INTRODUCTION

To simplify application procedures and reduce technical errors, two-step and one-step self-etch adhesives (all-in-one) have been developed. These adhesives are widely accepted by dentists since they show a reliable clinical performance1-4). This is due to the acidic monomers that are utilized in self-etch adhesives playing a key role in their enamel and dentin bonding performance5-15).

Generally, self-etch adhesives can be classified into four categories: “strong” (pH<1), “intermediately strong” (pH≈1.5), “mild” (pH≈2) and “ultra-mild” (pH≥2.5)5).

It is well understood that the ability to solubilize the smear layer and the depth of the underlying demineralized dentin are strongly dependent on the pH value of the self-etch adhesive used16,17). This is due to the smear layer causing the early neutralization of the acidic monomer utilized in self-etch adhesives18-21), since the smear layer consists of the debris of the shattered and cracked dentin apatite and the dentinal collagen fibers22).

All-in-one are generally diluted with a solvent, such as an ethanol or acetone, to prevent the phase separation of water from all-in-one adhesive. However, there is no study to quantitatively investigate the effect of the concentration of water in a solvent aqueous solution used as a diluent for all-in-one adhesives on the etching efficacy of the acidic monomer, although the solvent limits the ionization of the acidic group in the acidic monomer in contrast to water.

Recently, Teshima23) in our group reported the effects of the amount of water added in the experimental 10-methacryloyloxydecyl dihydrogen phosphate (MDP)-based all-in-one (EX) adhesives containing different amounts of water (46.6, 93.2 and 208.1 mg/g). The enamel and dentin reactants of EX adhesives were analyzed using solid-state phosphorus-31 nuclear magnetic resonance and X-ray diffraction. Increased amount of water led to increases in the efficacy by which MDP demineralizes the enamel and dentin surfaces. However, the rate of calcium salts of MDP produced slowed down at the water concentrations above 93.2 mg/g. The dentin yielded greater amounts of di-calcium salts of the MDP monomer and dimer than the enamel, which develops a different type of layered structure of MDP from the enamel.

Keywords: All-in-one adhesive, MDP, Demineralization efficacy of MDP, MDP-Ca salt, 31P NMR
MATERIALS AND METHODS

All chemical reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan), unless otherwise indicated.

Preparation of EX adhesives

We designed three types of EX adhesives containing different amounts of water as the same as the previous study\(^2\). In brief, 6.0 g MDP (purity=97.0%) was mixed with the base monomer, which consists of 10.0 g urethane dimethacrylate (Negamikogyo, Ishikawa, Japan), 10.0 g triethylene glycol dimethacrylate (Shin-Nakamura Chemical, Wakayama, Japan) and 9.4 g 4-methacryloyloxyethyl trimellitic anhydride (purity=97.0%). One mass% of camphorquinone and dimethylamino benzoic acid ethyl ester, and 2,000 ppm hydroquinone monomethyl ether were then dissolved in the mixed monomer, respectively. Colloidal silica (4.26 g, R-972, Nihon Aerosil, Tokyo, Japan) and 10.0 g triethylene glycol dimethacrylate (Negamikogyo, Ishikawa, Japan) were suspended in each EX adhesive (1.000 g), mixed with the base monomer, which consists of 10.0 g urethane dimethacrylate (Negamikogyo, Ishikawa, Japan), 10.0 g triethylene glycol dimethacrylate (Shin-Nakamura Chemical, Wakayama, Japan) and 9.4 g 4-methacryloyloxyethyl trimellitic anhydride (purity=97.0%). One mass% of camphorquinone and dimethylamino benzoic acid ethyl ester, and 2,000 ppm hydroquinone monomethyl ether were then dissolved in the mixed monomer, respectively. Colloidal silica (4.26 g, R-972, Nihon Aerosil, Tokyo, Japan) was then added to the 35.4 g mixed monomer.

