Effect of calcium salt of 10-methacryloyloxydecyl dihydrogen phosphate produced on the bond durability of one-step self-etch adhesive

（10-メタクリロイロキシデシルジハイドロジェンホスフェイトのカルシウム塩の生成量がワンステップセルフエッチボンディング材の接着耐久性に及ぼす影響）

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ABSTRACT

Five experimental 10-methacryloyloxydecyl dihydrogen phosphate (MDP)-based one-step self-etch adhesives were designed by varying amounts of MDP. The aim of this study was to examine the effect of the quantity of calcium salt of MDP (MDP-Ca) salt produced by demineralization on the bond durability between experimental one-step adhesives and enamel or dentin.

Bond strengths of experimental adhesives to the enamel and dentin were measured, before and after 30,000× thermocycling. The fractured enamel and dentin samples as well as the fractured adhesive surfaces obtained during adhesion test were analyzed by a scanning electron microscope and energy-dispersive X-ray microscope.

An increase in the amount of MDP-Ca salt to above 37.2 mg/g drastically decreased the dentin bond strength and changed the fracture type during the thermocycling process. In contrast, the enamel bond strength remained unchanged, although the fracture type changed to an interfacial failure with increasing the amount of MDP-Ca salt to 78.3 mg/g.

INTRODUCTION

To understand the mechanisms by which the acidic monomers of two-step and one-step self-etch adhesives bind to enamel and dentin, many researchers have focused on the acid-base interaction between acidic monomers and tooth apatite[1-15].

Yoshida *et al*.[3] and Yoshioka *et al*.[4] established the "adhesion-decalcification" concept for self-etch adhesives applied to tooth apatite. Consequently, the acidic monomer, such as 10-methacryloyloxydecyl dihydrogen phosphate (MDP), 4-methacryloyloxyethyl trimellitic acid (4-MET), or 2-methacryloyloxyethyl hydrogen phosphate (Phenyl-P) used in self-etch adhesives yielded its calcium salt and also dicalcium phosphate dehydrate (DCPD) during demineralization of tooth apatite[12,13].

Inoue *et al*. reported that Clearfil SE Bond, in which MDP yielded its calcium salt with lower water-solubility than 4-MET and Phenyl-P, exhibited higher dentin bond durability than the 4-MET-based Unifil Bond and Phenyl-P-based Clearfil Liner Bond II[16]. In contrast, Itoh *et al*. reported that MDP-based Clearfil Tri-S Bond and glycerol phosphate dimethacrylate-based OptiBond All-In-One exhibited greater reductions in the dentin bond strength than methacryloyloxyalkyl acid phosphate-based Bond Force after one-year water storage[17].

However, these reports have no quantitative information on 1) how much amounts of acidic monomer used in commercial self-etch adhesives yield its calcium salt through

demineralization of tooth apatite, and 2) remain within the adhesive layer created at the enamel or dentin surface during hardening of self-etch adhesive, as an unreactant residual with calcium. These quantitative analyses are of importance since the reactant and unreactant residues of acidic monomer with calcium will be included within the created adhesive layer[9,11,14,15]. It is anticipated that the reactant and/or unreactant residual of acidic monomer with calcium may cause degradation of the created adhesive layer during the aging period, if the reactant of acidic monomer with calcium was unable to copolymerize with other monomers in the self-etch adhesive, and/or water that had permeated in the created adhesive layer weakened the mechanical properties of the created adhesive layer[18,19].

Previously, our group designed a series of five MDP-based one-step self-etch adhesives containing different amounts of MDP, to investigate the effect of the quantity of calcium salt of MDP (MDP-Ca salt) produced by demineralization on the enamel and dentin bonding performance of experimental one-step adhesives after one-day water storage[14]. We reported that the enamel and dentin bond strengths increased with an increase in the amount of MDP-Ca salt produced, but further increases slightly decreased both bond strengths.

The aim of this study was to examine the effect of the quantity of MDP-Ca salt produced on the bond durability between experimental one-step self-etch adhesives and enamel or dentin. The null hypotheses tested were that the amount of MDP-Ca salt produced (1) has no effect on the enamel and dentin bond durability of experimental one-step adhesives and (2) is

not a useful indicator for estimation of the enamel and dentin bond durability.

