Development of MDP-based one-step self-etch adhesive —Effect of additional 4-META on bonding performance—

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We designed three experimental 10-methacryloyloxydecyl dihydrogen phosphate (MDP)-based one-step (EX) adhesives consisting of MDP, urethane dimethacrylate, and triethylene glycol dimethacrylate adhesives with different water contents (98.4, 196.8, and 294.4 mg/g), and 4-methacryloyloxyethyl trimellitic anhydride (4-META) or 2-hydroxyethyl methacrylate (HEMA)-containing one-step adhesive. The effect of the amount of MDP-calcium (MDP-Ca) salt produced through demineralization of enamel and dentin on the bonding performance was examined. The efficacy of 4-META and HEMA was then discussed. When the amount of water in EX adhesive was increased, the production amount of MDP-Ca salt of enamel increased, but not the dentin. The enamel bond strength slightly increased with increasing the production amount of MDP-Ca salt, in contrast to the dentin. However, addition of 4-META in the EX adhesive (water content=98.4 mg/g) increased both bond strengths, although the production amounts of MDP-Ca salt significantly decreased. The 4-META enhances both bond strengths more effectively than the HEMA.

Keywords: One-step adhesive, Demineralization efficacy, Calcium salt of MDP, Bond strength

INTRODUCTION

Two- and one-step self-etch adhesives are widely utilized, because of their simplified application procedures and low technique sensitivity, compared with etch and rinse adhesives¹-⁶. Investigators have performed studies to understand the adhesion mechanism of one- and two-step self-etch adhesives to the tooth through acidic monomers. They have been examined using electron spectroscopy for chemical analysis⁷-¹⁰, X-ray diffraction ¹¹-¹³, carbon 13 nuclear magnetic resonance (¹³C NMR)¹⁴-¹⁷, phosphorus 3¹ NMR¹⁸, and scanning electron microscope (SEM) observation of adhesive-enamel or -dentin¹⁹,²⁰. Yoshida et al.⁷,¹⁰ established the “adhesive decalcification” concept, suggestive of the bonding potential of 10-methacryloyloxydecyl dihydrogen phosphate (MDP) to the synthesized hydroxyapatite (HAp) higher than the 4-methacryloyloxyethyl trimellitic acid (4-MET) or 2-methacryloyloxyethyl phenyl hydrogen phosphate (Phenyl-P). The chemical nature of the calcium salt of the acidic monomer, specifically the solubility in water directly relates to the long-term dentin bond durability²¹. In addition, Li et al.¹⁹ and Nikaido et al.²⁰ reported that the “acid-base resistant zone” created by two and one-step selfetching adhesives plays a key role in prevention of secondary caries, sealing of restoration margins and promotion of restoration durability.

Recently, 2-hydroxyethyl methacrylate (HEMA)-free one-step adhesive has been developed, since HEMA is notorious for its high allergenic potential, associated to allergic reaction type IV²²-²⁴. These HEMA-free adhesives generally contain a dentin adhesion-promoting monomer, such as 4-methacryloyloxyethyl trimellitic anhydride (4-META) or 2-acryloxyethyl trimellitic acid (4-AET). However, the efficacy of 4-META or 4-AET utilized in the HEMA-free adhesive has not been clarified yet.

In order to clarify the efficacies of amount of water and of addition of 4-META utilized in the one-step self-etch adhesive on the bonding performance, we designed a series of three experimental MDP-based one-step (EX) adhesives consisting of MDP, urethane dimethacrylate (UDMA), and triethyleneglycol dimethacrylate (TEGDMA) with different water contents (98.4, 196.8, and 294.4 mg/g), and a series of two 4-META-containing and HEMA-containing one-step adhesives by adding 4-META or HEMA in EX adhesive (water content=98.4 mg/g). The effect of amount of water in EX adhesive on the amount of MDP-Ca salt produced through demineralization of HAp, bovine enamel and dentin was examined using the ¹³C NMR technique.¹⁶,¹⁷ Further, the effect of the production amount of MDP-Ca salt on the enamel and dentin bonding performance was examined. The efficacy of 4-META was then discussed. The null hypotheses were that: (1) the amount of water in the EX adhesive has no effect on the production amount of MDP-Ca salt of bovine enamel and dentin, (2) the production amount of MDP-Ca salt has no effect on the enamel and dentin bonding performance, and (3) the addition of 4-META has no effect on the production amount of MDP-Ca salt of bovine enamel and dentin.
MDP-Ca salt and on the enamel and dentin bonding performance.

