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## Prime-and-rinse approach for improving the enamel micro-tensile bond strengths of self-etch adhesives

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### ABSTRACT

The study investigated the effects of prime-and-rinse approach using 15% MDP (10-methacryloyloxydecyl dihydrogen phosphate)-containing primer on the enamel micro-tensile bond strengths (MTBS) of (ultra-) mild self-etch adhesives, enamel surfaces and enamel-resin interfaces. The buccal enamel surfaces of 69 human third molars were polished and randomly assigned to three groups: Group A (control, self-etch approach): Polished enamel surfaces were not further pre-treated. The enamel surfaces were acid-etched (Group B, (selective) enamel etching) or primed with 15% MDP-containing primer (Group C, prime-and-rinse approach) for 15 s and thoroughly water-sprayed. The enamel surfaces were applied with self-etch adhesives and placed with composite resins (Adper Easy One + Filtek Z350 (3 M ESPE); Clearfil S3 Bond + Clearfil Majesty (Kuraray-Noritake Co.); G Bond + Gradia Direct (GC); iBond + Charisma (Heraeus-Kulzer)), respectively. The specimens were prepared for MTBS test and scanning/transmission electron microscopy observations. Compared with group A, groups B and C produced significantly higher enamel MTBS ( $p < .01$ ), regardless of the adhesives used. Groups B and C possessed similar enamel MTBS ( $p > .05$ ). The SEM findings showed that smear layer remained on the polished enamel surface was completely removed by acid etching and almost completely removed by prime-and-rinse approach. The TEM microphotographs reveal that smear layer was detectable at the resin-enamel interface in group A, not in groups B and C. The novel prime-and-rinse approach using MDP-containing primer before the application of (ultra-) mild self-etch adhesives could greatly increase the enamel MTBS. That might be an alternative to selective enamel etching.

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bond strength; self-etch  
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## Introduction

Self-etch adhesive systems have been widely used in the clinic due to the lower technique sensitivity, shorter clinical application time and less incidence of postoperative sensitivity when compared to the etch-and-rinse adhesive systems [1–5]. However, some concerns have been raised with regard to their effectiveness of bonding to enamel, especially when the (ultra-) mild self-etch adhesives are used [6–8]. Previous reports demonstrated the low enamel bond strengths of (ultra-) mild self-etch adhesives [9–14].

In order to increase the enamel bond strengths, selective enamel etching with phosphoric acid has been strongly recommended to be used prior to application of (ultra-) mild self-etching adhesives [15–17]. However, selective enamel etching is extremely difficult to confine within peripheral enamel margin surrounding a cavity, since etchant may unintentionally flow over enamel-dentin junction (EDJ) to over-etch dentin [5,18,19]. That definitely deteriorates the dentin bonding effectiveness of (ultra-) mild self-etch adhesives [20]. That can be explained by fact that acid-etching could completely demineralize dentin surface, exposing a mineral-depleted collagen network [21], and subsequent application of self-etch adhesive will produce an over-etching layer at resin–dentin interface, weakening the dentin bond.

Previous researches showed that some functional monomers such as phosphoric acid esters (PAEs) and carboxylic acids have the capability of decalcifying and adhering to HAp simultaneously [22–26]. We demonstrated that the chemical interaction of phosphoric acid esters (PAEs) with HAp produced one water-soluble PAEs-HAp complex revealing etching ability and another water-insoluble PAEs-HAp complex possessing chemical bonding between them [27]. In other words, the less soluble the monomer-Ca salts, the more intense and stable the chemical bonding to the HAp—a main component of tooth hard tissues [28]. Likewise, self-etch adhesives applied on enamel and dentin surface will simultaneously produce some water-soluble, slightly water-soluble and water-insoluble monomer-Ca salts and various species of calcium phosphate. Afterward, they will deposit on the surface of the tooth hard tissues along with evaporation of the solvent by air-drying and subsequently be in situ polymerized with adhesive monomers in the hybrid layer. That might result in the weakest point of the self-etch adhesive systems and thus may affect the bonding performance [29,30].