A series of three types of EX adhesives was then prepared by diluting 39.66 g of the filled resin with 80.5 g acetone aqueous solution with different mass ratios of water to acetone (water/acetone [g/g]=5.6/74.9, 11.2/69.3 and 25.0/55.5). The quantities of water included in the EX adhesive were 46.6, 93.2 and 208.1 mg/g, but the quantity of MDP utilized in the EX adhesives was kept a constant at 49.9 mg/g. The pH values of the corresponding EX adhesives were 0.87, 1.42 and 1.68, respectively.

Preparation of enamel and dentin reactants of EX adhesives

In accordance with previous studies\(^2\), 0.200 g of enamel or dentin particles that had been prepared by cutting bovine crown teeth (2–2.5 years old) using an air turbine with a fine diamond bur (#105R, Shofu, Kyoto, Japan) were suspended in each EX adhesive (1.000 g), and the suspensions were then mixed with 80.5 g acetone aqueous solution with different mass ratios of water to acetone (water/acetone [g/g]=5.6/74.9, 11.2/69.3 and 25.0/55.5). The quantities of water included in the EX adhesive were 46.6, 93.2 and 208.1 mg/g, the quantity of MDP in the EX adhesives was kept a constant at 49.9 mg/g. The pH values of the corresponding EX adhesives were 0.87, 1.42 and 1.68, respectively.

Observation of \(^{31}\text{P} NMR spectra

The \(^{31}\text{P} NMR spectra of enamel and dentin reactants of each EX adhesive were observed using an NMR spectrometer (EX-270, JEOL, Tokyo, Japan). The contact, repetition and accumulation times were 2,000 μsec, 20.05 s and 120 times, respectively. Ammonium dihydrogen phosphate was used as an external reference and \(^{31}\text{P} NMR chemical shifts are expressed in ppm.

The curve-fitting analyses of the corresponding \(^{31}\text{P} NMR spectra were performed by assuming that DCPD with an amorphous phase was also produced along with several types of MDP-Ca salts as described previously\(^2\). To analyze the \(^{31}\text{P} NMR spectral patterns, we used OriginPro® 9.1 Data Analysis and Graphing Software (OriginLab, Northampton, MA, USA). The intensity of each simulated peak used for the curve-fitting analyses of enamel and dentin reactants was then determined for each experimental group.

Determination of amounts of MDP-Ca salts and DCPD with an amorphous phase developed by demineralization of enamel or dentin

The peak intensity of MDP that had been consumed yielding several types of MDP-Ca salts was determined by totaling the relative intensity ratios of the simulated peaks for each MDP-Ca salt in each experimental group. The amount of MDP consumed was then determined by assuming that the peak intensity for MDP-Ca salts was 2.641 when 116.1 mg MDP employed in 1.0000 g of EX adhesive had completely yielded several types of MDP-Ca salts in accordance to previous studies\(^2\). The amount of MDP that had been consumed yielding several types of MDP-Ca salts was then determined as the amount of MDP-Ca salts produced.

On the other hand, the peak intensity of DCPD with an amorphous phase was determined as the relative intensity ratio of the simulated peak for DCPD. The amount of DCPD with an amorphous phase produced was calculated by assuming that the peak intensity for MDP-Ca salts was 2.641 when 116.1 mg MDP employed in 1.0000 g of EX adhesive had completely yielded a mono-calcium salt of the MDP monomer\(^2\).

Observation of XRD patterns

The XRD patterns of enamel and dentin reactants of each EX adhesive were performed using an X-ray diffractometer (RINT2000, Rigaku, Tokyo, Japan). The experimental conditions were as follows: 50 kV accelerating voltage, 300 mA current, CuK\(_\alpha\) wavelength of 0.1542 nm, 1.0°/min scan speed, 1/2° divergence slit, 0.1542 nm, 1.0°/min scan speed, 1/2° divergence slit, 0.73 mm scattering slit, 0.3 mm receiving slit, 0.02° step width, 20.0 rpm rotation rate, 1.6–70° scanning range (20 degree) and a graphite crystal monochromator.

Statistical analysis

Significant differences were analyzed by one-way analysis of variance and Tukey-Kramer post-hoc test, and by standard t-test with Bonferroni correction. The level of statistical significance was set at 0.05.