**MATERIALS AND METHODS**

**Materials**

1. Preparation of experimental one-step adhesives

To prepare the experimental adhesive, we used MDP (Kuraray Noritake Dental Inc, Tokyo, Japan), UDMA (Negami Chemical Industrial, Ishikawa, Japan), and TEGDMA (Shin-Nakamura Chemistry, Tokyo, Japan). A series of three EX adhesives with different amounts of water (98.4, 196.8, and 294.4 mg/g) was prepared by varying mass ratios of water to acetone. The codes, components, and compositions of EX adhesives were summarized in Table 1. Consequently, after mixing these monomers for each adhesive in described in Table 1, 1 mass% of camphorquinone (Wako Pure Chemical Industries, Ltd, Osaka, Japan), dimethylamino benzoic acid ethyl ester (Wako Pure Chemical Industries), and 2,000 ppm of hydroquinone monomethyl ether (Wako Pure Chemical Industries) were dissolved in each mixed monomer as a photo-initiator, accelerator, and inhibitor, respectively. Further, 10 mass% of colloidal silica (R-972, Nihon Aerosil, Tokyo, Japan) to each mixed monomer were filled as a filler. The EX adhesives were prepared after each resin paste was diluted by acetone aqueous solution with different mass ratios of water to acetone.

Next, the 4-META-containing one-step (EX1-M) adhesive was prepared after adding 4-META in the EX1 adhesive. There, we adjusted the mole number of additional 4-META to that of MDP utilized in the EX1 adhesive. With a comparison purpose with the EX1-M adhesive, the HEMA-containing one-step (EX1-H) adhesive was also prepared by adding HEMA. A series of two EX1 adhesives (EX1 adhesives: EX1-M and EX1-H adhesives) were also listed in Table 1. There, the 4-META in the EX1-M adhesive probably represents as the 4-MET, since the water employed induces a hydrolysis of the carboxylic acid anhydride group in the 4-META.

The pH value of each experimental adhesive was measured three times using pH meter (HM-30V, TOA-DKK, Tokyo, Japan). The pH value of each adhesive was combined in Table 1.

2. Preparation of enamel and dentin particles

After removal of pulp, the bovine crown enamel was cut by an air-turbine with a diamond point under a stream of cooling water. The cut enamel particles were obtained by decantation of the collected cooling water. The cut enamel particles were obtained by decantation of the collected cooling water. The bovine crown dentin particles were also prepared according to the procedures as mentioned above. The primary particle sizes of enamel or dentin were 5–20 µm. In addition, the HAp particles (HAP-200, Taihei Chemistry, Osaka, Japan) were used as a model for the enamel. The primary particle sizes of enamel or dentin were 5–20 µm.

**Methods**

1. 13C NMR observations of EX1, EX2, EX3, EX1-M or EX1-H adhesive before and after the reaction with HAp, bovine enamel and bovine dentin

Bovine enamel or bovine dentin particles (0.200 g) were suspended in each adhesive (1.000 g) and the suspensions were vibrated for 10 min. After centrifugation, the supernatants of each adhesive were obtained as a

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**Table 1** The code and components, compositions of experimental adhesive.