Ten-methacryloyloxydecyl dihydrogen phosphate (10-MDP) is a promising functional acidic monomer used in some (ultra-) mild self-etch adhesives. MDP can partially demineralize the enamel/dentin surface, simultaneously resulting in water-soluble and water-insoluble monomer-Ca (MDP-Ca) salts in the own acidic solution on the enamel/dentin surfaces [31]. Prime-and-rinse using MDP-containing primer can demineralize enamel/dentin surface and leave some water-insoluble monomer-Ca salts on the enamel/dentin surface, however, the etch-and-rinse approach only demineralizes the enamel/dentin surface without any formation of soluble and insoluble monomer-Ca salts. Therefore, we proposed prime-and-rinse approach for distinguishing from the etch-and-rinse approach [30]. In our previous research, a prime-and-rinse approach using MDP-containing primer replaced phosphoric acid etching before application of etch-and-rinse adhesive, or after acid, etching could increase

**Table 1.** All the materials and steps of application used in the study.

Materials (Batch, code)	Manufacturers	pH	Compositions	Steps of application
Clearfil S3 Bond (C90008, S3)	Kuraray-Noritake, Tokyo, Japan	≈2.7	10-MDP, HEMA, Bis-GMA, ethanol, water, silanized colloidal silica, camphorquinone	Apply and leave for 20 s, strongly air-blow for approximately 5 s, and light-cure for 10 s
Clearfil Majesty A3 (5F0004)		–	Bis-GMA, TEGDMA, hydrophobic aromatic dimethacrylate, silanated barium glass filler, Pre-polymerized organic filler, initiators, accelerators, pigments.	Place 2 mm in two increments and light-cure for 40 s, respectively.
G Bond (1504021, GB)	GC, Tokyo, Japan	≈2	4-MET, phosphoric ester-monomer, UDMA, TEGDMA, acetone, water, silica filler, photo-initiator, stabilizer	Apply and leave undisturbed for 10 s, strongly air-blow for approximately 5 s, and light-cure for 10 s.
Gradia Direct A3 (1409011)		–	UDMA, silica powder, alumino-silicate glass, organic filler	Place 2 mm in two increments and light-cured for 40 s, respectively.
Adper Easy One (576919, AEO)	3M ESPE, Seefeld, Germany	≈2.4	phosphoric acid-methacryloxy-hexylesters, copolymer of acrylic and itaconic acid, Bis-GMA, HDDMA, HEMA, DMAEMA, ethanol, water, silane-treated silica, phosphine oxide, CQ	Apply for 20 s, gently air-blow for approximately 5 s, and light-cured for 10 s.
Filtek Z250 XT A3 (N588669 )	3M ESPE, Paul, USA	–	inorganic filler (zirconia/silica), resins (Bis-GMA, UDMA, Bis-EMA, PEGDMA, TEGDMA)	Place 2 mm in two increments and light-cure for 40 s, respectively.
iBond (010706,IB)	Heraeus-Kulzer, Hanau, Germany	≈2	4-MET, UDMA, glutaraldehyde, acetone, water, photoinitiators, stabilizers	Apply for 20 s, gently air-blow for approximately 5 s, and light-cure for 10 s.
Charisma A3 (62002)		–	Bis-GMA, Silicon dioxide	Place 2 mm in two increments and light-cure for 40 s, respectively.

Abbreviations: 10-MDP: 10-methacryloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; Bis-GMA: Bisphenol A diglycidylmethacrylate; 4-MET: 4-methacryloxyethyl trimellitate anhydride; HDDMA:1,6-hexanediol dimethacrylate; DMAEMA: 2-dimethyl amino ethyl methacrylate; UDMA: Urethane Dimethacrylate; Bis-EMA: Bisphenol A ethoxylated dimethacrylate; PEGDMA: (ethylene glycol) dimethacrylate; TEGDMA: triethylene glycol dimethacrylate.

enamel bond strength [25,30,32]. Furthermore, the prime-and-rinse approach using MDP-containing primer could greatly increase the dentin bond strength of mild self-etch adhesives [33]. However, the prime-and-rinse approach using MDP-containing primer has never been studied for enamel bonding before application of (ultra-) mild self-etch adhesives.

The purpose of this study was to investigate the effects of prime-and-rinse approach using MDP-containing primer on enamel bond strength of (ultra-) mild self-etch adhesives and enamel-adhesive bond surfaces. The null hypotheses tested in

this study were that (1) prime-and-rinse approach using MDP-containing primer before application of (ultra-) mild self-etch adhesives could not increase the enamel bond strengths when compared with self-etch approach, and (2) even worse when compared with (selective) enamel etching.

