DENTAL MATERIALS XXX (2016) XXX–XXX

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Layering mechanism of MDP-Ca salt produced in demineralization of enamel and dentin apatite

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ARTICLE INFO

Article history:
Received 12 May 2016
Received in revised form
10 September 2016
Accepted 27 September 2016
Available online xxx

Keywords:
One-step adhesive
Decalcification
MDP-Ca salt
^{31}P NMR
X-ray diffraction

ABSTRACT

Objective. The 10-methacryloyloxydecyl dihydrogen phosphate (MDP) (EX adhesives)-based one-step self-etch adhesives have become widely utilized due to their simplified application procedures. The aim of this study was to determine the type of the molecular species of calcium salts of MDP (MDP-Ca salts) that form a layered structure and to understand the layering mechanism of MDP-Ca salts.

Methods. The EX adhesives were prepared by varying the amounts of MDP (25.6, 49.9, 80.5 and 116.1 mg) added in 1 g of the EX adhesive. Enamel and dentin reactant residues were obtained after the reaction of each EX adhesive to enamel or dentin particles for 30 s. The chemical analyses of both reactant residues were then performed.

Results. The molecular species of MDP-Ca salts that form a layered structure were determined as mono-calcium salt (MCS-MD) and di-calcium salts of the MDP dimer (DCS-MD). The dentin sample showed two types of characteristic XRD peaks assigned to the layer structure, since the dentin produced DCS-MD along with MCS-MD in contrast to the enamel sample. A mono-calcium salt of the MDP monomer (MCS-MM), a predominant molecular species, was not contributed to a layered-structure formation, since the intensities of characteristic XRD peaks are limited by the production of DCS-MD and MCS-MD.

Significance. The self-assembled layering of MCS-MD and DCS-MD is associated by a hydrophobic bond between two 10-methylene groups in MCS-MD and DCS-MD. The MCS-MD may form a more tightly-packed layered structure than DCS-MD by the hydrogen bonded interaction between hydroxy groups bonded to each phosphorous atom.

1. Introduction

Two- and one-step self-etch adhesives have been developed to simplify application procedures and reduce technique sensitivity. These self-etch adhesives have been widely accepted by dentists, since they show good bonding performance [1–6].

Studies have been performed to understand the adhesion mechanism of self-etch adhesives to the tooth through acidic monomers, such as 10-methacryloyloxydecyl dihydrogen phosphate (MDP), 2-methacryloyloxyethyl phenyl hydrogen phosphate (Phenyl-P) or 4-methacryloyloxyethyl trimellitic acid (4-MET) [7–12]. Yoshida et al. [7] reported that MDP yields a more chemically stable calcium salt than 4-MET and phenyl-P and bonds electrostatistically to hydroxyapatite. Fukegawa et al. [9] established that the MDP bonded to the hydroxyapatite surface is accompanied by the formation of an intermediary layer of MDP. It consists of two MDP molecules with their methacrylate groups directed toward each other and their phosphate groups directed away from each other

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http://dx.doi.org/10.1016/j.dental.2016.09.037

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and calcium salts were deposited between the layers of their phosphate groups. Furthermore, Yoshihara et al. [11] reported that the higher bonding performance of MDP-based self-etch adhesive is contributed to the formation of an intermediary layer of MDP on the hydroxyapatite and its thickness. However, these studies have lack on which type of the molecular species of MDP-Ca salts produced forms a layered structure and why the molecules of MDP-Ca salts form a layered structure.

To gain an insight on the type of the molecular species of MDP-Ca salts that had been produced during the application of the EX adhesive to enamel and dentin, the enamel and dentin reactant residues of the experimental one-step self-etch adhesives containing different amounts of MDP (EX adhesive) were analyzed using phosphorous-31 nuclear magnetic resonance (^{31}P NMR) and X-ray diffraction (XRD) techniques. The molecular species of MDP-Ca salt that form a layered structure was determined and the layering mechanism of MDP-Ca salts was then discussed. The null hypotheses tested were that: the type of molecular species of MDP-Ca salts (1) that has been produced, and (2) that form a layered structure, differs between enamel and dentin.

2. Materials and methods

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

2.1. Preparation of EX adhesives

A series of 4 types of EX adhesives was prepared by varying the amount of MDP added. In brief, the EX adhesives consisted of MDP (purity = 97.0%), a base monomer, catalysts, inhibitor, filler, acetone and water [13]. The base monomer was prepared by mixing 10.0 g urethane dimethacrylate (Negamikogyo, Ishikawa, Japan), 10.0 g triethylene glycol dimethacrylate (Shin-Nakamura Chemical Co., Wakayama, Japan) and 9.4 g 4-methacryloyloxyethyl trimellitic anhydride (Sun Medical, Shiga, Japan). The mixed monomer was then prepared by adding different amounts of MDP (3.0, 6.0, 10.0 or 15.0 g) as an acidic monomer to 29.4 g of the base monomer. Thereafter, 4.26 g of colloidal silica (R-972, Nihon Aerosil, Tokyo, Japan) were filled in each mixed monomer.

A series of 4 types of EX adhesives was then prepared after each resin past was diluted with an acetone aqueous solution, consisting of 69.3 g acetone and 11.2 g distilled water. The quantities of MDP in 1.0000 g of the EX adhesive were 25.6, 49.9, 80.5 and 116.1 mg. The pH values of these EX adhesives were 1.75, 1.59, 1.44 and 1.38, respectively.