<table>
<thead>
<tr>
<th>Code</th>
<th>Component and Composition</th>
<th>MDP</th>
<th>UDMA</th>
<th>TEGDMA</th>
<th>HEMA</th>
<th>4-META</th>
<th>Filler</th>
<th>Water</th>
<th>Acetone</th>
<th>Total amount</th>
<th>Amount of water (mg/g)</th>
<th>pH value (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EX adhesives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EX1</td>
<td></td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>0</td>
<td>0</td>
<td>3.27</td>
<td>11.2</td>
<td>69.3</td>
<td>113.8</td>
<td>98.4</td>
<td>1.91 (0.06)</td>
</tr>
<tr>
<td>EX2</td>
<td></td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>0</td>
<td>0</td>
<td>3.27</td>
<td>22.4</td>
<td>58.1</td>
<td>113.8</td>
<td>196.8</td>
<td>2.21 (0.04)</td>
</tr>
<tr>
<td>EX3</td>
<td></td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>0</td>
<td>0</td>
<td>3.27</td>
<td>33.5</td>
<td>47.0</td>
<td>113.8</td>
<td>294.4</td>
<td>2.28 (0.05)</td>
</tr>
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<td>EX1 adhesives</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>EX1-M</td>
<td></td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>0</td>
<td>9.4</td>
<td>4.26</td>
<td>11.2</td>
<td>69.3</td>
<td>124.2</td>
<td>90.2</td>
<td>1.49 (0.05)</td>
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<tr>
<td>EX1-H</td>
<td></td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>4.0</td>
<td>0</td>
<td>3.69</td>
<td>11.2</td>
<td>69.3</td>
<td>118.2</td>
<td>94.8</td>
<td>1.48 (0.06)</td>
</tr>
</tbody>
</table>

Amount of each component were expressed by gram. (): SD

Amount of water was calculated as an amount of water utilized in 1.000 g of the respective adhesive.

MDP: 10-methacryloyloxydecyl dihydrogen phosphate; UDMA: urethane dimethacrylate; TEGDMA: triethyleneglycol dimethacrylate; HEMA: 2-hydroxyethylmethacrylate; 4-META: 4-methacryloyloxyethyl trimellitic anhydride
reacted EX1, EX2, EX3, EX1-M or EX1-H with bovine enamel or bovine dentin, respectively. The NMR spectra of each reacted adhesive were then observed using an EX-270 spectrometer (JEOL, Tokyo, Japan). The accumulation and repetition times were 3,000 times and 9 s, respectively. NMR samples for each reacted adhesive were prepared after respective supernatant (0.300 g) was dissolved in dimethyl-sulfoxide-d$_6$ (0.200 g). Further, NMR spectrum of the unreacted EX1, EX2, EX3, EX1-M or EX1-H was observed immediately after each adhesive was prepared. Preparations of reacted and unreacted EX1, EX2, EX3, EX1-M or EX1-H and NMR observations were performed 3 times. With a comparison purpose with bovine enamel, we measured NMR spectra of the EX adhesives after the reaction with HAp.

2. Determination of production amount of MDP calcium salt
The relative intensity ratios of NMR peak of the vinyl methylene carbon of MDP to that of UDMA were determined before and after the reaction with HAp, bovine enamel or bovine dentin. The reduction of the NMR peak of the vinyl methylene carbon of MDP was determined by dividing the difference between the relative intensity ratios obtained before and after the reaction by the relative intensity ratio obtained before the reaction. The amount of MDP-Ca salt produced through demineralization of HAp, bovine enamel or bovine dentin was then calculated by multiplying the reduction obtained from each adhesive to the quantity of MDP utilized in 1.000 g of respective adhesive. This was due to the MDP-Ca salt produced was insoluble in experimental adhesives$^{16,17}$.

3. Preparation of specimens for the adhesion test
After pulp removal, the facial enamel surface of the bovine crown was grounded with a sequence of 100-, 600- and 1,000-grit silicon carbide papers under water irrigation. To adjust the adhesive area, we placed 80-µm double-faced tape with a circular hole (internal diameter=3.2 mm, Nichiban, Tokyo, Japan) onto the ground enamel or dentin surface. The enamel or dentin surface inside the circular hole was conditioned with each adhesive for 20 s and then air-blown with a high-pressure airflow for 5 s. Light-irradiation was then applied to the adhesive for 10 s with a Light Curing Unit (XL3000, 3M ESPE, Graftenau, Germany). A 1-mm-thick silicone ring mold with a circular hole (internal diameter=3.2 mm) was mounted onto the double-faced tape. The hole was then immediately filled with resin composite (Clearfil AP-X, Kuraray Noritake Dental Inc) and then irradiated with light for 20 s. After removal of the mold and tape, the bonded specimens were immersed in water at 37°C.