RESULTS

\(^{31}\text{P} NMR analyses of enamel and dentin reactants of EX adhesives and curve-fitting analysis results of the corresponding \(^{31}\text{P} NMR spectra

Figure 1 shows typical \(^{31}\text{P} NMR spectra of enamel (1a) and dentin (1b) reactants of EX adhesives containing different amounts of water. Based on previous studies\(^2\), each \(^{31}\text{P} NMR peak detected in both spectra was assigned to the phosphorous atoms for several types of MDP-Ca salts.
Typical $^{31}$P NMR spectra of enamel (a) and dentin (b) reactants of EX adhesives containing different amounts of water.

From top to bottom in each panel, the amounts of water added in the EX adhesive were 46.6, 93.2 and 208.1 mg/g. The NMR peak “α” was assigned to the phosphorus atoms for the enamel apatite, the NMR peak “β” was assigned to the phosphorus atoms for the dentin apatite, the NMR peak “1” was assigned to the phosphorus atom with two ionized hydroxy groups for the di-calcium salt of the MDP monomer (DCS-MM), the NMR peak “3” was assigned to the two phosphorus atoms with two ionized hydroxy groups for the di-calcium salt of the MDP dimer (DCS-MD), the NMR peak “5” was assigned to the phosphorus atom with one ionized hydroxy group for the mono-calcium salt of the MDP monomer (MCS-MM), and the NMR peak “6” was assigned to the two phosphorus atoms with one ionized hydroxy group for the mono-calcium salt of the MDP dimer (MCS-MD).

The curve-fitting analysis results of the corresponding $^{31}$P NMR spectra of enamel (a) and dentin (b) reactants of EX adhesives containing different amounts of water.

From top to bottom in each panel, the amounts of water added in the EX adhesive were 46.6, 93.2 and 208.1 mg/g. The black line in each panel represents the observed $^{31}$P NMR spectra of enamel or dentin reactants, the green line shows the simulated peak “α” or “β” used for the curve-fitting of enamel or dentin apatite respectively, the sky-blue lines show the simulated peaks “1”, “3”, “5” and “6” used for the curve-fitting of several types of MDP-Ca salts, the pink line shows the simulated peak “γ” used for the curve-fitting of DCPD with an amorphous phase, the red line shows the synthetic spectrum obtained by the curve-fitting analysis of enamel or dentin reactants and the blue line was obtained after subtracting the synthetic spectrum from the original $^{31}$P NMR spectrum of the respective enamel or dentin reactant.

As shown in Fig. 1a, the enamel developed a broad NMR peak, which consisted of the NMR peak “5” assigned to the mono-calcium salt of the MDP monomer (MCS-MM) and NMR peak “6” assigned to the mono-calcium salt of the MDP dimer (MCS-MD). The intensity of this broad NMR peak increased with increasing the amount of water added in the EX adhesive.

On the other hand, the dentin, as shown in Fig. 1b, developed NMR peak “1” assigned to the di-calcium salt of the MDP monomer (DCS-MM) and NMR peak “3” assigned to the di-calcium salt of the MDP dimer (DCS-MD) along with the broad NMR peak, consisting of NMR peaks “5” and “6”. The intensities of NMR peaks “1” and “3” increased with increasing the amount of water added, but in contrast, that of the broad peak decreased.

Based on the NMR analysis results, the curve-fitting analyses of the corresponding $^{31}$P NMR spectra are performed as shown in Fig. 2.