MATERIALS AND METHODS

Preparation of experimental one-step self-etch adhesives

A series of five types of MDP-based one-step self-etch adhesives containing different amounts of MDP, for which the amounts of MDP-Ca salt produced by demineralization of the cut enamel and dentin particles were previously characterized[14], was used in this study. The code, components, compositions of each one-step adhesive, and the amount of MDP-Ca salt produced are listed in Table 1. The codes of the five types of MDP-based self-etch adhesives are EX-0, EX-3, EX-6, EX-10, and EX-15.

Preparation of specimens for the adhesion test

The facial enamel surface of a bovine crown was ground with a sequence of 100-, 600-, and 1,000-grit silicon carbide papers under water irrigation. Eighty-μm-thick double-faced tape with a circular hole (internal diameter=3.2-mm, Nichiban, Tokyo, Japan) was placed onto the ground enamel or dentin surface. The enamel or dentin surface inside the circular hole was conditioned with each one-step adhesive for 20 s and then blown out with a high-pressure airflow for 5 s. Light irradiation was applied to each one-step adhesive for 10 s using a Light Curing Unit (XL3000, 3M ESPE, Grafenau, 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, Tokyo, Japan) and irradiated with light for 20 s. After the mold and tape were removed, the bonded specimens were immersed in water at 37ºC. The number of specimens for each adhesive group was 30.

Measurement of shear bond strength

After the bonded specimens were stored in water at 37ºC for 1 day, they were divided into two experimental groups: before or after thermocycling group. The specimens in the thermocycling group were cycled between 5°C and 55°C in water baths for 30,000 cycles. The dwell time in each water bath and the transfer time were 60 and 7 s, respectively. The shear bond strength of each one-step adhesive to the enamel or dentin surface was measured under a crosshead speed of 1.0 mm/min using a universal testing machine (TG-5KN, Minebea Co, Nagano, Japan). The number of specimens for each adhesive group was 15.

Type of fracture mode

After the adhesion test, fifteen enamel and dentin samples for each one-step adhesive were dehydrated by 50, 60, 70, 80, 90, and 100 vol% ethanol aqueous solutions, immersed in *tert*-butyl alcohol, and then freeze-dried under vacuum (FDU-1200, EYELA, Japan). The samples were then mounted onto aluminum stubs and sputter-coated with a gold-palladium alloy. Thereafter, each specimen was observed with a scanning electron microscope (SEM, S-2150, Hitachi, Japan) at numerous magnifications at 15 kV, to classify the fracture type into

the four categories[14,15]. The four categories were as follows: category 0, no adhesive remained on the enamel or dentin surface; category 1, less than half of the adhesive remained on the enamel or dentin surface; category 2, more than half of the adhesive remained on the enamel or dentin surface; and category 3, all of the adhesive remained on the enamel or dentin surface.

X-ray spectroscopy analysis of the elemental distribution on the adhesive surface fractured from enamel or dentin

The EX-6 or EX-15 adhesives that had fractured from the enamel or dentin surface during the adhesion test were mounted onto aluminum stubs and sputter-coated with a gold-palladium alloy. The energy-dispersive X-ray microscope (EDX; Field Emission SEM, JSM-7001F, JEOL, Tokyo, Japan) was used to determine the presence of silicon, phosphorous, and calcium on the fractured EX-6 and EX-15 adhesive surfaces at numerous magnifications at 10 kV. After this, SEM observations of the same area of each fractured adhesive surfaces analyzed by EDX were performed.

Statistical analysis

The bond strengths of each one-step adhesive obtained before or after thermocycling were analyzed using one-way analysis of variance (ANOVA) and Tukey multiple comparison tests. The effect of thermocycling on the bond strengths for each amount of MDP-Ca salt was analyzed using a *t*-test. The level of statistical significance was set at 0.05.