4. Measurement of shear bond strength
After storage of bonded specimens for 1 day, the shear bond strength of experimental adhesives to the enamel or dentin was measured under a crosshead speed of 1.0 mm/min by a conventional testing machine (TG-5KN, Minebea, Nagano, Japan). The number of specimens for each experimental adhesive was 15.

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### Table 2

<table>
<thead>
<tr>
<th>Code</th>
<th>Quantity of MDP (mg/g)</th>
<th>HAp</th>
<th>Enamel</th>
<th>Dentin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Reduction (%)</td>
<td>Reacted MDP (mg/g)</td>
<td>Reduction (%)</td>
</tr>
<tr>
<td>EX adhesives</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EX1</td>
<td>87.9</td>
<td>5.4 (2.3)</td>
<td>4.7 (2.0)</td>
<td>68.9 (7.1)</td>
</tr>
<tr>
<td>EX2</td>
<td>87.9</td>
<td>8.2 (0.5)</td>
<td>7.2 (0.4) **</td>
<td>77.3 (3.2)</td>
</tr>
<tr>
<td>EX3</td>
<td>87.9</td>
<td>15.8 (1.3)</td>
<td>13.9 (1.2) **</td>
<td>81.4 (1.3)</td>
</tr>
<tr>
<td>EX1 adhesives</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EX1</td>
<td>87.9</td>
<td>—</td>
<td>—</td>
<td>68.9 (7.1)</td>
</tr>
<tr>
<td>EX1-M</td>
<td>80.5</td>
<td>—</td>
<td>—</td>
<td>58.3 (1.9)</td>
</tr>
<tr>
<td>EX1-H</td>
<td>84.6</td>
<td>—</td>
<td>—</td>
<td>63.7 (0.8)</td>
</tr>
</tbody>
</table>

$: SD

1. The quantity of MDP utilized in 1.000 g of the respective adhesive
2. The reduction in the NMR peak of the vinyl methylene carbon of MDP was determined by dividing the difference between the relative intensity ratios in the NMR peak of the vinyl methylene carbon for MDP obtained before and after the reaction by the relative intensity ratio obtained before the reaction.
3. The amount of MDP reacted with calcium through demineralization of enamel or dentin was determined by multiplying the reduction in the NMR peak of the vinyl methylene carbon of MDP to quantitative amount of MDP utilized in 1.000 g of each adhesive.

*: $p<0.05$, **: $p<0.01$
5. Adhesive Remnant Index (ARI)
After adhesion test, fifteen enamel and dentin samples were dehydrated by 50, 60, 70, 80, 90, and 100-vol% ethanol aqueous solutions, immersed in tertiary-butyl alcohol, and then freeze-dried under vacuum (FDU-1200, EYELA, Japan). Samples were then mounted onto aluminum stubs, and sputter-coated with a platinum-palladium alloy. In order to classify the fracture mode into the four categories of the Adhesive Remnant Index (ARI)\(^{25}\), the scanning electron microscope (SEM, S-2150, Hitachi, Japan) observations of each specimen were examined at numerous magnifications at 15 kV.

The four categories of the ARI were as follows: ARI=0: no adhesive remained on the enamel or dentin surface; ARI=1: less than half of the adhesive remained on the enamel or dentin surface; ARI=2: more than half of the adhesive remained on the enamel or dentin surface; ARI=3: all of the adhesive remained on the enamel or dentin surface. There, we noted the number of specimens which had exhibited an enamel crack or fracture in the ARI score 2 in the parentheses.

6. Statistical analysis
To understand the demineralization aspect of HAp, enamel and dentin, the regression line between the amount of MDP utilized and the amount of MDP-Ca salt produced was determined using a least-square method. The regression slopes were compared between the HAp and enamel and the enamel and dentin by Student’s \(t\)-test.

