European Journal of Oral Sciences

Eur J Oral Sci 2019; 1–9
DOI: 10.1111/eos.12660
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European Journal of Oral Sciences

Effect of a novel prime-and-rinse approach on short- and long-term dentin bond strength of self-etch adhesives

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Li M, Xu J, Zang L, Wang C, Jin X, Hong Y, Fu B, Hannig M. Effect of a novel prime-and-rinse approach on short- and long-term dentin bond strength of self-etch adhesives.
Eur J Oral Sci 2019; 00: 1–9. © 2019 Eur J Oral Sci

This study investigated the effects of the prime-and-rinse approach, using a 10-methacryloyloxydecyl dihydrogen phosphate (MDP)-containing primer, on the short- and long-term dentin microtensile bond strengths (MTBSs) of mild self-etch adhesives. Half of sixty human midcoronal dentin surfaces were polished as control (self-etch approach), and the other half were polished and further treated with a 15% MDP-containing primer and thoroughly sprayed with water as prime-and-rinse approach. The dentin surfaces were treated with a self-etch adhesive, and a composite resin was placed on the surfaces. The following materials were used: Clearfil S3 Bond+Clearfil Majesty; G-Bond+Gradia Direct; Adper Easy One+Z250; and i Bond+Charisma. The MTBS was examined after 24 h and 14 months in water storage. The resin–dentin interfaces were analysed using scanning electron microscopy/transmission electron microscopy. Pretreated dentin surfaces were further analysed using scanning electron microscopy and micro-Raman spectroscopy. Compared with the self-etch approach, the prime-and-rinse approach significantly increased the dentin MTBS, regardless of the duration of storage. The scanning electron microscopy/transmission electron microscopy findings revealed that the prime-and-rinse approach removed most of the dentin smear layer. The Raman spectra of the MDP-treated dentin reveal the characteristic spectra of collagen, hydroxyapatite, and the monomer. Therefore, the prime-and-rinse approach using MDP-containing primers prior to the application of mild self-etch adhesives significantly increases the short- and long-term MTBS of dentin.

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Key words: resin–dentin interface; scanning electron microscopy; smear layer; transmission electron microscopy

Accepted for publication August 2019

Although dentin adhesives and their bond durability have been greatly improved in recent years, the resin–dentin interface remains the weakest link of composite restorations (1). Degradation of the resin–dentin interface eventually leads to failure of composite restorations, and replacement of these failed restorations accounts for nearly 60% of clinical filling procedures (2). Contemporary dental-adhesive strategies are divided into the following two categories (depending on the method used to treat the enamel and dentin smear layer and the application steps): the etch-and-rinse approach/adhesive; and the self-etch approach/adhesive (3, 4). Recently, universal adhesives have been developed that can be used in either an etch-and-rinse approach or a self-etch approach (3). Etch-and-rinse adhesives can achieve stable and long-term enamel bond durability; however, dentin bond durability is not as predictable as enamel bond durability when the etch-and-rinse approach is used (5).

The etch-and-rinse approach typically involves phosphoric acid etching to demineralize the underlying dentin, remove the smear layer, and expose collagen fibrils. Subsequently, the demineralized collagen fibrillar spaces are infiltrated with adhesive monomers to form a hybrid layer (6). Ideally, adhesive monomers would completely infiltrate inter- and intrafibrillar spaces and encapsulate denuded collagen fibrils. Unfortunately, this goal has not been fully achieved because of elusive limitations associated with the nanoscale structures of the dentin collagen fibrils, the molecular sizes of the adhesive monomers (7), and the hydrophilic/hydrophobic domains of the adhesive at the adhesive–dentin interface (8). Hydrolysis of unprotected collagen fibrils in demineralized dentin results in degradation of the resin–dentin interface (1, 9). In addition, most of the etch-and-rinse adhesives are technique-sensitive when the wet-bonding technique is used. Furthermore, minor errors occurring during complicated clinical application procedures may jeopardize

the effectiveness of the dentin bond (10). Tay *et al.* (11) introduced the ethanol wet-bonding technique to eliminate the water and expand the compartments among the exposed denuded collagen networks resulting from acid etching. Using the ethanol wet-bonding technique, water-saturated demineralized dentin matrices could be converted to ethanol-saturated demineralized dentin matrices. This could lower the hydrophilicity of dentin matrices and promote infiltration of hydrophobic resins into the interfibrillar spaces and dentinal tubules (12). The hydrophobic resin makes the polymerized adhesive more hydrolytically stable, and better resin sealing of the collagen matrix could minimize endogenous collagenolytic activities on dentin. This can preserve the integrity of the hybrid layer, with minimal to nearly zero nanoleakage (13). However, the clinical outcome of the ethanol wet-bonding technique is still controversial because of technique sensitivity and the effect of dentinal fluid (14).

Self-etch adhesives are classified into four categories (ultra-mild, mild, intermediately strong, strong) depending on the pH of the self-etch solutions (15). Some two-step self-etch adhesives possess a bonding performance similar to that of etch-and-rinse adhesives (16). However, mild one-step self-etch adhesives cannot completely remove the weak smear layer (17), and the water-soluble calcium salts of monomers resulting from the interaction of acidic monomers with the tooth hard-tissues are not rinsed off (18). The water-soluble calcium salts are precipitated along with the volatilization of solvents and then are polymerized within the adhesive resin at the resin–dentin interface (19). This process may compromise the durability of the dentin bond because the water-soluble calcium salts are expected to be unstable in a moist environment (15).