The $^{31}$P NMR spectra of enamel (2a) and dentin (2b) reactants were successfully curve-fitted by adding the simulated peak “γ” for DCPD with an amorphous phase to the simulated peak “1” for DCS-MM, the simulated peak “3” for DCS-MD, the simulated peak “5” for MCS-MM, the simulated peak “6” for MCS-MD and the simulated peak “α” for enamel apatite or the simulated peak “β” for dentin apatite respectively. This was due to the synthetic spectrum (red line) obtained by the curve-fitting analysis being most likely overlapped with the observed $^{31}$P NMR spectrum (black line) of each enamel.
Table 1  Chemical structure of several types of MDP-Ca salts produced during the 30-s application of EX adhesives to the enamel and dentin samples

|                      | Monomer Structure                                                                 |
|----------------------|----------------------------------------------------------------------------------|
| Di-calcium salt of   | \[\text{CH}_2\text{O}_2\text{P}_2\text{O}_2\text{Ca}_2\text{OH} \]              |
| the MDP monomer      | \( \text{O-Ca-OH} \)                                                             |
| (DCS-MM)             |                                                                                  |
| Di-calcium salt of   | \[\text{CH}_2\text{O}_2\text{P}_2\text{O}_2\text{Ca}_2\text{O} \]              |
| the MDP dimer        | \( \text{O-Ca-O} \)                                                             |
| (DCS-MD)             | \( \text{P-O-CH}_2 \)                                                            |
| Mono-calcium salt of | \[\text{CH}_2\text{O}_2\text{P}_2\text{O}_2\text{Ca}_2\text{OH} \]              |
| the MDP monomer      | \( \text{OH} \)                                                                  |
| (MCS-MM)             |                                                                                  |
| Mono-calcium salts   | \[\text{CH}_2\text{O}_2\text{P}_2\text{O}_2\text{Ca}_2\text{O} \]              |
| of the MDP dimer     | \( \text{P-O-CH}_2 \)                                                            |
| (MCS-MD)             | \( \text{OH} \)                                                                  |

Table 2  Relative intensity ratios of each simulated peak “1”, “3”, “5”, “6” or “\( \gamma \)” to the simulated peak “\( \alpha \)” or “\( \beta \)” by varying the amount of water in the EX adhesive

| Amount of water | Enamel reactant residues | Dentin reactant residues |
|-----------------|--------------------------|--------------------------|
|                 | 46.6 mg/g | 93.2 mg/g | 208.1 mg/g | 46.6 mg/g | 93.2 mg/g | 208.1 mg/g |
| Peak “1”        | 0       | 0         | 0.008 (0.005) | 0 | 0.046 (0.021) | 0.134 (0.010) |
| Peak “3”        | 0       | 0         | 0.055 (0.014) | 0 | 0.086 (0.025) | 0.166 (0.026) | 0.319 (0.037) |
| Peak “5”        | 0.077 (0.021) | 0.180 (0.008) | 0.211 (0.035) | 0.061 (0.007) | 0.227 (0.064) | 0.158 (0.023) |
| Peak “6”        | 0.016 (0.008) | 0.167 (0.035) | 0.159 (0.029) | 0.023 (0.010) | 0.130 (0.028) | 0.014 (0.015) |
| Peak “\( \gamma \)”   | 0.184 (0.055) | 0.282 (0.032) | 0.244 (0.048) | 0.168 (0.037) | 0.128 (0.027) | 0.561 (0.037) |

a: quantity of water employed in 1 g of the EX adhesive.
Peak “1”: phosphorus atom with one ionized hydroxy group for DCS-MM; Peak “3”: two phosphorus atoms with two ionized hydroxy groups for DCS-MD; Peak “5”: phosphorus atom with one ionized hydroxy group for MCS-MM; Peak “6”: two phosphorus atoms with one ionized hydroxy group for MCS-MD; Peak “\( \gamma \)” : phosphorus atom for DCPD with an amorphous phase.
( ): Standard deviation

and dentin reactant.

The relative intensity ratio of each simulated peak “1”, “3”, “5”, “6” and “\( \gamma \)” to the simulated peak “\( \alpha \)” or the simulated peak “\( \beta \)” was determined in each experimental group and summarized in Table 2, respectively.

Effect of the concentration of water in the EX adhesive on the amounts of MDP-Ca salts and DCPD with an amorphous phase produced

Figure 3 shows the effects of the concentration of water in the EX adhesive on the amounts of MDP-Ca salts (3a) and DCPD with an amorphous phase (3b) produced during the 30-s application of EX adhesive to the enamel and dentin.

