Breschi, L.; Pashley, D. H.; Tay, F. R. Effect of nanolayering of calcium salts of phosphoric acid ester monomers on the durability of resin-dentin bonds. *Acta Biomater.* **2016**, *38*, 190–200.

- (13) Zhang, L.; Wang, W.; Wang, C.; Li, M.; Wang, Z.; Su, Z.; Fu, B. Interaction of ACP and MDP and its effect on dentin bonding performance. *J. Mech. Behav. Biomed. Mater.* **2019**, *91*, 301–308.
- (14) Bernales Sender, F. R.; Castañeda Vía, J. A.; Tay, L. Y. Influence of different phosphoric acids before application of universal adhesive on the dental enamel. *J. Esthet. Restor. Dent.* **2020**, *32*, 797–805.
- (15) Van Meerbeek, B.; Yoshihara, K.; Yoshida, Y.; Mine, A.; De Munck, J.; Van Landuyt, K. L. State of the art of self-etch adhesives. *Dent. Mater.* **2011**, *27*, 17–28.
- (16) Yao, C.; Ahmed, M. H.; Yoshihara, K.; Mercelis, B.; Parise Gré, C.; Van Landuyt, K. L.; Huang, C.; Van Meerbeek, B. Bonding to enamel using alternative enamel conditioner/etchants. *Dent. Mater.* **2019**, *35*, 1415–1429.
- (17) Erickson, R. L.; Barkmeier, W. W.; Kimmes, N. S. Bond strength of self-etch adhesives to pre-etched enamel. *Dent. Mater.* **2009**, *25*, 1187–1194.
- (18) de Paris Matos, T.; Perdigão, J.; de Paula, E.; Coppla, F.; Hass, V.; Scheffer, R. F.; Reis, A.; Loguercio, A. D. Five-year clinical evaluation of a universal adhesive: a randomized double-blind trial. *Dent. Mater.* **2020**, *36*, 1474–1485.
- (19) Takeda, M.; Takamizawa, T.; Imai, A.; Suzuki, T.; Tsujimoto, A.; Barkmeier, W. W.; Latta, M. A.; Miyazaki, M. Immediate enamel bond strength of universal adhesives to unground and ground surfaces in different etching modes. *Eur. J. Oral Sci.* **2019**, *127*, 351–360.
- (20) Van Meerbeek, B.; Yoshihara, K.; Van Landuyt, K.; Yoshida, Y.; Peumans, M. From Buonocore's pioneering acid-etch technique to self-adhering restoratives. A status perspective of rapidly advancing dental adhesive technology. *J. Adhes. Dent.* **2020**, *22*, 7–34.
- (21) Qian, M.; Lu, Z.; Chen, C.; Zhang, H.; Xie, H. Alkaline nanoparticle coatings improve resin bonding of 10-methacryloyloxydecyl-dihydrogenphosphate-conditioned zirconia. *Int. J. Nanomed.* **2016**, *11*, 5057–5066.
- (22) Xie, H.; Tay, F. R.; Zhang, F.; Lu, Y.; Shen, S.; Chen, C. Coupling of 10-methacryloyloxydecyl-dihydrogenphosphate to tetragonal zirconia: Effect of pH reaction conditions on coordinate bonding. *Dent. Mater.* **2015**, *31*, e218–e225.
- (23) Suda, S.; Tsujimoto, A.; Barkmeier, W. W.; Nojiri, K.; Nagura, Y.; Takamizawa, T.; Latta, M. A.; Miyazaki, M. Comparison of enamel bond fatigue durability between universal adhesives and two-step self-etch adhesives: Effect of phosphoric acid pre-etching. *Dent. Mater. J.* **2018**, *37*, 244–255.
- (24) Fukegawa, D.; Hayakawa, S.; Yoshida, Y.; Suzuki, K.; Osaka, A.; Van Meerbeek, B. Chemical interaction of phosphoric acid ester with hydroxyapatite. *J. Dent. Res.* **2006**, *85*, 941–944.
- (25) Wang, R.; Shi, Y.; Li, T.; Pan, Y.; Cui, Y.; Xia, W. Adhesive Interfacial Characteristics and the related bonding performance of four self-etching adhesives with different functional monomers applied to dentin. *J. Dent.* **2017**, *62*, 72–80.