10-Methacryloyloxydecyl dihydrogen phosphate (MDP) has been successfully used as a functional monomer in mild self-etch adhesives (15, 20). The MDP, as well as some other phosphoric acid esters, has been shown to possess the potential to chemisorb dentin as a result of the formation of water-insoluble calcium salts from the chemical interaction between MDP and hydroxyapatite in the tooth hard tissues (18, 21). We proposed a prime-and-rinse approach using an MDP-containing primer to distinguish from the etch-and-rinse approach using phosphoric acid and the self-etch approach. The prime-and-rinse approach not only partially dissolves and removes the dentin smear layer but also washes off most of the water-soluble calcium salts and retains some water-insoluble calcium salts, including monomer-calcium (MDP-Ca) salts, on the dentin surface (22, 23). Therefore, this approach might provide potential chemical bonding sites for the adhesive resin. The prime-and-rinse approach using MDP-containing primer has been reported to improve enamel bond strengths after acid etching and before application of an etch-and-rinse adhesive (24).

Recently, our research demonstrated that the prime-and-rinse approach, using MDP-containing primer, significantly increases the short-term enamel and dentin bond strengths of mild one-step self-etch adhesives (22, 23). However, whether the prime-and-rinse approach increases the long-term dentin-bond durability of mild self-etch adhesives has not yet been sufficiently investigated. Thus, the purpose of this study was to investigate the effects of the prime-and-rinse approach using the MDP-containing primer, prior to the application of self-etch adhesives, on the short- and long-term dentin microtensile bond strengths (MTBSs), the resin–dentin interfaces, and the dentin surfaces by scanning electron microscopy/transmission electron microscopy and micro-Raman spectroscopy. The following alternative hypotheses were tested: (i) the prime-and-rinse approach using the MDP-containing primer prior to application of mild self-etch adhesives does not improve the dentin bond effectiveness; and (ii) water ageing has no effect on the dentin MTBS of the self-etch adhesives when the prime-and-rinse approach is used.

Material and methods

Preparations of specimens and experimental primers

Sixty caries-free, crack-free, freshly extracted human third molars were collected from patients who provided informed consent. The research protocol was approved by the Institutional Ethics Committee and performed in accordance with the International Ethical Guidelines and Declaration of Helsinki. The teeth were stored in 0.5% Chloramine-T (Aladdin Reagent, Shanghai, China) solution at 4°C and used within 1 month after extraction. Midcoronal dentin was exposed using a slow-speed saw (IsoMet 1000; Buehler, Lake Bluff, IL, USA) and polished with 320-grit silicon carbide (SiC) paper under running water.

A 15 wt.% MDP-containing primer was prepared by dissolving MDP (Watson, Jiangsu, China, Lot # WI12090678) in ethanol-aqueous (1:1) solution (wt.%).

Microtensile bond-strength testing

Four commercially available mild self-etch adhesives and the respective composite resins from the same manufacturers [Clearfil S3 Bond+Clearfil Majesty (Kuraray-Noritake, Tokyo, Japan), G-Bond+Gradia Direct (GC, Tokyo, Japan), Adper Easy One+Z250 (3M ESPE, St Paul, MN, USA), and i Bond+Charisma (Heraeus Kulzer, Hanau, Germany)] were used in the present study.

Thirty-two specimens were used for MTBS testing. The dentin surfaces of half (n = 16) of these specimens were treated with the MDP-containing primer for 15 s and then sprayed with water for 30 s (prime-and-rinse approach; experimental group); the other half (n = 16) of the specimens were polished only (self-etch approach; control group). Subsequently, a self-etch adhesive was applied to the dentin surfaces treated using a prime-and-rinse approach and to the polished dentin surfaces, strictly following the manufacturer's instructions. Composite resin from the same manufacturer was placed over the adhesive-treated dentin surface in four, 1-mm-thick increments. Light curing was performed using a light-curing unit with a power output of 1,500 mW cm^{-2} (Radii Plus; SDI, Victoria, Australia). The compositions of the self-etch adhesives and their application steps are summarized in Table 1.

After 24 h of storage in distilled water at 37°C, all the dentin-bonded specimens were sectioned longitudinally

Table 1

Chemical composition and application steps of the self-etch adhesives used in this study

Product (manufacturer)	Composition	Steps of application
Clearfil S3 Bond (Kuraray-Noritake, Tokyo, Japan, Lot#:00176B)	MDP, Bis-GMA, HEMA, ethanol, water, silanized colloidal silica, dl-CQ	Apply adhesive to dentin and rub it for 20 sStrongly air blow for 5 s until the film no longer movesLight cure for 10 s
G Bond (Gradia Direct, GC, Tokyo, Japan, Lot#:1304261)	4-MET, phosphoric ester-monomer, UDMA, TEGDMA, acetone, water, silica filler, photoinitiator, stabilizer	Apply adhesive to dentin and leave undisturbed for 10 sStrongly air blow for 5 s until the film no longer movesLight cure for 10 s
Adper Easy One (3M ESPE, MN, USA, Lot#:513763)	Phosphoric acid-methacryloxy-hexylesters, polyalkenoic acid, Bis-GMA, HDDMA, HEMA, DMAEMA, ethanol, water, silane-treated silica, TPO, CQ	Apply adhesive to dentin and rub it for 20 sGently air blow for 5 s until the film no longer movesLight cure for 10 s
i Bond (Heraeus, Kulzer, Hanau, Germany, Lot#:010116)	4-META, UDMA, HEMA, glutaraldehyde, acetone, water, CQ, stabilizers	Apply adhesive to dentin and rub it for 20 sGently air blow for 5 s until the film no longer movesLight cure for 10 s