As shown in Fig. 3a, increasing the amount of water from 46.6 to 93.2 mg/g significantly increased the amount of MDP-Ca salts produced by the enamel or dentin from 4.1 to 15.2 mg and from 7.5 to 25.0 mg, respectively (\( p<0.05 \)). However, the enamel and dentin showed a knee-point in the rate of MDP-Ca salts produced. At the water concentrations above 93.2 mg/g, the production rate of MDP-Ca salts slowed down. The dentin produced a greater amount of MDP-Ca salts in each experimental group than the enamel (\( p<0.05 \)).

On the other hand, as shown in Fig. 3b, the amount of DCPD with an amorphous phase produced by the dentin tended to increase from 3.4 to 11.2 mg (\( p<0.05 \)) with increasing the amount of water from 93.2 to 208.1 mg/g, but in contrast, the amount of DCPD produced by the enamel ranged from 3.7 to 5.6 mg. At the water concentration 93.2 mg/g, the enamel produced a greater amount of DCPD with an amorphous phase than the dentin (\( p<0.05 \)). However, at the water concentration 208.1 mg/g, the dentin produced a greater amount of DCPD than the enamel (\( p<0.05 \)).
Fig. 3 Effect of the concentration of water on the amounts of MDP-Ca salts (a) and DCPD with an amorphous phase (b) produced during the 30-s application of EX adhesives to enamel and dentin particles. The number of samples in each experimental group was 3. The error bars show the SDs. The small characters (a and b) indicate a significant difference in the amounts of MDP-Ca salts and DCPD with an amorphous phase produced by the enamel, and the large character (A and B) indicate a significant difference in the amounts of MDP-Ca salts and DCPD with an amorphous produced by the dentin, respectively ($p<0.05$). The asterisks indicate a significant difference in the production amount of MDP-Ca salts or DCPD and DCPD with an amorphous between the enamel and dentin ($p<0.05$). In Fig. (a), the circles show the amounts of MDP-Ca salts produced by enamel, and the squares show the amounts of MDP-Ca salts produced by dentin. The amount of MDP that had been consumed yielding several types of MDP-Ca salts was determined by the following equation: $116.1 \text{ mg} \times \text{peak intensity for MDP-Ca salts determined in each experimental group} / 2.641$. The dashed lines show that 100% MDP in 1.0000 g of the EX adhesive was consumed yielding MDP-Ca salts. The difference between the 100% MDP in 1.0000 g of the EX adhesive and the amount of MDP-Ca salts produced indicates the amount of MDP remaining in the EX adhesive as an unreactant residue. In Fig. (b), the circles show the amounts of DCPD and DCPD with an amorphous produced by enamel, and the squares show the amounts of DCPD with an amorphous phase produced by dentin. The amount of DCPD with an amorphous phase produced was calculated by the following equation: $116.1 \text{ mg} \times \text{peak intensity for DCPD determined in each experimental group} / 2.641 \times 172.09 \text{ (molecular weight of DCPD)/378.25 (molecular weight of the mono-calcium salt of the MDP monomer)}$.

**Effect of the concentration of water in the EX adhesive on the amount of each MDP-Ca salt produced**

Figure 4 shows the effects of the concentration of water in the EX adhesive on the amount of each MDP-Ca salt produced by the demineralization of enamel and dentin. The amount of each MDP-Ca salt was determined by multiplying the amount of MDP-Ca salts to the ratio of each MDP-Ca salt produced in each experimental group (Fig. 5).

In the enamel reactants (Fig. 4a), the amount of MCS-MM produced significantly increased from 3.7 to 10.4 mg until the amount of water reached 93.2 mg/g ($p<0.05$), and then gradually increased to 12.3 mg. In contrast, the amount of MCS-MD produced levelled off at approximately 4.7 mg at the water concentrations above 93.2 mg/g. When the amount of water was increased to 208.1 mg/g, the enamel produced DCS-MM and DCS-MD, however, the amounts of DCS-MM and DCS-MD produced were only 0.5 and 1.6 mg, respectively.