## Materials and methods

### *Specimen preparation*

An experimental primer containing 15% (w/w) of MDP was prepared by dissolving 10-MDP (Watson International Ltd, Jiangsu, China, Lot #WI12090678) in ethanol-aqueous (1:1) solution. Sixty-nine extracted, non-cariou human third molars stored in 0.5% chloramine-T solution at 37 °C were used within one month after extraction in this study. The research protocol was approved by the Institutional Ethics Committee and performed in accordance with the international Ethical Guideline and Declaration of Helsinki [34]. The teeth were stored in tap water for 24 h before the buccal enamel surfaces of the teeth were polished with 320-grit SiC paper under running water in order to form a uniform smear layer. Sixty teeth were randomly divided into three groups according to application approaches ( $n=20$ ). Group A (control): The enamel surfaces were not further pre-treated serving as self-etch approach. The enamel surfaces were etched with 37% phosphoric acid for 15 s (Group B: (selective) enamel etching) or primed with 15% of MDP-containing primer for 15 s (Group C: prime-and-rinse approach). Subsequently, they were all water-sprayed for 30 s and gently dried. The enamel surfaces were applied with one of four one-bottle self-etch adhesives and placed with the respective composite resins from the same manufacturer strictly according to the manufacturer's instructions. They included Clearfil S3 Bond (S3) + Clearfil Majesty, Kuraray-Noritake, Tokyo, Japan; G Bond (GB) + Gradia Direct, GC, Tokyo, Japan; Adper Easy One (AEO), 3 M ESPE, Seefeld, Germany + Filtek Z250, 3 M ESPE, Paul, USA and i Bond (IB) + Chrisma, Heraeus-Kulzer, Hanau, Germany. All the materials and the steps of application used in the study are summarized in Table 1. The composite resin was placed on the pre-treated enamel surfaces in two 2-mm thick increments, and each light-cured for 40 s. Light-curing was performed using a light-curing unit with an output of 1500 mW/cm<sup>2</sup> (Radii Plus, SDI, Victoria, Australia).

### *Micro-tensile bond strength (MTBS) tests*

After storage in distilled water for 24 h at 37 °C, forty-eight enamel-bonded specimens were perpendicularly sectioned through the resin-enamel interfaces using a low-speed saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) under continuous water cooling. They were prepared into multiple beams with a cross-section area of approximately 1.0 mm<sup>2</sup>. A Micro Tensile Tester (Bisco Inc. Schaumburg, IL, USA) was used to perform the MTBS tests at a crosshead speed of 1 mm/min until fracture. The dimension of the fractured surface was measured with a resolution of 0.01 mm using a pair of digital vernier calipers (MNT-150, Meinaite, China). The specimens of pre-testing

**Table 2.** Mean enamel micro-tensile bond strengths (Means  $\pm$  SD [median, *n*], MPa) in this study.

Adhesives	Control	Enamel etching	Prime-and-rinse
IB	17.79 $\pm$ 7.84(16.33, 22) <sup>Aa</sup>	27.2 $\pm$ 7.84(26.84, 20) <sup>Ba</sup>	26.54 $\pm$ 6.21(27.03, 28) <sup>Ba</sup>
GB	17.87 $\pm$ 5.37(17.49, 28) <sup>Aa</sup>	29.76 $\pm$ 6.79(28.73, 33) <sup>Bab</sup>	27.77 $\pm$ 8.03(25.81, 32) <sup>Ba</sup>
S3	19.48 $\pm$ 6.71(17.69, 31) <sup>Aa</sup>	34.36 $\pm$ 8.86(37.60, 29) <sup>Bc</sup>	32.75 $\pm$ 10.06(31.85, 27) <sup>Bab</sup>
AEO	17.54 $\pm$ 6.88(17.61, 21) <sup>Aa</sup>	34.11 $\pm$ 10.43(30.73, 24) <sup>Babc</sup>	37.28 $\pm$ 9.28(35.17, 33) <sup>Bbc</sup>

Notes: The same lowercase/uppercase superscript letters in a vertical column/a horizontal row indicate no significant differences ( $p > .05$ ). The different lowercase/uppercase superscript letters in a vertical column/a horizontal row indicate significant differences ( $p < .01$ ). IB: I Bond; GB: G Bond; S3: Clearfil S3 Bond; AEO: Adper Easy One. *n*: the numbers of the beams for MTBS test.

failures (PTFs) were excluded in this study. The MTBS were calculated in megapascals (MPa).