4-MET, 4-methacryloyloxyethyl trimellitic acid; 4-META, 4-methacryloxyethyl trimellitate anhydride; Bis-GMA, bisphenol A glycerolate dimethacrylate; CQ, camphorquinone; DMAEMA, 2-dimethyl amino ethyl methacrylate; HDDMA, 1,6-hexanediol dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloxydecyl dihydrogen phosphate; TEGDMA, triethylene glycol dimethacrylate; TPO, 2,4,6-trimethylbenzoyldiphenyl phosphine oxide; UDMA, urethane dimethacrylate.

through the resin–dentin interfaces into multiple beams with a cross-sectional area of approximately 1 mm^2. Half of the beams from each group were subjected to tensile loading with a microtensile tester (Bisco, Schaumburg, IL, USA), at a crosshead speed of 1 mm min^{-1}, until failure. The other halves of the beams from each group were subjected to the same microtensile test after 14 months of storage at 37°C in distilled water that was changed weekly. Specimens that experienced pretesting failure during specimen sectioning were excluded from this study.

Failure modes

After the MTBS tests, the modes of failure were determined by stereomicroscopy at ×50 magnification, according to the following conditions previously reported by Armstrong and colleagues (25): cohesive failure (failure occurred within dentin or the composite resin); adhesive failure (failure occurred between the resin–adhesive interface and the bottom of the hybrid layer); and mixed failure [failure occurred within the adhesive joint and adherend(s)].

Scanning electron microscopy observations

The surfaces of six polished dentin specimens were treated with and without MDP-containing primers, as mentioned above. The specimens were split through the middle. Pretreated dentin surfaces, fractured dentin surfaces, and three representatively fractured specimens per subgroup after the MTBS tests, were analysed using scanning electron microscopy. All the specimens were desiccated in an ascending series of ethanol (50%–100%) and dried with hexamethyldisilazane (HMDS) for 10 min before they were sputter-coated with gold. Finally, the specimens were analysed using a scanning electron microscope (Zeiss Ultra 55, Oberkochen, Germany).

Micro-Raman spectroscopy

The surfaces of six polished dentin specimens were treated with and without MDP-containing primers, as mentioned above. Raman spectroscopy was performed using a micro-Raman spectrometer (Labram HR Evolution, Horiba, France) equipped with an HeNe laser (633 nm) and a power output of 17 mW. The system was focused through a ×100 Olympus Plan objective (numerical aperture = 0.75) that was set to a beam diameter of approximately 1.5 μm. For comparison, the Raman spectrum of MDP was also recorded.

Transmission electron microscopy analysis

Additionally, 16 resin-bonded dentin specimens (one from each subgroup) were prepared as mentioned above. Each specimen was sectioned into three, 0.5-mm-thick slabs that included the resin–dentin interface. Half of each dentin slab was fixed with 2.5% glutaraldehyde and post-fixed with 1% osmium tetroxide. After fixation, all slabs were desiccated in an ascending series of ethanol (50%–100%), immersed in propylene oxide as a transition fluid, and then embedded in transmission electron microscopy-grade epoxy resin. After the embedding resin had set, non-demineralized ultra-thin sections with a thickness of 70–90 nm were obtained with a diamond knife (Diatome, Biel, Switzerland). The ultra-thin sections were analysed using a transmission electron microscope (JEOL JEM-1230; JEOL, Tokyo, Japan) at 100 kV. The other half of each dentin slab was stored at 37°C for 14 months in distilled water that was changed weekly, then the dentin slabs were prepared and analysed by transmission electron microscopy, as mentioned above.

Statistics

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 22.0 (Released 2013; IBM, Armonk, NY, USA). The MTBS data were log-transformed to follow a normal distribution that was determined by a normal Q-Q plot. A multiple linear regression analysis was performed to determine the effects of self-etch adhesives, storage time, and bonding approaches on the dentin bond strengths. Adper Easy One served as the reference, while the effects of the other adhesives were

estimated. The normal distribution of residuals, the scatter plot of the standardized residuals, and the standardized predicted values were used to verify that the final model fulfilled the assumptions for linear regression. Failure mode data were analysed using the chi-square test.

Results

The results of the multiple linear regression analysis of the MTBS data are shown in Table 2. All explanatory variables were statistically significant ($P < 0.001$). The adhesive, Adper Easy One, had a significantly higher dentin MTBS than the other three self-etch adhesives ($P < 0.001$). Compared with the self-etch approach, the prime-and-rinse approach resulted in a significant increase of the short- and long-term dentin MTBS ($P < 0.001$). After storage of the specimens in water for 14 months, the general dentin MTBS of the adhesives significantly decreased ($P < 0.001$). The predominant failure modes in this study were mixed failures (Fig. 1). The incidence of adhesive failure was similar for both the prime-and-rinse approach and the self-etch

Table 2

Results of multiple linear regression of the microtensile bond strength observations as a function of the bonding approach (self-etch vs. prime-and-rinse), storage time (24 h vs. 14 months), and self-etch adhesive used (Adper Easy One, Clearfil S3 Bond, G Bond, and i Bond)