In contrast to the enamel, the dentin produced DCS-MM and DCS-MD more preferentially than MCS-MM and MCS-MD (Fig. 4b). The amounts of DCS-MM and DCS-MD produced linearly increased from 0 to 8.2 mg and from 2.7 to 9.2 mg with the increased amounts of water added, respectively ($p<0.05$). In contrast, the amount of MCS-MM and MCS-MD produced decreased from 13.4 to 9.6 mg and from 3.9 to 0.5 mg with increasing the amount of water added from 93.2 to 208.1 mg, respectively ($p<0.05$). The dentin developed greater amounts of DCS-MM and DCS-MD in each experimental group than the enamel ($p<0.05$).

The XRD analysis confirmed the NMR analysis results on the amount of each MDP-Ca salt produced by the demineralization of the enamel and dentin as shown in Fig. 6.

The enamel reactants (Fig. 6a) show the “A”-labeled three characteristic XRD peaks ($2\theta=2.17\pm0.02^\circ$, $4.48\pm0.00^\circ$, $6.73\pm0.02^\circ$) assigned to the layered structure of MCS-MD. The intensities of “A”-labeled three characteristic XRD peaks levelled off at the water
concentrations above 93.2 mg/g.

In contrast, the dentin reactants (Fig. 6b) show the second and third characteristic XRD peaks ($2\theta = 4.48 \pm 0.01^\circ, 6.74 \pm 0.01^\circ$) of the “A”-labeled three characteristic XRD peaks for the layered structure of MCS-MD and the “B”-labeled three characteristic XRD peaks ($2\theta = 2.30 \pm 0.02^\circ, 4.89 \pm 0.01^\circ, 7.35 \pm 0.01^\circ$) assigned to the layered structure of DCS-MD.[20] The intensities
Fig. 6  Typical XRD patterns of enamel (a) and dentin (b) reactants of EX adhesives containing different amounts of water. From top to bottom in each panel, the amounts of water in the EX adhesive were 46.6, 93.2 and 208.1 mg/g. The “A”-labels indicate 3 characteristic XRD peaks assigned to the layered structure of the mono-calcium salt of the MDP dimer (MCS-MD), and the “B”-labels indicate 3 characteristic XRD peaks assigned to the layered structure of the di-calcium salt of the MDP dimer (DCS-MD). Furthermore, we combined the production amounts of MCS-MD and DCS-MD, the layered structure model of the respective molecular species, and their d-spacing values in this Figure, respectively.

DISCUSSION

In this study, to characterize the mechanism by which MDP demineralizes the enamel and dentin surfaces prepared by an air turbine with a diamond bur, we used $^{31}$P NMR analysis technique. This is because this technique gives us useful information on not only the molecular species of MDP-Ca salts but also the amount of each MDP-Ca salt and DCPD with an amorphous phase, which have been produced by the demineralization of the smear layer and underlying enamel or dentin surface$^{24-26}$. These analysis results are essential to understand the bonding mechanism of commercially available MDP-based all-in-one adhesives to both surfaces of the wall and floor of the cavity that have been prepared for direct resin composite restoration, since both cavity surfaces are also covered with the smear layer.

Increasing the amount of water added in the EX adhesive from 46.6 to 93.2 mg/g significantly increased the amount of MDP-Ca salts produced by the demineralization of both surfaces of the enamel or dentin particles. This is due to the decalcification and calcification of tooth apatite by MDP depending upon the chemical equilibrium, since the solubilizability of both ions of calcium and phosphate released by the demineralization of tooth apatite into the EX adhesive is limited by the water included in the EX adhesive.