### Failure mode analysis

After the MTBS tests, the modes of failure were determined by stereomicroscopy (OLYMPUS, SZ61, Japan) at a magnification of 50-fold. Failure modes were categorized into (a) interfacial failure occurring either between the enamel and adhesive or between adhesive and composite resin; (b) cohesive failure in composite resin (cohesive resin); (c) cohesive failure occurring in the enamel or at the dentin-enamel junction (DEJ) (cohesive DEJ/enamel); (d) mixed failure occurring in adhesive, enamel and composite resin [35].

### Scanning electron microscopy (SEM)

Nine enamel segments (3 mm  $\times$  3 mm  $\times$  1 mm) were obtained from buccal enamel surfaces of another 9 teeth. The enamel surfaces were pre-treated as the above-mentioned three approaches (3 teeth each group) without applications of adhesives and placement of composite resins. All the specimens were split through the middle of the segments. The pre-treated enamel surfaces, the split enamel surfaces and two randomly-selected, de-bonded specimens each subgroup after the MTBS tests were analyzed by an SEM (SU8010, Hitachi, Japan) after they were dehydrated with a series of ascending concentrations of ethanol (30 ~ 100%) and gold-sputtered.

### Transmission electron microscopy (TEM)

Twelve resin-bonded enamel specimens, one per subgroup, were each cut into an ~0.5-mm thick slab including the resin-enamel interface during specimen sectioning. All the slabs were fixed in Karnovsky's fixative and post-fixed in 1% osmium tetroxide. After fixation, they were desiccated in an ascending ethanol series (30–100%), immersed in propylene oxide as a transition fluid for 4 h, and finally embedded in a TEM grade epoxy resin. After the embedding resin was completely set, ultra-thin non-demineralized sections (~70–90 nm thick) were obtained with a diamond knife (Diatom, Biel, Switzerland). They were analyzed by TEM (JEOL JEM-1230, Tokyo, Japan) at 100 kV.

**Table 3.** The pairwise comparisons between the three different enamel pretreatments.

Pairwise Comparisons	<i>p</i> values
Control vs. Enamel etching	<.001
Control vs. Prime-and-rinse	<.001
Enamel etching vs. Prime-and-rinse	=1.000

### Statistical analysis

The normality and homoscedasticity assumption of the MTBS data was violated (Shapiro–Wilk Test,  $p = .025$  & Levene Test,  $p = .001$ ). Statistical analysis was performed using the Kruskal-Wallis test followed by post-hoc pairwise comparisons with Bonferroni correction [36,37]. Chi-square ( $\chi^2$ ) test was used to analyze the failure modes. The statistical analysis was performed with statistical analysis software package (SPSS 22.0, IBM Corp. New York, USA). The significance level was set  $\alpha = 0.05$ .

## Results

### Micro-tensile bond strength (MTBS)

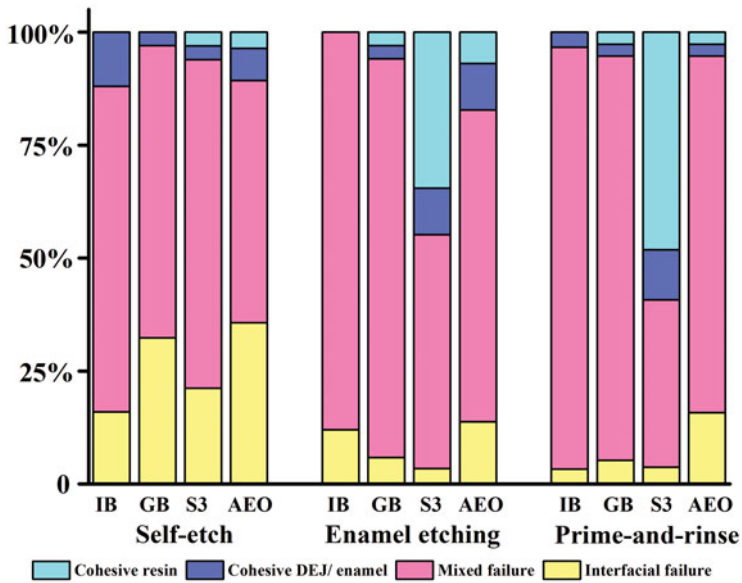
All the MTBS data are summarized in Table 2. There was no significant difference between the enamel MTBS among the four adhesives in the control group ( $p > .05$ , Table 2). Compared with the control group (Group A, self-etch approach), (selective) enamel etching approach (Group B) and prime-and-rinse approach using 15% MDP-containing primer (Group C) could significantly increase the enamel MTBS, regardless of the different adhesives used ( $p < .01$ , Tables 2 and 3). However, there was no significant difference between the latter two ( $p > .05$ , Table 3).