Further, the production amount of MDP-Ca salt and the bond strengths obtained from each EX and EX1 adhesive were analyzed by one-way analysis of variance (ANOVA) and Turkey multiple comparison tests. The level of statistical significance was set at 0.05.

**RESULTS**

_Determination of production amount of MDP-Ca salt by \(^{13}\)C NMR technique_

The \(^{13}\)C NMR spectra of the vinyl methylene region of the EX1 before and after the reaction with HAp, enamel or dentin are shown in Fig. 1. Based on our previous papers, the NMR peaks “a”, “b”, and “c” were assigned to the vinyl methylene carbon for UDMA, TEGDMA, and MDP, respectively.

The relative intensity of the NMR peak “c” of the vinyl methylene carbon of MDP to the NMR peak “a” to that of UDMA decreased after the reaction with the HAp, bovine enamel or bovine dentin. However, degree of deterioration in the relative intensity of the NMR peak “c” differed among substrates reacted.

The reductions in the relative intensity ratio of the NMR peak “c” to that of the NMR “a” were determined. The amounts of MDP-Ca salt produced through demineralization of HAp, bovine enamel or bovine dentin were calculated for each experimental adhesive (Table 2).

![Spectrum A](image1)

![Spectrum B](image2)

![Spectrum C](image3)

![Spectrum D](image4)

**Etching efficacy and bonding performance of EX adhesives**

1. Effects of amount of water in EX adhesives on the production amount of MDP-Ca salt

Figure 2 shows the effect of amount of water utilized in EX adhesive on the amounts of MDP-Ca salt produced through demineralization of HAp, bovine enamel and bovine dentin.

When the amount of water in the EX adhesive was increased from 98.4 to 294.4 mg/g, the pH value of EX adhesives increased from 1.91 to 2.28. However, the...
production amounts of MDP-Ca salt of HAp or bovine enamel increased from 4.7 to 13.9 mg \((p<0.001)\) and from 60.6 to 71.6 mg \((p=0.035)\), respectively. The bovine enamel produced greater amounts of MDP-Ca salt than the HAp.

In contrast, the bovine dentin exhibited different demineralization aspect from the bovine enamel. The production amounts of MDP-Ca salt ranged at 75 mg, although the amount of water was increased.

2. Effects of production amount of MDP-Ca salt on the bonding performance of EX adhesives

The effect of the production amount of MDP-Ca salt on the bovine enamel and dentin bonding performance of EX adhesives and the ARI scores as well as the SEM views of typical fractured enamel and dentin surfaces are shown in Figs. 3 and 4, respectively.

The enamel bond strength slightly increased from 19.8 to 20.6 MPa with increases in the production amount of MDP-Ca salt (Fig. 3). The number of specimens where the ARI score was 2 increased from 6 to 11 (Fig. 4). When the amount of water was increased, the scratches, developed during a grinding with carbide papers, became broadened.

In contrast, the dentin bond strength decreased from 12.4 to 10.9 MPa \((p=0.062)\), although the production amounts of MDP-Ca salt were the same. The number of specimen where the ARI score was 2 decreased from 8 to 5. The dentinal tubes were opened and the residual resin tags were not commonly observed inside the dentinal tubes.

Etching efficacy and bonding performance of EX1 adhesives

1. Effects of amount of water in EX1 adhesives on the amount of MDP-Ca salt produced

Figure 5 shows the effect of amount of water utilized in EX1 adhesive on the amounts of MDP-Ca salt produced through demineralization of bovine enamel and dentin.