2.2. Preparation of enamel and dentin particles

The pulp was removed from 2 to 2.5 years old bovine teeth. The bovine crown enamel and dentin was then cut by using an air turbine with a diamond point (#105R, Shofu, Kyoto, Japan) under a stream of cooling water, respectively. After being cut from the 100 bovine teeth, the enamel and dentin particles were obtained by decanting the cooling water collected and then rinsed 3 times with distilled and deionized water. After being dried at 20° C for 1 day, the crown enamel and dentin particles were stored in freezer at −80 °C.

2.3. Preparation of enamel and dentin reactant residues with the EX adhesive

Preparation of enamel and dentin reactants was described previously [14,15]. In brief, 0.200 g of the enamel and dentin particles were, after being thawed, suspended in each EX adhesive (1.000 g), and the suspensions were then vibrated for 30 s at 20 °C. After the reaction, 30 ml of ethanol (approximately 24 g) were added to each suspension to stop further reaction of MDP with the enamel and dentin. Each suspension was centrifuged at 3500 rpm for 20 min, and the supernatant was then decanted. Thereafter, each reactant residue was rinsed 3 times with 30 ml ethanol. The enamel and dentin reactant residues were then dried for 3 h at 20 °C. Enamel and dentin reactant residues were prepared 3 times.

2.4. Observation of solid-state ^{31}P NMR spectra

The ^{31}P NMR spectra of enamel and dentin reactant residues were observed using an NMR spectrometer (EX-270, JEOL, Tokyo, Japan). The contact, repetition and accumulation times were 2000 μs, 20.05 s and 120 times. The ^{31}P NMR chemical shifts are expressed in ppm, with 85% H_3PO_4 as an external reference.

The curve-fitting analyses of the corresponding ^{31}P NMR spectra were performed using OriginPro® 9.1 Data Analysis and Graphing Software (OriginLab Co., Northampton, MA, USA) in order to assume the DCPD is also produced along with several types of MDP-Ca salts as a byproduct as described previously [15]. The intensity of each simulated peak used for the curve-fitting analyses of the enamel and dentin reactant residues was then determined.

2.5. Determination of production amount of MDP-Ca salts

The peak intensity for MDP-Ca salts was determined by totaling the relative intensity ratio of the simulated peak assigned to each MDP-Ca salt in each experimental group. The amount of MDP that had been consumed yielding several types of MDP-Ca salts was determined by assuming that the peak intensity for MDP-Ca salts was 2.641 when the 116.1 mg MDP in 1.0000 g of the EX adhesive had completely yielded MDP-Ca salts as described previously [15].

2.6. Determination of production rate of MDP-Ca salts

The regression line between the amount of MDP added in the EX adhesive and the consumption amount of MDP for yielding MDP-Ca salts was determined using a least-square method. The slope of the regression line was determined as the production rate of MDP-Ca salts. The regression slopes were compared between the enamel and dentin by one-way ANOVA and Scheffé's multiple comparison tests. The level of statistical significance was set at 0.05.

2.7. Observation of XRD patterns

The XRD patterns of enamel and dentin reactant residues were recorded using an X-ray diffractometer (RINT2000,

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Rigaku Co., Tokyo, Japan). The experimental conditions were as follows: 50 kV accelerating voltage, 300 mA current, CuKα wavelength of 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 (2θ degree) and a graphite crystal monochromator.

2.8. Statistical analysis

The amount of MDP-Ca salts produced was analyzed by one-way analysis of variance and Tukey-Kramer post-hoc test and a standard t-test with Bonferroni correction. In addition, the amount of DCPD produced was also analyzed using the same procedure. The level of statistical significance was set at 0.05.

3. Results

3.1. ^{31}P NMR analyses of enamel and dentin reactant residues of EX adhesives and curve-fitting of corresponding ^{31}P NMR spectra

Fig. 1 shows the typical ^{31}P NMR spectra of enamel (a) and dentin (b) reactant residues of EX adhesives containing different amounts of MDP, respectively. In the ^{31}P NMR spectrum of each enamel or dentin reactant residue, the observed ^{31}P NMR spectrum is represented with the black line. The ^{31}P NMR peaks detected in both NMR spectra were assigned and listed in Table 1.