Variable	Unstandardized coefficient		Standardized coefficient		
	B	SE	*β*	*t*	*P*
Constant	1.603	0.026		62.179	<0.0001
Bonding approach					
Self-etch (ref.)					
Prime-and-rinse	0.157	0.011	0.424	14.114	<0.0001
Storage time					
24 h (ref.)					
14 months	−0.106	0.011	−0.286	−9.528	<0.0001
Self-etch adhesive					
Adper Easy One (ref.)					
Clearfil S3 Bond	−0.272	0.016	−0.646	−17.516	<0.0001
G Bond	−0.223	0.016	−0.515	−14.075	<0.0001
i Bond	−0.275	0.016	−0.643	−17.525	<0.0001

The unstandardized regression coefficient estimates the difference in microtensile bond strength from the reference (ref.) category.

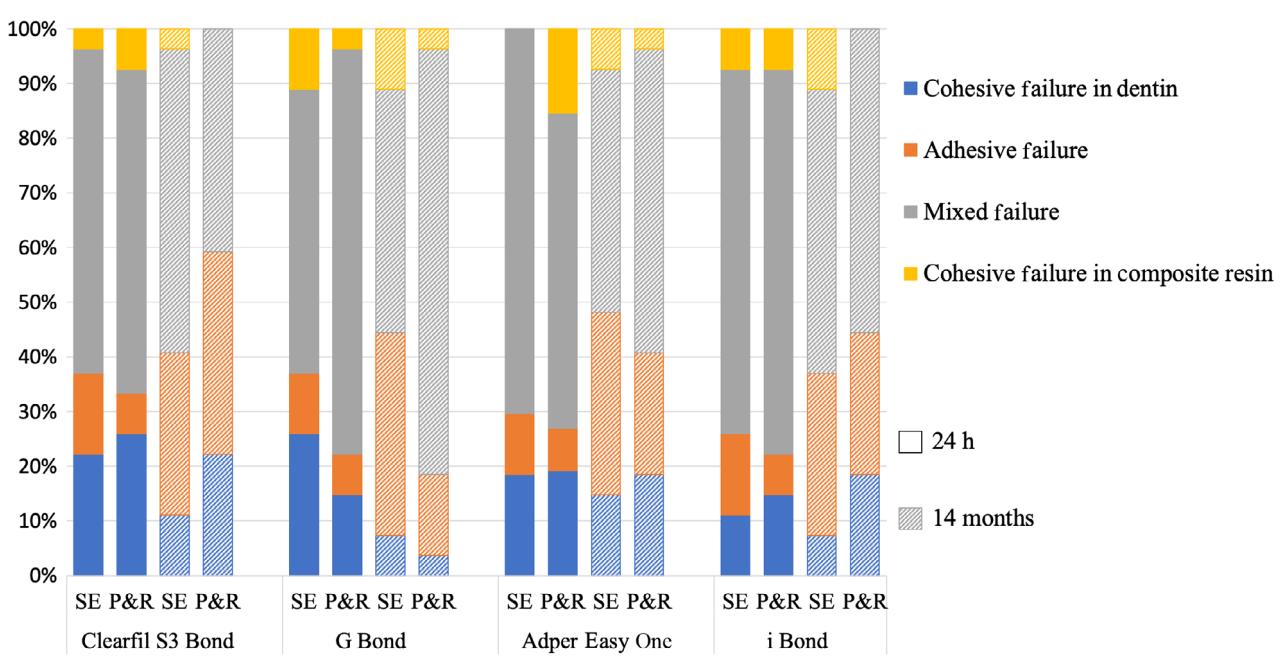

Fig. 1. Distribution of failure modes. The predominant failure modes in all groups were mixed failure and adhesive failure. Overall, the adhesive failures were significantly increased after 14 months of storage in water. P&R, prime-and-rinse approach; SE, self-etch approach.