The production amount of MDP-Ca salt of bovine enamel or dentin drastically decreased with decreases in the amount of water from 98.4 to 90.2 mg/g and to 94.8 mg/g, although addition of 4-META or HEMA in the EX1 adhesive decreased the pH value from 1.91 to 1.49 and to 1.48, respectively. The regression slope obtained between the amount of water utilized and the production amount of MDP-Ca salt of bovine enamel or dentin differed between the EX1 adhesives (the regression line was obtained from the EX1, EX1-H and
Fig. 4  ARI scores [0/1/2(#)/3] and typical SEM views of the fractured enamel and dentin surfaces of EX adhesives. There, we noted the number of specimen which exhibited the enamel crack or fracture in ARI score 2 in the parentheses. Upper SEM views show the enamel surfaces, and lower SEM views show the dentin surfaces. The first column of SEM views shows the grounded enamel and dentin surfaces, the second column of SEM views shows the fractured enamel and dentin surface for the EX1 adhesive, the third column of SEM views shows the fractured enamel and dentin surface for the EX2 adhesive, and the fourth column of SEM views shows the fractured enamel and dentin surface for the EX3 adhesive.

In the fractured enamel surface, the application of experimental adhesives allows for a broadening of scratches compared with the grounded enamel surface, since the hardened adhesive remains on the surface. When the amount of water is increased to 294.4 mg/g, the scratches are unable to see on the enamel surface, because the fracture occurs within adhesive layer. The number of specimens exhibiting the enamel crack increased with increases in the amount of MDP utilized. Furthermore, the change in the ARI score from 1 to 2 caused a development of the enamel crack (white arrow).

Similar to the enamel surface, remarkable scratches are observed on the grounded dentin surface and most of dentinal tubes are filled with the smear plug. When the amount of water is increased, smear plugs are removed and most of dentinal tubes are opened. The resin tags are not commonly observed inside the dentinal tubes.

EX1-M adhesives) and the EX adhesives (the regression line was obtained from the EX1, EX2 and EX3 adhesives) ($p=0.012$ for the enamel and $p=0.002$ for the dentin).

2. Effects of production amount of MDP-Ca salt on the bonding performance of EX1 adhesives

The effect of the production amount of MDP-Ca salt on the bovine enamel and dentin bonding performance of EX1 adhesives and the ARI scores as well as the SEM views of typical fractured enamel and dentin surfaces are shown in Figs. 6 and 7, respectively.

As shown in Fig. 6, addition of 4-META in the EX1 adhesive (EX1-M adhesive) resulted in increases in the enamel bond strength by 0.8 MPa and dentin bond strength by 2.6 MPa ($p=0.006$), reflecting increases in the number of specimens where the ARI score was 2 from 6 to 13 for the enamel and from 8 to 12 for the dentin (Fig. 7).

In contrast, when the EX1-H adhesive was applied, any enhancement in the enamel and dentin bond strength and any change in the ARI score were not observed. The mean values of enamel and dentin bond strengths and the ARI scores of the EX1-H adhesive were close to those obtained from the EX1 adhesive, respectively.

DISCUSSION

The pH value of the EX adhesive increased with increases in the amount of water in the EX adhesive, due to decreases in the apparent amount of MDP dissolved in water. However, the production amount of MDP-Ca salt of the HAp and bovine enamel increased by increasing the amount of additional water. This finding was due to increases in the amount of water resulting in the progress of dissociation of the phosphoric acid in the MDP. However, the production amounts of MDP-Ca salt of the HAp were below one-fifths of the bovine enamel. The observed differences in the production amount of MDP-Ca salt were due to the crystallinity of the HAp being higher than that of the bovine enamel$^{7,13}$. In contrast, the production amounts of MDP-Ca salt of the bovine dentin were the same at 75 mg/g, although the amount of water was increased. This was due to a
limitation to the amount of MDP that could capture the calcium ion produced through demineralization of dentin as reported by Yoshida et al.\textsuperscript{10}. Therefore, the null hypothesis that the amount of water in the EX adhesives has no effect on the amount of MDP-Ca salt through demineralization of bovine enamel only was rejected. The observed different amount of MDP-Ca salt produced by the enamel and the dentin was attributed to the crystallinity of the bovine enamel being higher than that of the bovine dentin\textsuperscript{7,13}).