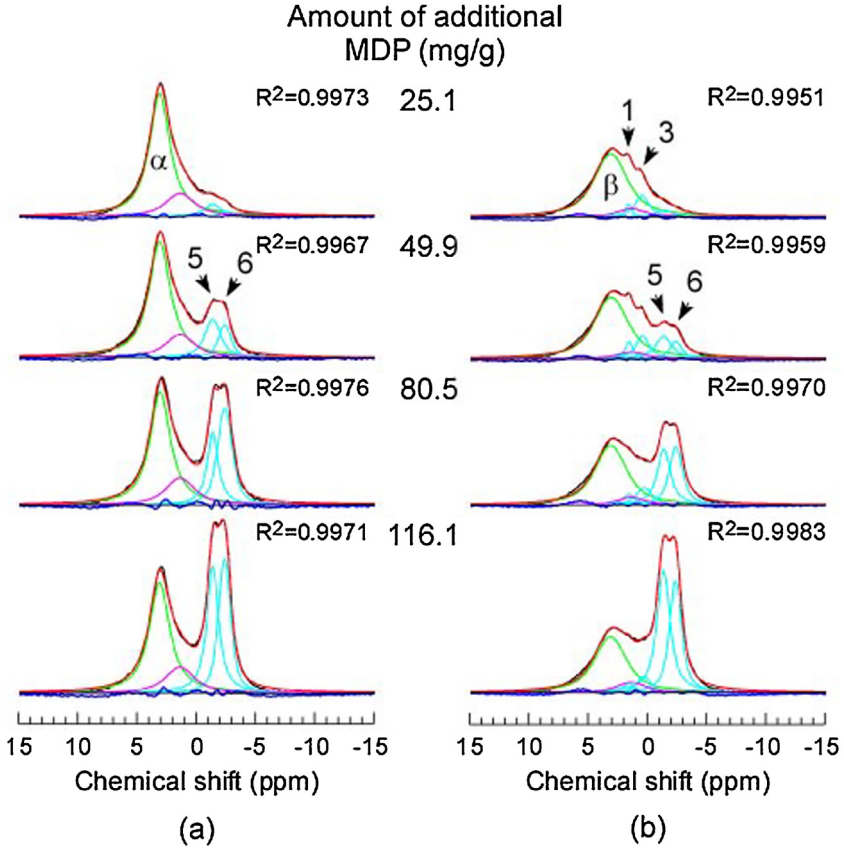

Fig. 1 – Typical ^{31}P NMR spectra of enamel (a) and dentin (b) reactant residues of EX adhesives containing different amounts of MDP and the curve-fitting analyses of the corresponding ^{31}P NMR spectra. From top to bottom in each panel, the amounts of MDP added in the EX adhesive were 25.6 mg, 49.9 mg, 80.5 mg and 116.1 mg.
The black line represents the observed ^{31}P NMR spectrum of enamel or dentin reactant, the green line shows the simulated peak used for the curve-fitting of enamel or dentin apatite, the sky-blue lines show the simulated peaks used for the curve-fitting of several types of MDP-Ca salts, the pink line shows the simulated peak 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 reactant and the blue line is obtained after subtracting the synthetic spectrum from the original ^{31}P NMR spectrum of the respective enamel or dentin reactant. The NMR peak "1" was assigned to the phosphorus atom with two ionized hydroxy group for a di-calcium salt of the MDP monomer (DCS-MM), NMR peak "3" was assigned to the two phosphorus atoms with two ionized hydroxy groups for a di-calcium salt of the MDP dimer (DCS-MD), NMR peak "5" was assigned to the phosphorus atom with one ionized hydroxy group for a mono-calcium salt of the MDP monomer (MCS-MM), and NMR peak "6" was assigned to the two phosphorus atoms with one ionized hydroxy group for a mono-calcium salt of the MDP dimer (MCS-MD). Furthermore, the NMR peaks "α" and "β" were assigned to the phosphorus atoms for enamel apatite or dentin apatite, respectively. (For interpretation of the references to color in the text, the reader is referred to the web version of this article.)

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The ^{31}P NMR spectra of enamel reactant residues showed a broad NMR peak (Fig. 1a). It consists of NMR peak "5" (−1.46 ppm) assigned to the phosphorous atom for a mono-calcium salt of the MDP monomer (MCS-MM) and NMR peak "6" (−2.22 ppm) assigned to the two phosphorous atoms for a mono-calcium salt of the MDP dimmer (MCS-MD). The intensity of this broad NMR peak increased with an increase in the amount of MDP added.

In contrast, the ^{31}P NMR spectra of dentin reactant residues showed the NMR peak "1" (1.67 ppm) assigned to the phosphorous atom for a di-calcium salt of the MDP monomer (DCS-MM) (Fig. 1b). The NMR peak "3" (0.60 ppm) assigned to the two phosphorous atoms for a di-calcium salt of the MDP dimer (DCS-MD) along with a broad NMR peaks, "5" (−1.46 ppm) and "6" (−2.22 ppm), as shown in Fig. 1b. With an increase in the amount of MDP, the intensity of the broad NMR peak increased, in contrast to the NMR peaks "1" and "3".

Reflecting the NMR analysis results of each enamel and dentin reactant residue in order to assume the production of DCPD with an amorphous phase, the curve-fitting analyses of corresponding ^{31}P NMR spectra were performed as shown in Fig. 1. In the ^{31}P NMR spectrum of each enamel or dentin reactant residue, the green line and the sky-blue lines show the simulated peak used for the curve-fitting of enamel or dentin apatite, and several types of MDP-Ca salts, respectively. The pink line shows the simulated peak used for the curve-fitting of DCPD with an amorphous phase, and the red line shows the synthetic spectrum obtained by the curve-fitting analysis of enamel or dentin reactant. The blue line is obtained after subtracting the synthetic spectrum from the original ^{31}P NMR spectrum of the respective enamel or dentin reactant. The chemical shifts of simulated peaks used for the curve-fitting analyses are listed in Table 1.

The ^{31}P NMR spectra of enamel reactant residues were successfully curve-fitted by adding the simulated peak "γ_2" for the DCPD with the simulated peak "5" for the MCS-MM, simulated peak "6" for the MCS-MD and simulated peak "α" for the enamel apatite (Fig. 1a).

Similar to the enamel reactant residues, the ^{31}P NMR spectra of dentin reactant residues were also completely curve-fitted by adding the simulated peak "γ_2" for the DCPD with the simulated peak "1" for the DCS-MM, the simulated peak "3" for the DCS-MD and the simulated peak "5" for the MCS-MM, the simulated peak "6" for the MCS-MD, and the simulated peak "β" for the dentin apatite (Fig. 1b).

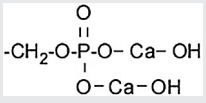
The relative intensity ratios of each simulated peak assigned to MDP-Ca salts and DCPD with an amorphous phase to the simulated peak assigned to enamel or dentin apatite were determined and summarized in Table 2.