RESULTS

Effect of the amount of MDP-Ca salt produced on enamel and dentin bond strengths during the thermocycling process

Figure 1 show the effect of the amount of MDP-Ca salt produced by demineralization on the enamel (1A) and dentin bond strengths (1B), before and after thermocycling. As shown in Figure 1A, when no MDP-Ca salt was produced (that is, when the EX-0 adhesive was applied), thermocycling led to a significant decrease in the bond strength ($p<0.05$). However, the production of MDP-Ca salt remained the enamel bond strength unchanged ($p>0.05$), although the amount of MDP-Ca salt produced was increased from 14.5 to 78.3 mg/g.

In contrast, increases in amounts of MDP-Ca salt to above 37.2 mg/g decreased the dentin bond strength during the thermocycling process (Figure 1B). A significant difference in the dentin bond strength was observed before and after thermocycling ($p<0.05$), when the amount of MDP-Ca salt was increased to above 57.9 mg/g.

Effect of the amount of MDP-Ca salt produced on the type of fracture mode and surface morphology of fractured enamel and dentin during the thermocycling process

Figure 2 shows the typical SEM images of fractured enamel (2A) and dentin surfaces (2B) with the fracture type (category) before and after thermocycling, respectively. The fracture types for each one-step adhesive before and after thermocycling are summarized in Table 2.

Increases in the amount of MDP-Ca salt changed the surface morphology of the fractured enamel surface (2A) and increased the number of specimen that had exhibited the category 1 (Table 2). When the amount of MDP-Ca salt produced was 78.3 mg/g, scratches caused by the grinding with the silicon carbide paper were noticeably more visible on the enamel surface after thermocycling than before thermocycling.

In contrast, dentin samples exhibited more drastic changes in the surface morphology than enamel samples (2B) and in the type of fracture mode during the thermocycling process (Table 2), when the amount of MDP-Ca salt was increased to above 57.9 mg/g. The large numbers of wide-open dentinal tubules were observed on the fractured dentin surface and the peritubular dentin appeared to be slightly etched at the circumference of the dentinal tubules. The scratches were observed on the fractured intertubular dentin surfaces more clearly than the fractured enamel surface.

Elemental distribution images of silicon, phosphorus, and calcium of fractured EX-6 and EX-15 adhesive surfaces from enamel or dentin surfaces after thermocycling

Figures 3 and 4 show the silicon, phosphorus, and calcium elemental distribution images for the fractured EX-6 and EX-15 adhesive surfaces from enamel or dentin surfaces after thermocycling, respectively. Both SEM views A show the ground enamel or dentin surfaces and both SEM images B and b show the same region of the EX-6 or EX-15 adhesive surface analyzed by EDX are also shown in each respective Figure.

As shown in Figure 3, increases in the amount of MDP-Ca salt from 35.4 (EX-6) to 78.3 mg/g (EX-15) led to changes in the morphology of the fractured adhesive surface from enamel (image b) and in the elemental distributions of silicon, phosphorous, and calcium (images c, d, and e). Both calcium and phosphorus element signals largely overlapped each other over the fractured adhesive surface (images d and e). The overlapped area of both element signals indicates the presence of MDP-Ca salt and/or DCPD produced by demineralization of enamel and/or the fractured enamel debris remained on the fractured adhesive surface during adhesion test.

In contrast, as shown in Figure 4, increasing the amount of MDP-Ca salt from 37.2 (EX-6) to 96.4 mg/g (EX-15) triggered drastic changes in the morphology of the fractured adhesive surface from dentin surface (image b). In particular, 2–3-μm-thick resin tags that had been completely peeled off from the dentinal tubules, dentinal collagen fibers that had not been impregnated by EX-15 at the bases of the resin tags, and scratches on the adhesive surface that had fractured from the intertubular dentin surface were observed. Both calcium and phosphorus element signals were overlapped more intensely at the resin tag portions peeled off from the dentinal tubules than those at the fractured adhesive surface from the intertubular dentin surface (images d and e).

DISCUSSION

It is well known that a scanning electron microscope (SEM) is a useful tool to obtain an insight on the adhesion and fracture mechanisms of adhesives to the tooth. The morphological analyses of the adhesive-enamel and adhesive-dentin interfaces as well as the fractured enamel and dentin surfaces obtained during adhesion test are often performed using SEM[20-23].