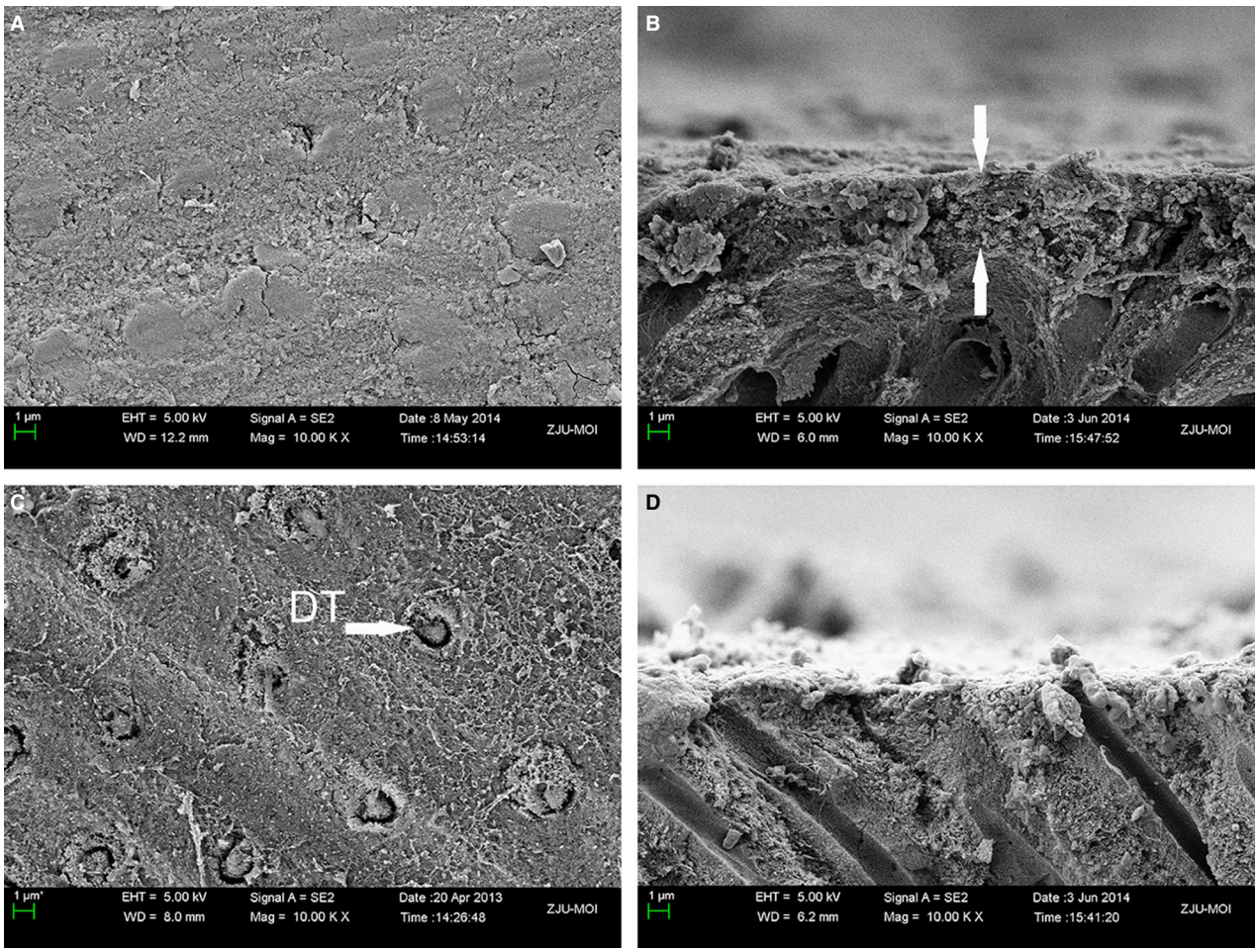

Fig. 2. Scanning electron microscopy of the dentin surfaces and the split dentin specimens. (A) The polished dentin surface shows a smear layer with characteristic scratched lines. (C) Most of the smear layer was removed by application of the 10-methacryloxydecyl dihydrogen phosphate (MDP)-containing primer, exposing the dentinal tubules (DTs); the remnants of smear plugs and the remaining scratch lines can be observed. The thickness of the smear layer of the split specimens of the polished dentin was approximately 2 μm (the area between the white arrows) (B), while the smear layer became nearly invisible after application of the MDP-containing primer (D).

approach ($P = 0.114$), but the incidence of adhesive failure was significantly higher after 14 months of storage than after 24 h ($P < 0.001$).

The scanning electron microscopy findings in this study revealed that polishing scratches remained on the dentin surfaces (Fig. 2A), just as smear layers approximately 2 μm thick (Fig. 2C) remained on top of the fractured specimens. After the dentin surfaces were pretreated with the MDP-containing primer, most of the smear layer was removed, exposing the remnants of smear plugs and dentin structures, such as dentinal tubules, peri- and inter-tubular dentin, and thin fibril-like networks (Fig. 2B,D).

The Raman spectra of MDP, dentin, and MDP-treated dentin are shown in Fig. 3. The spectra of the MDP-treated dentin reveal the characteristic peaks of the amides of dentin collagen (amide III, 1,245 cm^{-1}; amide II, 1,452 cm^{-1}; and amide I, 1,670 cm^{-1}), the double carbon bond of the monomer-MDP (1,640 cm^{-1}), and hydroxyapatite (431 cm^{-1}, 591 cm^{-1}, 960 cm^{-1}, and 1,071 cm^{-1}).

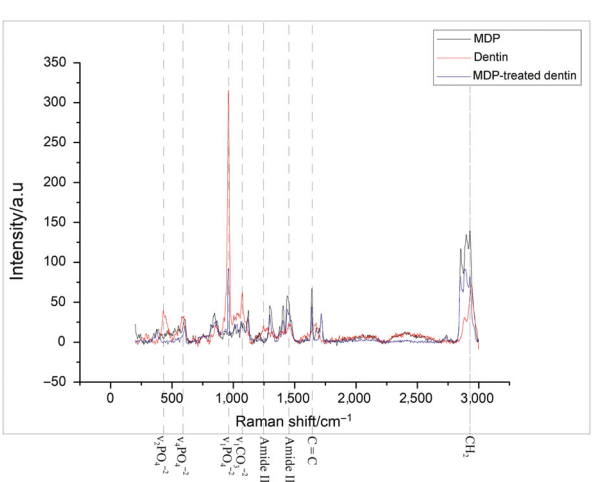

Fig. 3. Raman spectra of the dentin surfaces, 10-methacryloxydecyl dihydrogen phosphate (MDP)-treated dentin surfaces, and MDP.