- (26) Yoshida, Y.; Yoshihara, K.; Hayakawa, S.; Nagaoka, N.; Okihara, T.; Matsumoto, T.; Minagi, S.; Osaka, A.; Van Landuyt, K.; Van Meerbeek, B. HEMA inhibits interfacial nano-layering of the functional monomer MDP. *J. Dent. Res.* **2012**, *91*, 1060–1065.
- (27) Yoshihara, K.; Nagaoka, N.; Okihara, T.; Kuroboshi, M.; Hayakawa, S.; Maruo, Y.; Nishigawa, G.; De Munck, J.; Yoshida, Y.; Van Meerbeek, B. Functional monomer impurity affects adhesive performance. *Dent. Mater.* **2015**, *31*, 1493–1501.
- (28) Young, A.; Smistad, G.; Karlsen, J.; Rölla, G.; Rykke, M. Zeta potentials of human enamel and hydroxyapatite as measured by the Coulter DELSA 440. *Adv. Dent. Res.* **1997**, *11*, 560–565.
- (29) Zhang, Z.; Wang, X.; Zhang, L.; Liang, B.; Tang, T.; Fu, B.; Hannig, M. The contribution of chemical bonding to the short- and long-term enamel bond strengths. *Dent. Mater.* **2013**, *29*, e103–e112.
- (30) Feitosa, V. P.; Sauro, S.; Oglari, F. A.; Oglari, A. O.; Yoshihara, K.; Zanchi, C. H.; Correr-Sobrinho, L.; Sinhoretto, M. A.; Correr, A. B.; Watson, T. F.; Van Meerbeek, B. Impact of hydrophilicity and length of spacer chains on the bonding of functional monomers. *Dent. Mater.* **2014**, *30*, e317–e323.
- (31) Zhou, L.; Wang, Y.; Yang, H.; Guo, J.; Tay, F. R.; Huang, C. Effect of chemical interaction on the bonding strengths of self-etching adhesives to deproteinised dentine. *J. Dent.* **2015**, *43*, 973–980.
- (32) Yoshihara, K.; Nagaoka, N.; Yoshida, Y.; Van Meerbeek, B.; Hayakawa, S. Atomic level observation and structural analysis of phosphoric-acid ester interaction at dentin. *Acta Biomater.* **2019**, *97*, 544–556.
- (33) Yaguchi, T. Layering Mechanism of MDP-Ca salt produced in demineralization of enamel and dentin apatite. *Dent. Mater.* **2017**, *33*, 23–32.
- (34) Moszner, N.; Salz, U.; Zimmermann, J. Chemical aspects of self-etching enamel-dentin adhesives: A systematic review. *Dent. Mater.* **2005**, *21*, 895–910.
- (35) Shen, J.; Xie, H.; Wang, Q.; Wu, X.; Yang, J.; Chen, C. Evaluation of the interaction of chlorhexidine and mdp and its effects on the durability of dentin bonding. *Dent. Mater.* **2020**, *36*, 1624–1634.
- (36) Yoshihara, K.; Nagaoka, N.; Hayakawa, S.; Okihara, T.; Yoshida, Y.; Van Meerbeek, B. Chemical interaction of glycerophosphate dimethacrylate (GPDM) with hydroxyapatite and dentin. *Dent. Mater.* **2018**, *34*, 1072–1081.
- (37) Bertacci, A.; Lucchese, A.; Taddei, P.; Gherlone, E. F.; Chersoni, S. Enamel structural changes induced by hydrochloric and phosphoric acid treatment. *J. Appl. Biomater. Funct. Mater.* **2014**, *12*, 240–247.
- (38) Zhang, Y. F.; Zheng, J.; Zheng, L.; Zhou, Z. R. Effect of adsorption time on the adhesion strength between salivary pellicle and human tooth enamel. *J. Mech. Behav. Biomed. Mater.* **2015**, *42*, 257–266.
- (39) Mazzoni, A.; Scaffa, P.; Carrilho, M.; Tjäderhane, L.; Di Lenarda, R.; Polimeni, A.; Tezvergil-Mutluay, A.; Tay, F. R.; Pashley, D. H.; Breschi, L. Effects of etch-and-rinse and self-etch adhesives on dentin MMP-2 and MMP-9. *J. Dent. Res.* **2013**, *92*, 82–86.