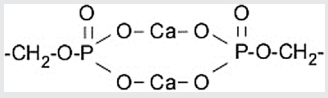
3.2. *Effect of the concentration of MDP in the EX adhesive on the production ratios of each MDP-Ca salt*

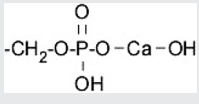
Fig. 2 shows the effect of the concentration of MDP on the production ratio of each MDP-Ca salt during the 30-s application of the EX adhesive to enamel (a) and dentin (b). The produc-

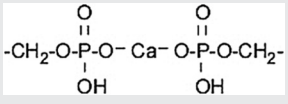
Table 1 – Chemical shifts of simulated peaks used for the curve-fitting analyses of enamel and dentin reactants with EX adhesive.

Simulated peak	Chemical shift of the NMR peak (ppm)		Molecular species
	Observed NMR	Curve-fitted NMR	
"α"	3.18	3.18	$Ca_{10}(PO_4)_6(OH)_2$ for enamel apatite
"β"	3.18	3.18	$Ca_{10}(PO_4)_6(OH)_2$ for dentin apatite
"1"	1.67	1.63	DCS-MM
"3"	0.60	0.50	DCS-MD (layered structure)
"5"	−1.46	−1.30	MCS-MM
"6"	−2.22	−2.32	MCS-MD (layered structure)
"γ_2"		1.45[a]	DCPD ($CaHPO_4{\cdot}2H_2O$)

"1": DCS-MM: dicalcium salt of the MDP monomer: 

"3": DCS-MD: dicalcium salts of the MDP dimer: 

"5": MCS-MM: monocalcium salt of the MDP monomer: 

"6": MCS-MD: monocalcium salts of the MDP dimer: 

"γ_2": DCPD with an amorphous phase: dicalcium phosphate dihydride.

[a] Referred from the previous paper (reference number in the manuscript: 17: Yokota Y and Nishiyama N, Dental Material Journal 2015;34:270–279).

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Table 2 – Relative intensity ratios of each simulated peak "1", "3", "5", "6" or "γ_2" to the intensity of the simulated peak "α" or "β" by varying the amount of MDP in EX adhesive.

Amount of MDP (mg)[a]	Enamel reactants			Dentin reactants				
	Peak "5"	Peak "6"	Peak "γ_2"	Peak "1"	Peak "3"	Peak "5"	Peak "6"	Peak "γ_2"
25.6	0.088 (0.022)	0.022 (0.011)	0.259 (0.022)	0.034 (0.003)	0.149 (0.006)	0.059 (0.011)	0.011 (0.009)	0.116 (0.003)
49.9	0.195 (0.035)	0.179 (0.033)	0.301 (0.041)	0.057 (0.007)	0.151 (0.014)	0.244 (0.020)	0.152 (0.014)	0.103 (0.005)
80.5	0.380 (0.028)	0.297 (0.011)	0.263 (0.013)	0.079 (0.010)	0.138 (0.010)	0.411 (0.042)	0.389 (0.030)	0.119 (0.007)
116.1	0.664 (0.022)	0.507 (0.105)	0.289 (0.028)	0.066 (0.006)	0.123 (0.009)	0.859 (0.081)	0.795 (0.111)	0.172 (0.003)

NMR peak "1": the phosphorus atom with two ionized hydroxy group for DCS-MM; NMR peak "3": the two phosphorus atoms with two ionized hydroxy groups for DCS-MD; NMR peak "5": the phosphorus atom with one ionized hydroxy group for MCS-MM; NMR peak "6": the two phosphorus atoms with one ionized hydroxy group for MCS-MD; NMR peak "γ_2": the phosphorus atom for DCPD with an amorphous phase.
(): standard deviation.
[a] The quantity of MDP employed in 1 g of the EX adhesive.

tion ratios of each MDP-Ca salt were determined, based on the relative intensity ratio of each simulated peak assigned to DCS-MM, DCS-MD, MCS-MM and MCS-MD to the simulated peak assigned to enamel or dentin apatite (Table 2).

As shown in Fig. 2a, with an increase in the amount of MDP added, the production ratio of MCS-MD, in the enamel, increased from 0.11 to 0.28, but in contrast, the production ratio of MCS-MM decreased from 0.89 to 0.72. The production ratio of MCS-MM and MCS-MD then leveled off at 0.72:0.28.

Similar to the enamel, the production ratio of each MDP-Ca salt was, in the dentin, strongly dependent upon the MDP concentration in the EX adhesive. An increase in the amount of MDP added led to increases in the production ratio of MCS-MM and MCS-MD, in contrast to the DCS-MM and DCS-MD, respectively (Fig. 2b). The production ratio of DCS-MM, DCS-MD, MCS-MM and MCS-MD altered from 0.20:0.43:0.34:0.03 to 0.05:0.04:0.62:0.29.

3.3. *Effect of the concentration of MDP in the EX adhesive on the production amounts of MDP-Ca salts and DCPD with an amorphous phase*

Fig. 3 shows the effect of the concentration of MDP in the EX adhesive on the production amount of MDP-Ca salts during the 30-s application of the EX adhesive to enamel (a) and dentin (b). The consumption amount of MDP for yielding several types of MDP-Ca salts was calculated using the following equation: 116.1 mg × the peak intensity for MDP-Ca salts determined in each experimental group/2.641 and determined as the production amount of MDP-Ca salts.