### Failure mode analysis

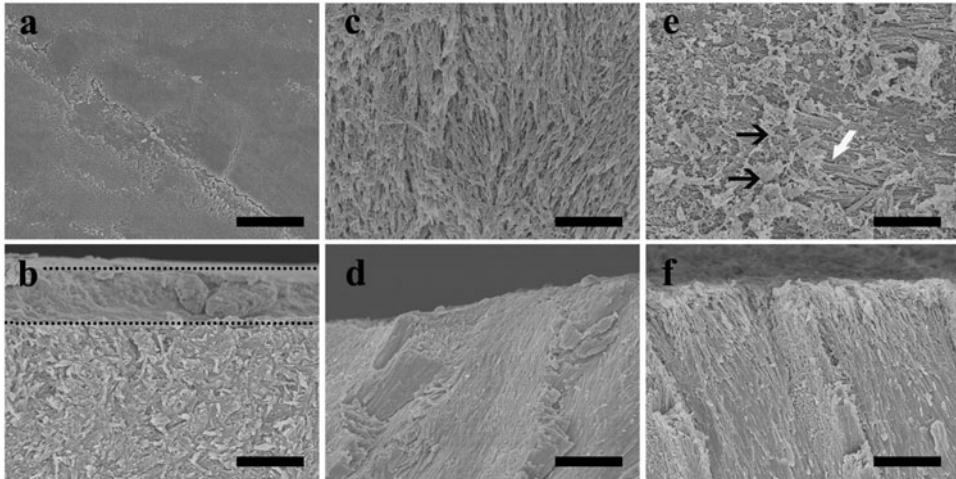
The failure modes in this study are shown in Figure 1. Most of the failure modes were the mixed failure in this study ( $p < .001$ ) except for adhesive S3 with the enamel pre-treatment by prime-and-rinse approach. There were no significant differences of the failure modes between (selective) enamel etching and prime-and-rinse approach ( $p > .05$ ). Overall, self-etch approach produced more adhesive failures than (selective) enamel etching and prime-and-rinse approach ( $p < .001$ ).

## SEM

The micro-morphology of the differently-treated enamel surfaces is shown in Figure 2. The polishing scratches are clearly observed on the polished enamel surface (Figure 2(a)) and approximately 1.5–2  $\mu\text{m}$  of smear layer is on the split enamel surface (Figure 2(b)) in the control group (Group A). In the (selective) enamel etching (Group B), the etching enamel surface reveals typical enamel prism and interprism (Figure 2(c)) without any visible enamel smear (Figure 2(d)). The enamel smear layer was almost eliminated, enamel HAp crystallites were exposed and some monomer-Ca

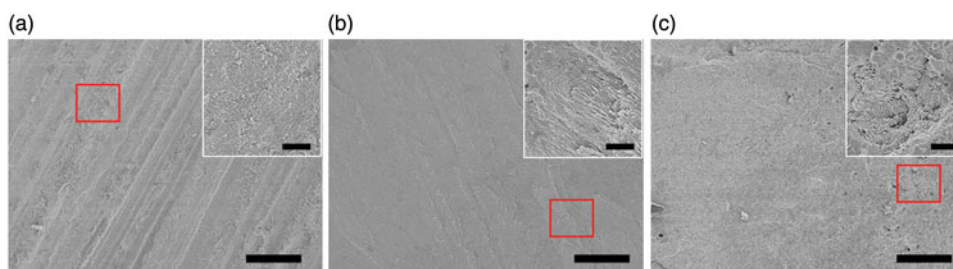


**Figure 1.** Failure modes analysis in this study. The predominant failure modes in all groups are mixed failure except for adhesive S3 with the enamel pre-treatment by prime-and-rinse approach. IB: I Bond; GB: G Bond; S3: Clearfil S3 Bond; AEO: Adper Easy One.