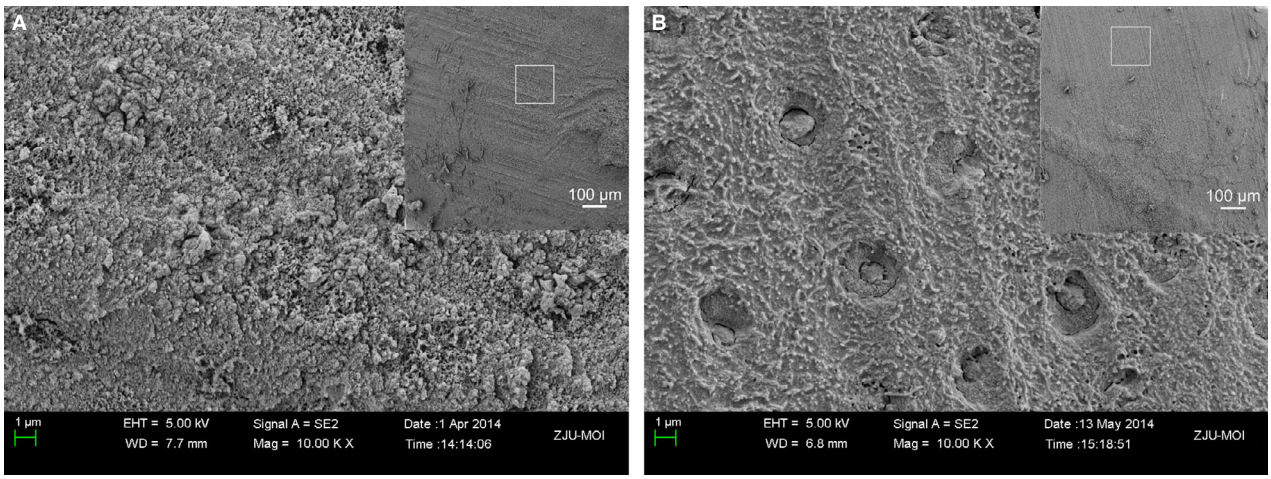

Fig. 4. Representative fractographs of the dentin side of the specimens after 24 h of storage in water. Some scratches were observed in the inserted images at low magnification (150×, scale bar = 100 μm). In the self-etch approach (A), the fractured dentin surface was covered with remnants of the hybrid layer or resin-impregnated smear debris, and the dentinal ultrastructures were not clearly visible at a high magnification (10,000×, scale bar = 1 μm). In the prime-and-rinse approach (B), the dentinal tubules, peri-tubular dentin, and resin tags were exposed (10,000×, scale bar = 1 μm).

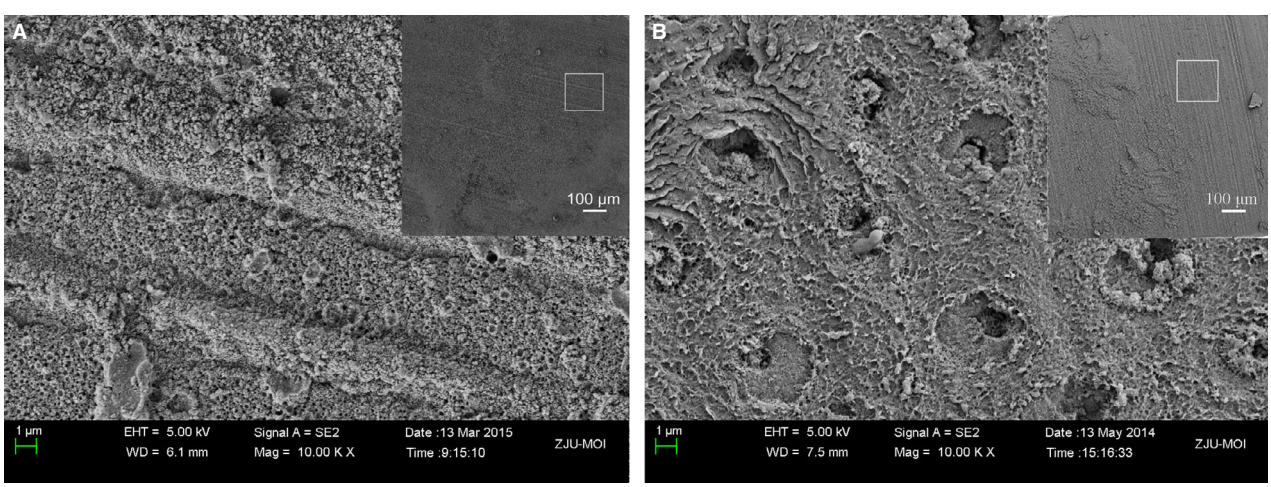

Fig. 5. Representative fractographs of the dentin side of the specimens after 14 months of storage in water. The dentinal ultrastructures were not clearly visible on the fractured surface of the dentin side after the self-etch approach (A), whereas the dentinal tubules, peri-tubular dentin, and resin tags were exposed after the prime-and-rinse approach (B). The high-magnification image (10,000×, scale bar = 1 μm) is the image within the white box inserted in the panel of the low-magnification image (150×, scale bar = 100 μm).

Figures 4 and 5 show representative scanning electron microscopy images of fractured surfaces of the dentin side. For the self-etch approach (Figs 4A and 5A), the dentin surfaces were covered by resin-impregnated smear debris, and the dentinal ultrastructures were invisible. For the prime-and-rinse approach (Figs 4B and 5B), dentinal tubules, peri-tubular dentin, and resin tags were observed on the fractured surfaces.