As shown in Fig. 3a and b, the production amount of MDP-Ca salts linearly increased from 4.8 to 51.5 mg for the enamel ($y = 0.7634x + -1.12$, $r^2 = 0.9810$) and from 11.1 to 81.0 mg for the dentin ($y = 0.5104x + -9.10$, $r^2 = 0.9939$) with an increase in the amount of MDP added, respectively. The dentin showed a greater production amount of MDP-Ca salts, thus showed

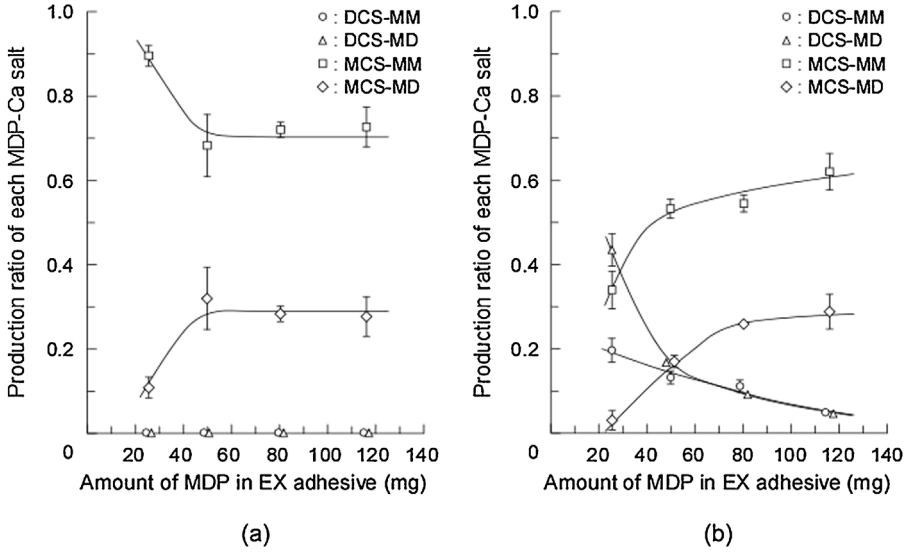

Fig. 2 – The effect of the concentration of MDP on the production ratio of each MDP-Ca salt during the 30-s application of EX adhesives to enamel (a) and dentin (b).
The circles show the production ratio of the di-calcium salt of the MDP monomer (DCS-MM); the triangles show the production ratio of the di-calcium salt of the MDP dimer (DCS-MD); the squares show the production ratio of the mono-calcium salt of the MDP monomer (MCS-MM); the diamonds show the production ratio of the mono-calcium salt of the MDP dimer (MCS-MD).

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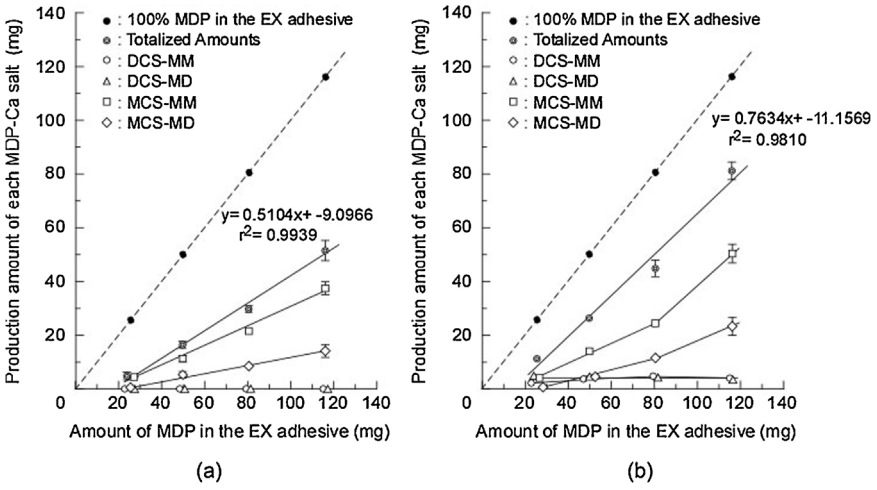

Fig. 3 – The effect of the concentration of MDP on the production amount of each MDP-Ca salt during the 30-s application of EX adhesives to enamel (a) and dentin (b).
The dabble circles show the totalized amounts of MDP-Ca salts produced; the white circles show the production amount of the di-calcium salt of the MDP monomer (DCS-MM); the triangles show the production amount of the di-calcium salt of the MDP dimer (DCS-MD); the squares show the production amount of the mono-calcium salt of the MDP monomer (MCS-MM); the diamonds show the production amount of the mono-calcium salt of the MDP dimer (MCS-MD); the black circles show the consumption amount of 100% of MDP in 1.0000 g of the EX adhesive. The difference between the consumption amount of 100% MDP in 1.0000 g of the EX adhesive and the consumption amount of MDP for yielding MDP-Ca salts indicates the amount of MDP remained in the EX adhesive as an unreactant residue.

a faster production rate of MDP-Ca salts than the enamel ($p < 0.05$).

Next, the production amount of each MDP-Ca salt was also determined by multiplying the peak intensity ratio of each MDP-Ca salt by the production amount of MDP-Ca salts determined in each experimental group.

As shown in Fig. 3a, the production amounts of MCS-MM and MCS-MD, in the enamel, increased from 4.3 to 37.3 mg and from 0.3 to 7.1 mg with an increase in the amount of MDP added in the EX adhesive, respectively. The predominant molecular species of MDP-Ca salt was an MCS-MM.

Similar to the enamel, the production amount of each MDP-Ca salt was, in the dentin, strongly affected by the amount of MDP added (Fig. 3b). With an increase in the amount of MDP, the production amounts of MCS-MM and MCS-MD increased from 3.8 mg to 50.2 mg and from 0.2 mg to 11.6 mg, respectively. However, the production amounts of DCS-MM and DCS-MD leveled off at 2.2–3.8 mg and at 1.8–2.4 mg, respectively.