In this study, the element distribution analyses of the fractured EX-6 and EX-15 adhesive surfaces from the enamel or dentin during adhesion test were performed. The EDX analysis of both calcium and phosphorous elements probably give us an insight on the demineralization aspect of enamel or dentin by one-step adhesives and also the degradation mechanism of the bonds between one-step adhesives and enamel or dentin during the thermocycling process, since the amount of MDP-Ca salt determined is an useful indicator, that predicts the degree of demineralization of enamel or dentin. Specifically, both calcium and phosphorous element signals that has been overlapped indicate a distribution of byproducts, such as MDP-Ca salt and DCPD produced by demineralization. This analysis is possible since MDP-Ca salt and DCPD produced probably exist at the fractured adhesive surface, if the fracture occurred at the adhesive-enamel or -dentin interface. This was because that these byproducts did not directly bind to the enamel and dentin surfaces, since they were removed from the enamel or dentin surface by water rinsing[9,11].

However, we need to exclude the overlapped calcium and phosphorous element signals

evidenced by the fractured enamel or dentin debris that has bind to the fractured adhesive surface from the obtained analysis result, since the overlapped signals not only show the distribution of these byproducts, but also the existence of the fractured enamel or dentin debris. The exclusion was possible since the fractured enamel or dentin debris existed at the fractured adhesive surface drastically reduced the signal intensity of silicon element, due to a limitation of the escape depth of the characteristic X-ray.

The analysis results demonstrate clearly that an increase in the amount of MDP-Ca salt led to a change in the fracture type of both enamel and dentin samples, since water infiltrated into the adhesive interface and weakened the bonds between experimental one-step adhesives and enamel or dentin during the thermocycling process. Specifically, the dentin samples exhibited drastic change in the fracture type. This was probably due to the infiltration rate of water into the adhesive-dentin interface being faster than that into the adhesive-enamel interface, since the dentin involves not only 10 mass% of water and 20 mass% of hydrophilic dentinal collagen fibers, but also has the dentinal tubes which is able to pass through water.

If the observed drastic change in the fracture type of the dentin samples was due to the weakening of mechanical properties of the adhesive layer created at the dentin surface by water sorption[18,19], the enamel samples should exhibit more drastic change in the fracture type than the dentin samples. Thus, the greater amounts of the unreacted MDP polymer with calcium remains within the adhesive layer created on the enamel surface than the dentin

surface (Table 1), since the phosphate group in the unreacted MDP polymer enhances the water sorption[18].

In accordance to the faster infiltration rate of water into the adhesive-dentin interface than the adhesive-enamel interface, increases in the quantity of MDP-Ca salt decreased the dentin bond strength more drastically than the enamel bond strength during the thermocycling process. The MDP-Ca salt had different effects on the shear bond strength between experimental one-step adhesives and enamel or dentin. The null hypothesis that the amount of MDP-Ca salt produced has no effect on the enamel bond durability was accepted, but on the dentin bond durability was rejected.

The observed greater reductions in the dentin bond strength than the enamel bond strength were probably due to a hydrolysis of the dentinal collagen fibers within the demineralized dentin zone created at the peritubular dentin[22-24]. This was possibly because the fractured peritubular dentinal collagen fibers that had not impregnated with one-step adhesive were clearly seen at the circumference inside the dentinal tubules[25] and at the bases of the resin tags peeled off from the dentinal tubules. These SEM analyses suggest that the experimental one-step adhesives that had produced MDP-Ca salt more than 37.2 mg/g led to an increase in the probability that the demineralized dentin zone will be developed at the peritubular dentin. This was because both calcium and phosphorous element signals assigned to the byproducts produced by demineralization were concentrated at the peeled resin tag

portions more intensely than the fractured adhesive surface from the intertubular dentin surface, since the peritubular dentin was demineralized by these adhesives more strongly than the intertubular dentin[25].