**Figure 2.** The SEM microphotographs of enamel surfaces and split enamel surface (magnification = 10000 fold, bar = 2 μm). The polishing scratches (a) are visible on the polished enamel surface with ~1.5–2 μm thick smear layer (between the dotted lines) on the split enamel surface (b). The acid-etching enamel surface (c) reveal a distinct etching pattern with clear exposure of enamel prism rods and no smear layer is visible on the split enamel surface (d). The smear layer is nearly completely removed by prime-and-rinse approach using the MDP-containing primer (e and f), exposing some HAp crystallites (white arrow), and scattered patches of MDP-Ca salts (black arrows) remained on the enamel surfaces (e) resulting from the chemical interaction of MDP with enamel HAp.





**Figure 3.** Representative SEM images of the fractured surfaces. The inset images are the higher magnifications of the marked areas in red boxes. In the control group, the failure mode is interfacial failure and the polishing scratches remained on the fractured surfaces of enamel site (a). Higher magnification micrographs (inset in a) show remnants of smear debris with little exposure of enamel HAp crystallites. As for the enamel surfaces pre-treated with acid-etching (b), or prime-and-rinse approach using 15% MDP primer (c), the failure modes are mixed failure, higher magnification micrographs (insets in b and c) show lots of enamel HAp crystallites on the fractured surfaces. (a: control group; b: enamel etching group; c: prime-and-rinse group; magnification = 1000 fold, bar = 20  $\mu\text{m}$ ; inset magnification = 10,000 $\times$ , bar = 2  $\mu\text{m}$ ).

salts remained on the enamel surfaces (Figure 2(e,f)) after the enamel surface was treated with prime-and-rinse approach using MDP-containing primer (Group C).

The SEM micromorphology of the fractured surfaces of the de-bonded specimens is shown in Figure 3. The SEM image shows the adhesive failure in the control group and polishing scratches remained on the fractured surfaces of enamel site (Figure 3(a)). Higher magnification micrographs show remnants of smear debris with little exposure of enamel HAp crystallites. As for the enamel surfaces treated either with acid-etching (Figure 3(b)) and the MDP-containing primer (Figure 3(c)), the SEM images reveal mixed failures and higher magnification micrographs show lots of enamel HAp crystallites on the fractured surfaces.