Furthermore, the production amount of DCPD with an amorphous phase was determined by assuming that the peak intensity for MDP-Ca salts was 2.641 when the 116.1 mg MDP employed in 1.0000 g of the EX adhesive had completely yielded MCS-MM. The amount of DCPD produced was calculated using the following equation: 116.1 mg × the peak intensity for DCPD determined in each experimental group/2.641 × 172.09 (molecular weight of DCPD)/322.34 (molecular weight of MDP). There, the peak intensity for DCPD was determined as a relative intensity ratio of the simulated peak assigned to DCPD (Table 2). The dentin produced greater amounts of MCS-MD and DCS-MD than the enamel ($p < 0.05$).

As shown in Fig. 4, the production amount of DCPD with an amorphous phase leveled off at approximately 6.5 mg for enamel (4a) and at approximately 3.5 mg for dentin (4b). The enamel yielded a greater amount of DCPD in each EX adhesive than did the dentin ($p < 0.05$).

3.4. *XRD analyses of enamel and dentin reactant residues of EX adhesives*

Fig. 5 shows the typical XRD patterns of enamel (a) and dentin (b) reactant residues of EX adhesives containing different amounts of MDP, respectively. The XDP peaks detected in both XRD patterns were assigned as shown in Fig. 5.

The enamel reactant residues showed the “A”-labeled 6 characteristic XRD peaks assigned to the layered structure of MCS-MD (Fig. 5a). The intensities of these characteristic peaks increased with increasing the amount of MDP added.

On the other hand, the dentin reactant residues showed the “B”-labeled second and third XRD peaks of the 3 characteristic XRD peaks assigned to the layered structure of DCS-MD along with the 6 characteristic XRD peaks assigned to the layered structure of MCS-MD (Fig. 5b). With an increase in the amount of MDP, the intensities of the 6 characteristic peaks for the MCS-MD with a layered structure increased. In contrast, the intensity of the 2 characteristic peaks for the DCS-MD with a layered structure decreased. The dentin shows the 6 characteristic XRD peaks more intensely than the enamel.

4. Discussion

In this study, the bovine teeth were used to determine the amount of MDP-Ca salts produced by the demineralization of bovine crown enamel and dentin particles and the molecu-

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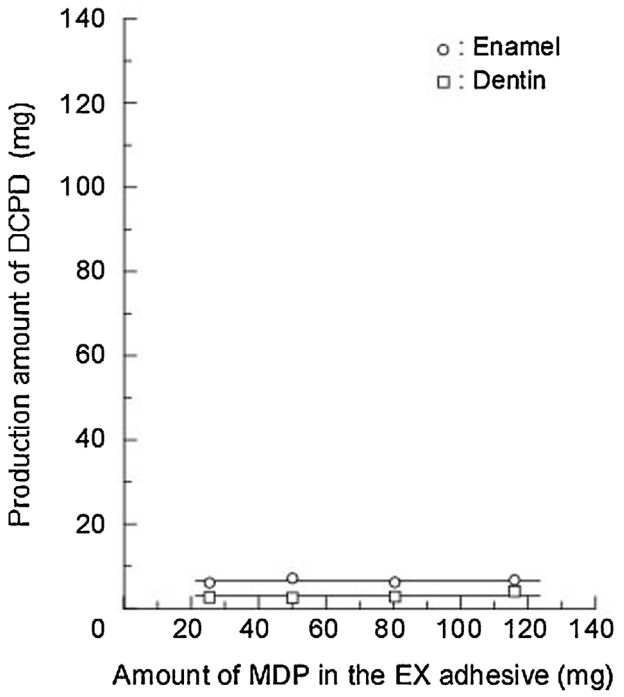

Fig. 4 – The effect of the concentration of MDP on the production amount of DCPD with an amorphous phase during the 30-s application of EX adhesives to enamel and dentin.
The circles show the production amounts of DCPD by the enamel; the squares show the production amounts of DCPD by the dentin.

lar species of the MDP-Ca salt that forms a layered structure. This is because the bovine teeth are easy to obtain in large quantities, in good condition and with a more uniform composition than that of human teeth [16], and the Ca/P ratio of the mineral removed from the enamel surfaces during demineralization, as well as the remineralization characteristics, were the same in both human and bovine enamel [17]. Since the bovine central permanent incisors attain full development at about 2 years, the bovine teeth at 2–2.5 years old were used in this study. Moreover, to reduce the individual difference of the bovine teeth, the enamel and dentin particles that have been prepared by cutting the crown enamel and dentin were homogeneously mixed during the rinsing procedure of these particles with water. It is conceivable to state that the analysis data benefited from the type of the molecular species of MDP-Ca salts.

NMR analyses of enamel and dentin reactant residues clearly suggest that the calcium salt formation of MDP and the calcification of calcium and phosphate ions occur in the EX adhesive solution. This was due to the calcium ions released into the EX adhesive solution yielding not only several types of MDP-Ca salts after the reaction with MDPs but also a DCPD with an amorphous phase after the reaction with phosphate ions, since the enamel and dentin released the calcium and phosphate ions into the EX adhesive solution through their demineralization. The observed faster production rate and greater production amount of MDP-Ca salts than DCPD was probably due to the MDP-Ca salts produced being immediately precipitated from the EX adhesive solution [13,18,19], in contrast to the DCPD with an amorphous phase.

The dentin showed a faster production rate and a greater amount of MDP-Ca salts than the enamel by 1.5 times (regulation slope = 0.7634/0.5104), although the mineral content of the dentin is lower than the enamel. The obtained result agreed with the previous study, which Iwai and Nishiyama had determined the production amount of MDP-Ca salt from the concentration change in MDP in the EX adhesive solution, before and after the reaction with enamel and dentin particles [13]. This was due to the demineralization rate of hydroxyapatite, thus the releasing rate of calcium and phosphate ions into the EX adhesive is strongly affected by the crystallinity and size of hydroxyapatite included in the dental substrate [20].