In contrast, any significant difference in the enamel bond strength was not observed before and after thermocyclying, even though the amount of MDP-Ca salt reached 78.3 mg/g. No changes observed in the enamel bond strengths was due to (1) the degradation period being limited by the number of thermocycles, i.e., 30,000 and (2) only a limited area of the created adhesive layer being exposed to water, *i.e.*, only the very thin round edge of the adhesive layer was exposed to water. The null hypothesis that the amount of MDP-Ca salt produced is not a useful indicator for estimation of the enamel was accepted, but on the dentin bond durability was rejected.

CONCLUSION

Despite the limitations of the present investigation, the following conclusion was established. The amount of MDP-Ca salt is a very useful indicator for predicting the dentin bond durability of MDP-based self-etch adhesives, and is not for the enamel bond durability.

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REFERENCES

1. Yoshida Y, Van Meerbeek B, Nakayama Y, Snauwaert J, Hellemans L, Lambrechts P, Vanherle G, Wakasa K. Evidence of chemical bonding at biomaterial-hard tissue interfaces. J Dent Res 2000; 79: 709-714.

2. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, Vanherle G. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. Oper Dent 2003; 28: 215-235.

3. Yoshida Y, Van Meerbeek B, Nakayama Y, Yoshioka M, Snauwaert J, Abe Y, Lambrechts P, Vanherle G, Okazaki M. Adhesion to and decalcification of hydroxyapatite by carboxylic acids. J Dent Res 2001; 80: 1565-1569.

4. Yoshioka M, Yoshida Y, Inoue S, Lambrechts P, Vanherle G, Nomura Y, Okazaki M, Shintani H, Van Meerbeek B. Adhesion/decalcification mechanisms of acid interactions with human hard tissues. J Biomed Mater Res 2001; 59: 56-62.

5. Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, Inoue S, Tagawa Y, Suzuki K, De Munck J, Van Meerbeek B. Comparative study on adhesive performance of functional monomers. J Dent Res 2004; 83: 454-458.

6. Nishiyama N, Suzuki K, Takahashi K, Nemoto K. The pKa effects of the carboxylic acid in *N*-methacryloyl-omega-amino acid on the demineralization and bond strengths to the teeth. Biomaterials 2004; 25: 5441-5447.

7. Nishiyama N, Fujita K, Ikemi T, Maeda T, Suzuki K, Nemoto K. Efficacy of varying the NMEP concentrations in the NMGly-NMEP self-etching primer on the resin-tooth bonding. Biomaterials 2005; 26: 2653-2661.

8. Fu B, Sun X, Qian W, Shen Y, Chen R, Hannig M. Evidence of chemical bonding to hydroxyapatite by phosphoric acid esters. Biomaterials 2005; 26: 5104-5110.

9. Fujita K, Nishiyama N. ^{13}C NMR analysis of the etching efficacy of acidic monomers in self-etching primers. J Dent 2006; 34: 123-133.

10. Nishiyama N, Aida M, Fujita K, Suzuki K, Tay FR, Pashley DH, Nemoto K. NMR study on the adhesion efficacy of experimental phosphonic acid monomers. Dent Mater J 2007; 26: 382-387.

11. Fujita K, Ma S, Aida M, Maeda T, Ikemi T, Hirata M, Nishiyama N. Effect of reacted acidic monomer with calcium on bonding performance. J Dent Res 2011; 90: 607-612.

12. Fukegawa D, Hayakawa S, Yoshida Y, Suzuki K, Osaka A, Van Meerbeek B. Chemical interaction of phosphoric acid ester with hydroxyapatite. J Dent Res 2006; 85: 941-944.

13. Yoshihara K, Yoshida Y, Nagaoka N, Fukegawa D, Hayakawa S, Mine A, Nakamura M, Minagi S, Osaka A, Suzuki K, Van Meerbeek B. Nano-controlled molecular interaction at adhesive interfaces for hard tissue reconstruction. Acta Biomater 2010; 6: 3573-3582.

14. Iwai H, Nishiyama N. Effect of calcium salt of functional monomer on bonding performance. J Dent Res 2012; 91: 1043-1048.

15. Iwai H, Fujita K, Iwai H, Ikemi T, Goto H, Aida M, Nishiyama N. Development of MDP-Based One-step Self-Etch Adhesive －Effect of Additional 4-META on Bonding Performance－. Dent Mater J 2013; 32: 1-9.