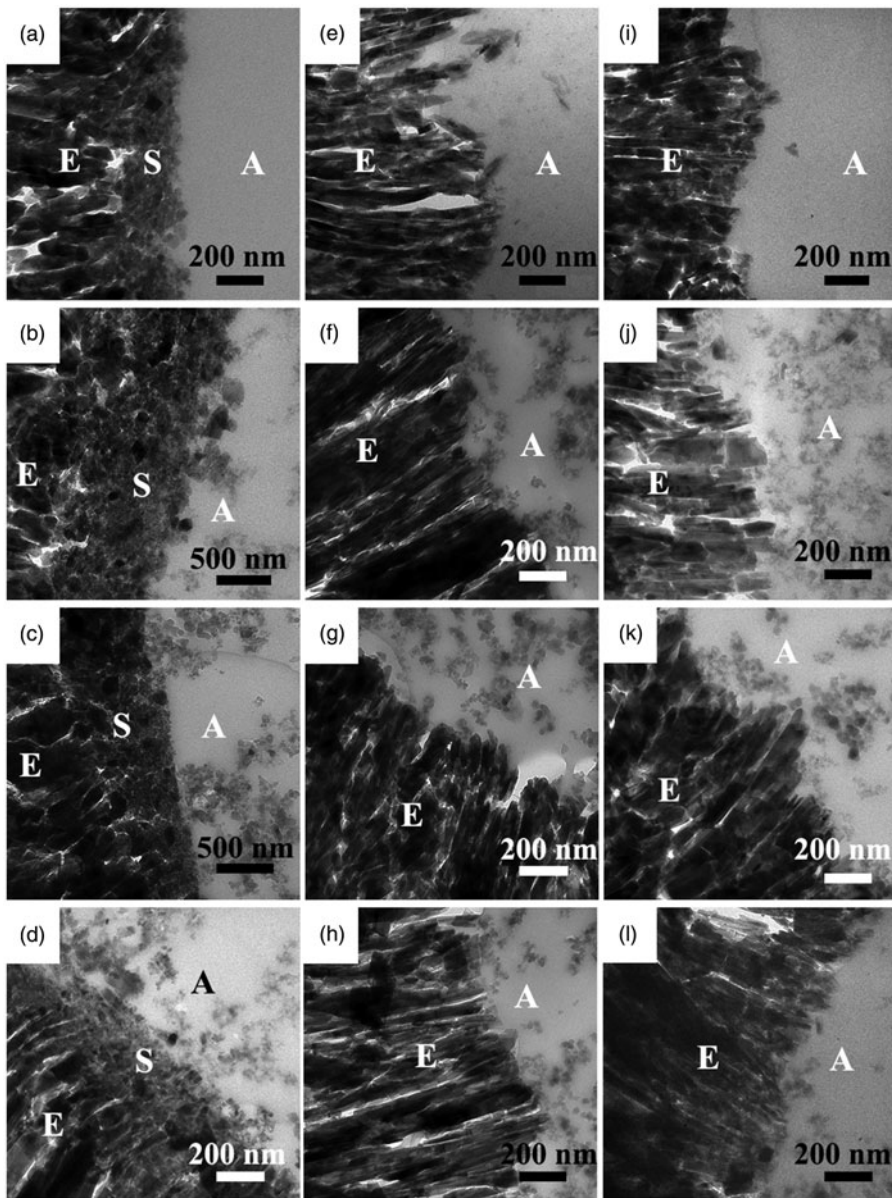
## TEM

The TEM microphotographs reveal that ‘resin-smear complex’ could be found at the adhesive-enamel interface in Group A (Figure 4(a-d)), but not in Group B (Figure 4(e-h)) and Group C (Figure 4(i-l)). No micro-gaps could be detected in all the groups.

## Discussion

The micro-tensile bond test is regarded as a reliable adhesion testing method that can be used to evaluate the bond strength between an adhesive and a bonding substrate [38]. The findings in this study revealed that all the adhesives used in self-etch approach yielded similar bond strengths (Table 2,  $p > .05$ ). The disparity of the enamel bond strengths of the four self-etch adhesives in the previous reports could be attributed to the different experimental conditions, different operators and different enamel surface treatments [39–41].

Up to date, mild self-etch adhesives bonded to enamel have not been directly demonstrated to resist the mechanical and chemical challenges in the oral cavity as the



**Figure 4.** TEM images of adhesive-enamel interface (control group (Group A): a–d; selective etching group (Group B): e–h; prime-and-rinse group (Group C): i–l; a, e, i: i Bond; b, f, j: Adper Easy One; c, g, k: Clearfil S3 Bond; d, h, l: G Bond; E: enamel; S: smear layer; A: adhesive; magnification = 100,000 $\times$ ). A smear layer can be detected at the adhesive-enamel interface in control groups (a–d) but cannot in groups B and C. No micro-gaps are detected in all the groups.

same as etch-and-rinse adhesives do [20]. The enamel bond durability of (ultra-) mild self-etch adhesives still remains to be solved [42]. Thus, the improvement of the enamel bond durability of mild and ultra-mild self-etch adhesives is still a great challenge for the dental materials researchers.

Compared with the control group, the outcomes of the enamel MTBS test clearly indicate that both prime-and-rinse approach and (selective) enamel etching could significantly increase the short-term enamel MTBS, irrespective of the adhesives used ( $p < .01$ , Tables 2 and 3), but there were no significant differences of the enamel MTBS between the latter two ( $p > .05$ , Tables 2 and 3). Thus, the null hypotheses that prime-and-rinse approach using MDP-containing primer prior to application of (ultra-) mild self-etch adhesives would not improve the enamel MTBS when compared with self-etch approach, and even worsen when compared with the (selective) enamel etching were totally rejected.