The transmission electron microscopy findings in this study revealed that the thickness of the hybrid layer was approximately 0.3–0.5 μm for the self-etch approach (control group) and approximately 0.8 μm for the prime-and-rinse approach (experimental group), except for the adhesive, i Bond (see Figure S3E,F). Hydroxyapatite crystallites remained abundant within the hybrid layers in both groups (Figs 6 and 7). After 14 months of storage in water, the hybrid layer remained intact in both groups. Remnants of the smear layer were observed in the control group (Fig. 7A) but not in the experimental group (Fig. 7B).

All dentin MTBS data and some similar scanning electron microscopy/transmission electron microscopy findings are shown in Table S1 and Figures S1–S4).

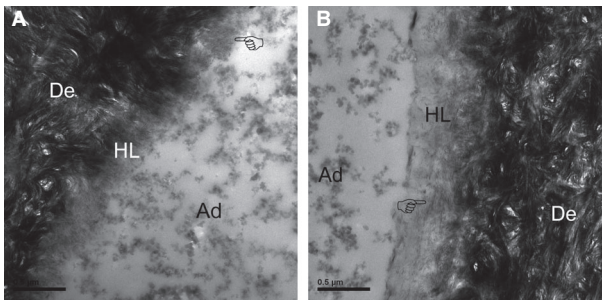

Fig. 6. Representative transmission electron microscopy photomicrographs of the specimens after 24 h of storage in water. The hybrid layers were thin (approximately 0.3–0.5 μm) in the self-etch approach (A) and became thicker (approximately 0.8 μm) in the prime-and-rinse approach (B). Needle-like hydroxyapatite crystallites were abundant in the hybrid layer. Ad, adhesive; De, dentin; HL, hybrid layer. Dentin magnification = 50,000×, scale bar = 0.5 μm.

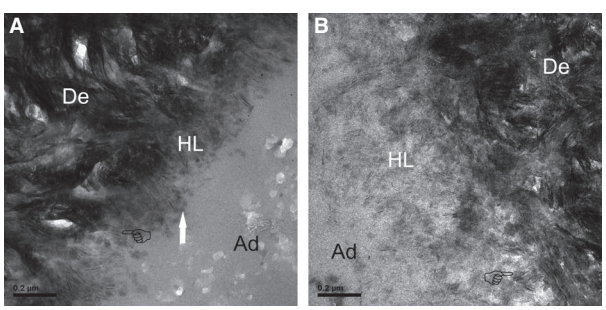

Fig. 7. Representative transmission electron microscopy photomicrographs of the specimens after 14 months of storage in water. The resin–dentin interfaces were tight and void-free, while filler de-bonding was observed in the adhesive layer. Remnants of the smear layer (white arrow) were observed in the self-etch approach (A) but not in the prime-and-rinse approach (B). The hybrid layers were thin in the self-etch groups and thick in the prime-and-rinse groups. Needle-like hydroxyapatite crystallites were observed in the hybrid layer of both the self-etch and prime-and-rinse groups. Ad, adhesive; De, dentin; HL, hybrid layer. Dentin magnification = 100,000×, scale bar = 0.2 μm.

Discussion

This study showed that the prime-and-rinse approach using a 15 wt.% MDP-containing primer prior to the application of self-etch adhesive systems significantly increases the short- and long-term dentin MTBS. The increase in the dentin MTBS might be attributed to removal of the weak dentin smear layer, which is composed of smashed dentin debris covering the bur-prepared or polished dentin surface. The thickness and ultrastructure of the dentin smear layer vary according to the use of different preparation instruments and techniques (26). Numerous studies have revealed that the dentin smear layer negatively influences the penetration of mild self-etch adhesives into the dentin substrate because the acidity of self-etch adhesives can be neutralized by its mineral content (15, 27–29). Residual dentin smear at the resin–dentin interface might compromise the dentin bonding effectiveness of contemporary mild self-etch adhesives (29, 30). The one-step self-etch adhesives used in this study are mild self-etch adhesives, and their demineralization capability is limited. The smear layer bonds weakly to the underlying intact dentin, but the resin–smear complex, which results from infiltration of resin into the smear layer, may lack the complete infiltration of the adhesive resin (17, 28, 30, 31). Thus, the fracture failure in this study probably originated at the transition between the residual smear layer and intact dentin in the self-etch approach (Figs 4 and 5). By contrast, the prime-and-rinse approach removed most of the dentin smear layers, and therefore the adhesives were in tight contact with the underlying dentin at the resin–dentin interfaces. This might explain why the adhesive failures of the prime-and-rinse approach in this study tended to occur at the bottom of the hybrid layer, exposing dentinal structures (Fig. 4). This was demonstrated by the scanning electron microscopy micrographs of the resin–dentin interface (Figs 4 and 5). The findings of this study are consistent with those of previous studies (26, 30).

Chemical bonding might greatly improve dentin bond effectiveness (15). The adhesion of the mild self-etch adhesives depends mainly on micromechanical interlocking through the hybrid layer and chemical bonds that are created by specific functional monomers (15). In this study, after the dentin surfaces were treated with the MDP-containing primer and thoroughly sprayed with water, the characteristic peak of double carbon was detected at 1,640 cm^{-1} on the dentin surface. This indicates that some of the MDP monomers were adsorbed on the dentin surfaces, even after spraying for 30 s with a high pressure of water (Fig. 3). The Raman spectra are consistent with the spectra reported in a previous study, indicating that MDP adheres to hydroxyapatite and produces monomer-Ca salts (32). Furthermore, the chemical interaction between MDP and hydroxyapatite could greatly improve the bonding effectiveness of resin adhesives (33).