However, the type of the molecular species of MDP-Ca salts produced differed between the enamel and dentin. Thus, the null hypothesis that the type of molecular species of MDP-Ca salts that has been produced differs between enamel and dentin was accepted. Yokota and Nishiyama [14] recently reported that the type of the molecular species of MDP-Ca salts that had been synthesized was strongly affected by the molar ratio of calcium ion to MDP in an ethanol aqueous solution. An increase in the molar ratio of calcium ion to MDP from 0.5/1 to above 1/1 allowed the production of di-calcium salts of the MDP monomer and dimer along with mono-calcium salts of the MDP monomer and dimer. The observed dentin producing the di-calcium salts of the MDP monomer and dimer was probably due to the molar ratio of calcium ion to MDP reaching to above 1, when the EX adhesive containing 25.5 mg of MDP was applied. This was due to the dentin releasing greater amounts of both calcium and phosphate ions into the EX adhesive solution than the enamel. However, the predominant molecular species of MDP-Ca salts produced by the dentin, at the MDP concentration of more than 49.9 mg, altered from di-calcium salts of the MDP monomer and dimer to mono-calcium salts of the MDP monomer and dimer, since an increase in the amount of MDP included in the EX adhesive led to a decrease in the molar ration of calcium ion to MDP.

The XRD analysis results clearly showed that the rapid layered-structure formation of MDP was limited by the production of mono- (d-spacing value = 3.65 nm) and di-calcium (d-spacing value = 3.94 nm) salts of the MDP dimer [14]. The dentin developed two types of the layered structure of MDP, in contrast to the enamel. Therefore, the null hypothesis that the type of molecular species of MDP-Ca salts that form a layered structure differs between enamel and dentin was accepted.

However, these findings are not attributed to the formation of an intermediary layer of MDP on the hydroxyapatite, even if two types of characteristic XRD peaks assigned to the layered structure of MDP showed similar d-spacing value to that of the intermediary layer of MDP (3.94 nm) [8–12], respectively. This consideration was possible since (1) no evidence that MDP had directly bonded to the hydroxyapatite was detected in the NMR spectra and XRD patterns as the same as the previous study [9–11] and (2) all of ^{31}P NMR and XRD peaks detected were completely assigned to several types of MDP-Ca salts and the DCPD with an amorphous phase [14,15].

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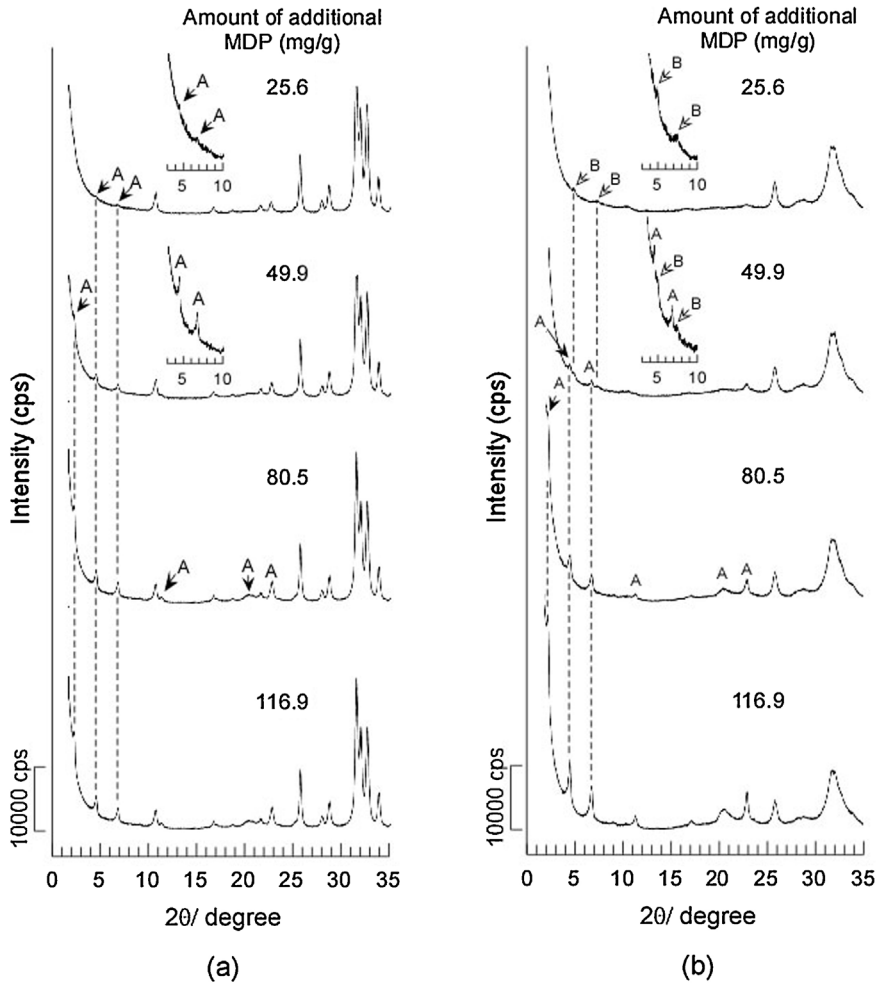

Fig. 5 – Typical XRD patterns of enamel (a) and dentin (b) reactant residues of EX adhesives containing different amounts of MDP (b). From top to bottom in each panel, the amounts of MDP in the EX adhesive were 25.6 mg, 49.9 mg, 80.5 mg and 116.1 mg.
The "A"-labels indicate 6 characteristic XRD peaks (enamel reactant residue: $2\theta = 2.21 \pm 0.03°$, $4.47 \pm 0.04°$, $6.75 \pm 0.01°$, $11.29 \pm 0.01°$, $20.39 \pm 0.02°$, and $22.87 \pm 0.01°$; dentin reactant residue: $2\theta = 2.19 \pm 0.02°$, $4.48 \pm 0.02°$, $6.74 \pm 0.02°$, $11.30 \pm 0.02°$, $20.39 \pm 0.02°$ and $22.87 \pm 0.01°$) assigned to the layered structure of the mono-calcium salt of the MDP dimer (MCS-MD), and the "B"-labels indicate second and third XRD peaks ($2\theta = 4.88 \pm 0.00°$ and $7.37 \pm 0.02°$) of the 3 characteristic peaks assigned to the layered structure of the di-calcium salt of the MDP dimer (DCS-MD).