However, the production rate of MDP-Ca salts slowed down at the water concentrations above 93.2 mg/g. The observed knee-point in the production rate of MDP-Ca salts may have contributed to a change in the calcium site of the ground enamel and dentin surfaces that MDP demineralizes from the smear layer to the underlying enamel or dentin surface, thus suggesting the solubilization of byproducts, MDP-Ca salts and DCPD with an amorphous phase produced by the demineralization of the enamel or dentin smear layer into the EX adhesive$^{23}$. Therefore, the hypothesis that the NMR technique is very useful to investigate the demineralization aspect of enamel and dentin surfaces prepared by an air turbine with a diamond bur was accepted. The observed slowing down in the production rate of MDP-Ca salts was due to the hydroxyapatite in the underlying enamel and dentin being less likely to be demineralized by MDP than that in the smear layer$^{18-21}$.

The curve-fitting analysis results clearly show that
the amount of each MDP-Ca salt produced by the enamel and dentin was strongly affected by the amount of water included in the EX adhesives, since the increased amounts of water led to increases in the amounts of di-calcium salts of the MDP monomer (DCS-MM) and dimer (DCS-MD) produced. This was probably due to not only decreases in the amount of MDP to dissolve in an aqueous solution in the EX adhesive but also increases in the concentration of calcium and phosphate ions released into an aqueous solution in the EX adhesive during the 30-s application of the EX adhesive to the enamel and dentin. In fact, Yokota and Nishiyama have previously reported that increasing the molar ratio of calcium ion to MDP from 0.5/1 to above 1/1 allows the production of DCS-MM and DCS-MD. Furthermore, the observed result that the dentin yielded greater amounts of DCS-MM and DCS-MD than the enamel in each experimental group was due to the dentin releasing greater amounts of both ions into the EX adhesive than the enamel, since the demineralization of tooth apatite is strongly affected by the crystal size and crystallinity of tooth apatite. These facts may, therefore, have contributed to an increase in the amount of DCPD with an amorphous phase produced by the dentin from 2.6 to 11.2 mg at the water concentration 208.1 mg/g.

The XRD analysis confirmed the curve-fitting analysis results, which showed that the enamel and dentin samples produced four types of the molecular species of MDP-Ca salts. However, no evidence on MCS-MM and DCS-MM that had formed a layered structure was detected in either XRD patterns, since the A"- and "B"-labeled characteristic XRD peaks detected were assigned to the MCS-MD and DCS-MD and the intensities of “A”- and “B”-labeled characteristic XRD peaks had completely contributed to the amounts of MCS-MD and DCS-MD produced by the enamel or dentin, respectively. These results suggest the molecular species of mono- and di-calcium salts of the MDP dimer may independently form a two-dimensionally layered structure on the x- and/or y-axis, respectively.

In addition, Teshima reported that increasing the amount of water added in the EX adhesive from 46.6 to above 93.2 mg/g triggered decreases in the dentin bond strengths before and after thermocycling by 2–3 MPa respectively, even the smear layer was completely removed from the ground dentin surface. This decreases in the dentin bond strength may have contributed to MDP-Ca salts produced by the demineralization of the smear layer of the ground dentin surface, since the calcium salt formation of MDP decreases the polymerizability of the vinyl group in MDP13,27. This was due to not only drastic increases in the amount of MDP-Ca salts solubilized into the EX adhesive from 7.5 to 25.0 mg (25.0/7.5=3.3 times) but also in the production ratio of MDP-Ca salts to DCPD with an amorphous phase from 2.2 times (MDP-Ca salts/DCPD=7.5/3.4) to 9.6 times (MDP-Ca salts/DCPD=25.0/2.6). Therefore, the hypothesis that the NMR spectroscopy is a powerful tool to determine the dentin bonding performance was accepted.

CONCLUSION

The amount of MDP-Ca salts produced by the demineralization of the enamel and dentin surfaces increased with increasing the amount of water in the EX adhesive. The dentin yielded greater amounts of di-calcium salts of the MDP monomer (DCS-MM) and dimer (DCS-MD) than the enamel, which develops a different type of layered structure of MDP from the enamel. However, both enamel and dentin samples showed a knee-point in the production rate of MDP-Ca salts, which indicates a change in the calcium site of the both enamel and dentin surfaces that MDP demineralizes from the smear layer to the underlying enamel and dentin surfaces.

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