Before the application of etch-and-rinse adhesives, the prime-and-rinse approach using MDP-containing primers, either following phosphoric acid etching or replacing phosphoric acid etching, could have dramatically increased the enamel bond strengths in our previous studies (24, 34). The findings in the present study further confirm the results of our previous study, showing that the prime-and-rinse approach, using MDP-containing primer, dramatically increases the dentin MTBS of one-step self-etch adhesive (22). Thus, the findings of the present study further demonstrate and support the chemical bonding of MDP around dentin hydroxyapatite crystallites, which could significantly increase the dentin bond strengths. This might be explained by the fact that MDP-Ca salts which are chemisorbed on the dentin substrate after the prime-and-rinse approach not only greatly improve the wetting ability of self-etch adhesives (35) but also provide a large number of potential chemical bonding sites on the MDP-treated

dentin surfaces (36). Moreover, the transmission electron microscopy findings in this study indicate that many residual dentin hydroxyapatite crystallites remained within the hybrid layer when the prime-and-rinse approach was used (Figs 6 and 7). This might contribute to the bonding effectiveness of dentin adhesives (37).

Iwai *et al*. (19) reported that an increase in the amount of MDP-Ca salts, up to 37.2 mg, greatly improved the dentin bond strengths; however, beyond this amount, the dentin bond strengths decreased. In the present study, the prime-and-rinse approach washed off most of the soluble calcium salts and retained the insoluble MDP-Ca salts on the MDP-treated dentin surfaces. Furthermore, the prime-and-rinse approach removed most of the dentin smear layer, and subsequently, the adhesives directly interacted with the underlying dentin covered by MDP. This might result in the production of a smaller amount of calcium salts because the intact dentin is less likely to be demineralized than the smear layer (38), resulting in increased quality of the resin–dentin interface.

The dentin MTBS data in this study are consistent with those of previous studies (39–41). According to the results obtained in the present study, Adper Easy One produced the highest dentin MTBS compared with the other adhesives. The dentin bond effectiveness of self-etch adhesives is dependent on the different compositions of the commercial products (42, 43). For one-bottle self-etch adhesives, acidic hydrophilic monomers are mixed with hydrophobic monomers in an aqueous organic solvent (35). Hydrophobic resins are properly cured via the initiation of polymerization of hydrophobic photoinitiators, whereas the hydrophilic domains might be suboptimally cured because of a lack of polymerization initiation (44). The addition of hydrophilic photoinitiators, such as 2,4,6-trimethylbenzoyldiphenyl phosphine oxide (TPO), has been proven to improve the degree of conversion of the hydrophilic domains of self-etch adhesives (45). The excellent dentin bond strength of Adper Easy One might be attributable to the hydrophobic [camphorquinone (CQ)] and hydrophilic (TPO) photoinitiators present in this adhesive; Clearfil S3 Bond and i Bond only contain hydrophobic photoinitiators (CQ) and the photoinitiator for G Bond is not disclosed (Table 1). In addition, a hydrophilic copolymer (such as polyalkenoic acid) in Adper Easy One could chemically interact with hydroxyapatite to form stable calcium salts of polyalkenoic acid, which could contribute to the stability of the dentin bond interfaces (46, 47). Moreover, adhesive failures significantly increased after prolongation of the storage time, indicating degradation of the resin–dentin interfaces over time.

Taken together, the prime-and-rinse approach using 15 wt.% MDP significantly improves the short- and long-term dentin bond effectiveness of mild self-etch adhesives. The dentin bond stability of self-etch adhesives is associated with the compositions of the self-etch adhesives. Thus, the alternative hypothesis – that pretreatment with MDP prior to the application of mild self-etch adhesives does not improve the dentin bond effectiveness – is completely rejected, and the alternative hypothesis – that water storage does not affect the dentin MTBS of one-step self-etch adhesives – is partially rejected.

In summary, the prime-and-rinse approach using MDP-containing primers prior to the application of mild self-etch adhesives not only removes the weak smear layer but also significantly increases the short- and long-term MTBS of dentin. The prime-and-rinse approach could supplement contemporary dentin-bonding strategies.

Acknowledgements – This work was funded by the National Natural Science Foundation of China (81371189) and the Natural Science Foundation of Zhejiang Province (LQ16H140002, LQ14H140002, and LQ15H140005).

Conflicts of interest – The authors (Mingxing Li, Ling Zhang, Chaoyang Wang, Xiaoting Jin, Yan Hong, and Matthias Hannig) declare that they have no conflicts of interest. The authors (Baiping Fu and Jingqiu Xu) declare that they have obtained the following patent in China: Novel dentin primers for prime-and-rinse approach (patent no. ZL 2014 1 0679144.4).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. The mean values and standard deviations of dentin MTBS for the groups examined.

Figure S1. Representative fractographs of the dentin side of the specimens after 24 h of storage in water.

Figure S2. Representative fractographs of the dentin side of the specimens after 14 months of storage in water.

Figure S3. Representative TEM photomicrographs of the specimens after 24 h of storage in water.

Figure S4. Representative TEM photomicrographs of the specimens after 14 months of storage in water.