The observed self-assembled layering of mono- and di-calcium salts of the MDP dimer is probably associated by a hydrophobic bond between two 10-methylene groups in mono- and di-calcium salts of the MDP dimer (Fig. 6). However, the mono-calcium salt of the MDP dimer may form a more tightly-packed layered structure than the di-calcium salt of the MDP dimer, since the hydrogen bonded interaction between hydroxy groups bonded to each phosphorous atom enhanced the bonding between molecular species of mono-calcium salt of the MDP dimer.

However, the mono-calcium salt of the MDP monomer that is predominantly produced will not be contributed to the layered-structure formation, since the intensities of characteristic XRD peaks assigned to the layered structure of MDP were related to the production amount of mono- and di-calcium salts of the MDP dimer, respectively. However, the mono-calcium salt of the MDP monomer may form a more tightly-packed crystal with an amorphous phase than the di-calcium salt of the MDP monomer by the hydrogen bonded interaction.

The MDP-Ca salts produced will randomly exist at the interphase consisting of the demineralized tooth and the adhesive layer. If the MDP-Ca salts were precipitated from the MDP-based one-step self-etch adhesive solution, they will exist at the adhesive-enamel or -dentin interface, in contrast, if not, they will be included within the adhesive layer of the MDP-based one-step self-etch adhesive. However, no evidence on (1) how many molecules of mono- or di-calcium salt of the MDP dimer will be aligned to form a layered structure and (2) how many crystals of mono- or di-calcium salt of the MDP dimer with a layered structure will be produced is detected in the ^{31}P NMR spectra and XRD patterns.

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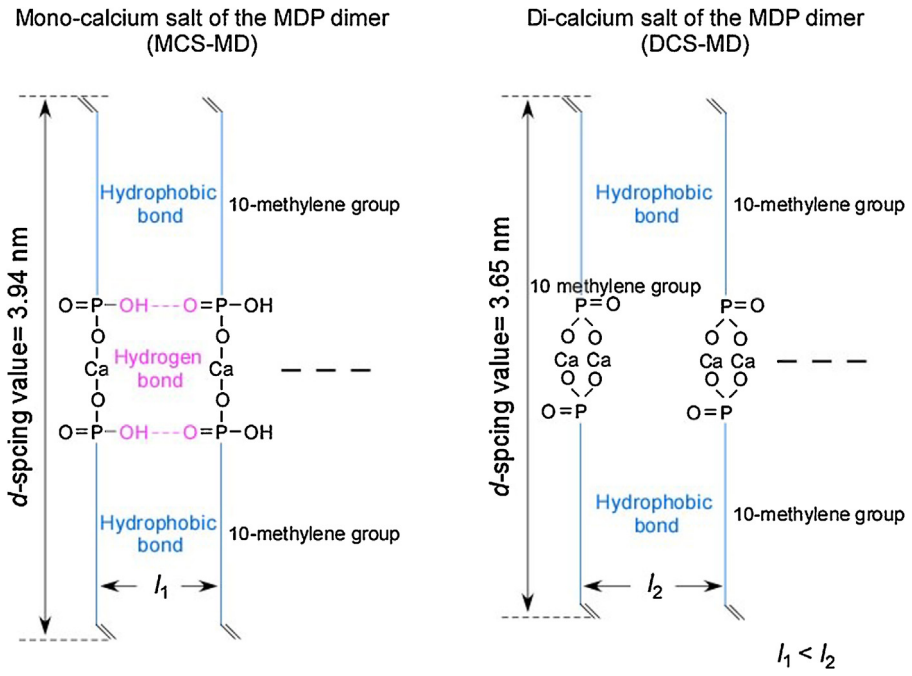

Fig. 6 – Model of the layered structure that mono-calcium salt of the MDP dimer (MCS-MD) and di-calcium salt of the MDP dimer (DCS-MD) form.

5. Conclusion

An increase in the MDP concentration allowed for an increase in the production amount of MDP-Ca salts during the application of the MDP-based one-step self-etch adhesive to enamel and dentin samples. The dentin produced greater amount of mono- and di-calcium salts of the MDP dimer that are able to form a layered structure than the enamel. However, the production ratio of mono- and di-calcium salts of the MDP dimer was less than 43% in each experimental group, since the enamel and dentin predominantly produced a mono-calcium salt of the MDP monomer.

Acknowledgements

This work was supported by a grant-in-aid for Developmental Scientific Research from the Ministry of Education, Science and Culture in Japan (No. 25462969) and by a grant from the Research Institute of Oral Science, Nihon University School of Dentistry at Matsudo. The XRD experiments were performed at the Laboratory for Electron Beam Research and Application (LEBRA), Nihon University.

I would like to express “my gratitude to” Professor Norihiro Nishiyama (Department of Dental Biomaterials, Nihon University School of Dentistry at Matsudo), and members of the Department of Dental Biomaterials for support, guidance, and invaluable advice.

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