When the amount of water in EX adhesive was increased, the enamel bond strength slightly increased from 19.8 to 20.6 MPa, due to the increases in the production amount of MDP-Ca salt of enamel from 60.6 to 71.6 mg/g. However, the increment in the enamel bond strength was only 0.8 MPa. This was due to the EX adhesives being unable to completely create a micro-mechanical inter-locking with enamel surface enough to cause a cohesive failure of adhesive or enamel, as same as the etch-rinse adhesive\textsuperscript{26}). In fact, we observed a mixed failure consisting of ARI score 1 and 2 in all of enamel specimens, since the etching potential of EX adhesives were lower than the etch-rinse adhesive. The amount of the residual adhesive after bracket debonding is frequently assessed in a qualitative manner, utilizing the ARI. Our results indicate that qualitative visual scoring using the ARI is capable of generating similar results with those assessed by quantitative image analysis technique. In contrast, increases in the amount of water in EX adhesive decreased the dentin bond strength, although the production amounts of MDP-Ca salt were the same (approximately 75 mg/g). This was possible since most of MDP in the EX adhesives were consumed by the production of MDP-Ca salt, and the dentin adhesion-promoting monomer besides MDP was not involved in the EX adhesives. Therefore, the null hypothesis that the production amount of MDP-Ca salt has no effect on the bonding performance of enamel and dentin was accepted. The observed different effect of the production amount of MDP-Ca salt on the enamel and dentin bond strengths was due to a principal adhesion mechanism of the adhesive differing between the enamel

Fig. 5 Effect of the amount of water in the EX1 adhesive on the amount of MDP-Ca salt produced through demineralization of enamel or dentin. Circles show the production amount of MDP-Ca salt of enamel, and squares show the production amount of MDP-Ca salt of dentin. White circle and square: the EX1 adhesive; gray circle and square: the EX1-H adhesive, and black circle and square: the EX1-M adhesive. The error bar shows the SD. Asterisks (*) indicate a significant difference (\(p<0.05\)). Asterisks (**) indicate a significant difference (\(p<0.01\)).

Fig. 6 Effect of the production amount of MDP-Ca salt on the enamel and dentin bond strength of the EX1 adhesives. Circles show the enamel bond strength and squares show the dentin bond strength. White circle and square: the EX1 adhesive; Gray circle and square: the EX1-H adhesive and black circle and square: the EX1-M adhesive. The error bar shows the SD. Asterisks (**) indicate a significant difference (\(p<0.01\)).
and the dentin\textsuperscript{27}.

Addition of HEMA or 4-META, as a dentin adhesion-promoting monomer, in the EX1 adhesive resulted in decreases in the production amounts of MDP-Ca salt of the enamel and dentin, although the pH value of EX1 adhesive decreased. Therefore, the null hypothesis that the addition of 4-META has no effect on the amount of MDP-Ca salt was rejected. The observed decreases in the production amount of MDP-Ca salt were probably due to the decreases in the amount of MDP and water in the EX1 adhesives. The regression slope obtained between the amount of water and the production amount of MDP-Ca salt of enamel or dentin differed between EX adhesives and EX1 adhesives. In a previous report, the 4-META is not involved in the demineralization of enamel or dentin\textsuperscript{28}.

However, specific decreases in the enamel and dentin bond strengths, caused by decreases in the production amounts of MDP-Ca salt of enamel and dentin, were not observed. Addition of 4-META in the EX1 adhesive (EX1-M adhesive) enhanced the enamel and dentin bond strength and increased the number of specimens exhibiting the ARI score=2 more effectively than the EX1-H adhesive. This may be due to the carboxylic acid in the 4-META promoting the bonding of the adhesive to the enamel and the dentinal collagen exposed by MDP through the chemical interaction more effectively than the HEMA. Therefore, the null hypothesis that the addition of 4-META has no effect on the bonding performance of enamel and dentin was rejected.

CONCLUSION

In spite of the limitations with the present investigation, the following conclusion was established.

The increase in the amount of water creates different effects on bond strength between the enamel and dentin. The 4-META enhances the enamel and dentin bond strengths of MDP-based one-step self-etch adhesive more effectively than the HEMA.

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