Smear layer is a layer of debris compacted on the surfaces of dental hard tissues created by bur-preparation and SiC-polishing [43,44]. This varies in roughness, density, thickness and weak attachment to the underlying tooth structures, depending on the dental instruments [42,44–47]. In order to create a uniform smear layer for the enamel/dentin bond strength study, polishing with SiC paper is one of the most used methods to prepare the tooth surfaces in numerous laboratory studies [48,49]. The etching potential of (ultra-) mild self-etch adhesives is not as aggressive as that of phosphoric acid etching, therefore, thick enamel smear layers remain a great challenge for mild self-etch adhesives [6]. This is consistent with the finding in this study (Figure 4(a–d)). Numerous studies have investigated the influences of smear layer on the enamel and dentin bonding performance of self-etch adhesives [42,50–56]. Smear layers remained in the enamel surfaces might be an obstacle in the achievement of reliable adhesion for mild self-etch adhesives [57–61]. Acid etching was able to completely eliminate the smear layer in this study (Figure 2(c–d)). The findings in this study are completely in agreement with previous studies [53,62]. Moreover, the enamel smear layer was almost completely removed by prime-and-rinse approach (Figure 2(e–f)). All the tested adhesives in this study are classified into mild or ultra-mild self-etch adhesives according to the aggressiveness (pH value) of self-etch adhesives [15]. The adhesives used in this study were incapable of completely dissolving the SiC-polishing enamel smear layer like acid etching. The TEM findings in this study further demonstrated that ‘resin-smear complex’ was detectable at the enamel-resin interface (Figure 4(a–d)). This may compromise the micromechanical interlocking between adhesive resin and the underlying enamel [63]. Previous reports have demonstrated that residual smear layer exists at the resin-enamel interface since (ultra-) mild self-etch adhesives only create a shallow etching pattern on enamel which results in a weak micromechanical retention [53,63]. The SEM microphotographs of the fractured surfaces in the control group reveal the remnants of smear debris with little exposure of enamel HAp crystallites (Figure 3(a)). However, the SEM microphotographs of the other two groups (enamel etching and prime-and-rinse approach) show lots of enamel HAp crystallites exposed on the fractured surfaces (Figure 3(b–c)). Furthermore, not only the smear layer has a rather weak bond to the underlying enamel, but also the ‘resin-smear complex’ (Figure 4(a–d)) might result from the resin incomplete infiltration into the smear layer [53]. Therefore, the ‘resin-smear complex’ may become an initial point of bond deterioration [42]. It is extremely important for resin monomers to completely penetrate the smear layers and to form chemical bonding with the tooth hard tissue in order to achieve a

reliable adhesion to enamel [42,62]. A firm micro- and nano-mechanical interlock results from the networks penetrating through the inter-crystallites [63]. The TEM microphotographs in this study reveal that the adhesives are tightly contacted with the underlying enamel without any 'resin-smear complex' at the resin-enamel interfaces when the prime-and-rinse approach and (selective) enamel etching used (Figures 4(e-l)).

Moreover, some phosphoric acid esters such as MDP has been proven to have a high chemical bonding potential to HAP, enamel and dentin within clinically reasonable application time [3,25,27,30,59]. This chemical bondability has been demonstrated to improve the short- and long-term enamel bond strength [25]. The previous publications demonstrated that the additional chemical bonding of MDP surrounding the enamel HAP crystallites could significantly increase the enamel bond strengths [64–66]. Therefore, the enamel MTBS increase in this study should be attributed to the elimination of the weak smear layer as well as the additional chemical bonding.

The selective enamel etching is strongly recommended to improve enamel bond durability when mild or ultra-mild self-etch adhesives used [15–17]. However, etchants would inadvertently flow over the peripheral enamel margins into dentin surfaces that would jeopardize the dentin bond [5,18,19]. Our preliminary study revealed that the prime-and-rinse approach using MDP-containing primer could improve the dentin bonding performance [33]. Furthermore, the prime-and-rinse approach using MDP-containing primer in this study could achieve the enamel MTBS similar to (selective) enamel etching.

Taken together, prime-and-rinse approach using the MDP-containing primer could nearly completely remove the enamel smear layer as well as produce some insoluble monomer-Ca salts (MDP-Ca salts) on the enamel. That might be explained by the fact that MDP-Ca salts chemisorbed on the enamel substrate could greatly improve the wetting ability of self-etching adhesives and create potential chemical bonding sites on the primed enamel surfaces. It might be the reason that prime-and-rinse approach could achieve a similar enamel MTBS to (selective) enamel etching. Therefore, prime-and-rinse approach using MDP-containing primer is an alternative to selective enamel etching before application of ultra-mild and mild self-etch adhesives. However, its long-term bond performance needs a further study.

## Conclusion

Within the limits of this *in vitro* study, prime-and-rinse approach using 15% MDP-containing primer could remove the enamel smear layer, and greatly improve the enamel bond strengths prior to application of (ultra-) mild self-etch adhesives. Hence, the novel prime-and-rinse approach might be an alternative to selective enamel etching when (ultra-) mild self-etch adhesive used.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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