16. Inoue S, Koshiro K, Yoshida Y, De Munck J, Nagakane K, Suzuki K, Sano H, Van Meerbeek B. Hydrolytic stability of self-etch adhesives bonded to dentin. J Dent Res 2005; 84: 1160-1164.

17. Itoh S, Nakajima M, Hosaka K, Okuma M, Takahashi M, Shinoda Y, Seki N, Ikeda M, Kishikawa R, Foxton RM, Tagami J. Dentin bond durability and water sorption/solubility of one-step self-etch adhesives. Dent Mater J 2010; 29: 623-630.

18. Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho RM, Yiu C, Rueggeberg FA, Foulger S, Saito T, Nishitani Y, Yoshiyama M, Tay FR, Pashley DH. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials 2005; 26: 6449-6459.

19. Hosaka K, Tagami J, Nishitani Y, Yoshiyama M, Carrilho M, Tay FR, Agee KA, Pashley DH. Effect of wet vs. dry testing on the mechanical properties of hydrophilic self-etching primer polymers. Eur J Oral Sci 2007; 115: 239-245.

20. Li N, Nikaido T, Takagaki T, Sadr A, Makishi P, Jihua Chen J, Tagami J. The role of functional monomers in bonding to enamel: Acid–base resistant zone and bonding performance. JOD 2010; 38: 722-730.

21. Nikaido T, Ichikawa C, Li N, Takagaki T, Sadr A, Yoshida Y, Suzuki K, Tagami J. Effect of functional monomers in all-in-one adhesive systems on formation of enamel/dentin acid-base resistant zone. Dent Mater J 2011; 30: 576-582.

22. Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG, Pashley DH. Nanoleakage: Leakage within the hybrid layer. Oper Dent 1995; 20: 18-25.

23. Hashimoto M, Ohno H, Sano H, Kaga M, Oguchi H. Degradation patterns of different adhesives and bonding procedures. J Biomed Mater Res Part B: Appl Biomater 2003; 66B: 324-330.

24. Hashimoto M, Tay FR, Ohno H, Sano H, Kaga M, Yiu C, Kumagai H, Kudou Y, Kubota M, Oguchi H. SEM and TEM analysis of water degradation of human dentinal collagen. J Biomed Mater Res Part B: Appl Biomater 2003; 66B: 287–298.

25. Semeraro S, Mezzanzanica D, Spreafico D, Gagliani M, Re D, Tanaka T, Sidhu SK, Sano H. Effect of different bur grinding on the bond strength of self–etching adhesives. Oper Dent 2006; 31: 317-323.

Table 1 The code, components, compositions of experimental adhesive, and amounts of reacted or unreacted MDP with calcium of enamel or dentin apatite[14].

	Component and Composition							MDP employed*	Enamel		Dentin	
Code	MDP	UDMA	TEGDMA	4-META	Filler	Water	Acetone		Reacted MDP**	Unreacted MDP#	Reacted MDP**	Unreacted MDP#
EX-0	0	10.0	10.0	9.4	4.26	11.2	69.3	0	0	0	0	0
EX-3	3.0	10.0	10.0	9.4	4.26	11.2	69.3	25.6	14.5 (0.6)	11.1	16.2 (2.5)	9.4
EX-6	6.0	10.0	10.0	9.4	4.26	11.2	69.3	49.9	35.4 (0.4)	14.5	37.2 (0.8)	12.7
EX-10	10.0	10.0	10.0	9.4	4.26	11.2	69.3	80.5	45.6 (2.2)	34.9	57.9 (2.0)	22.6
EX-15	15.0	10.0	10.0	9.4	4.26	11.2	69.3	116.1	78.3 (6.1)	37.8	96.4 (2.9)	19.7

Amounts of each component were expressed in grams.

MDP: 10-methacryloyloxydecyl dihydrogen phosphate; UDMA: urethane dimethacrylate;

TEGDMA: triethylene glycol dimethacrylate; 4-META: 4-methacryloyloxyethyl trimellitic anhydride

( ): SD

*: The quantity of MDP employed in 1.000 g of the respective adhesive (mg/g).

**: The amount of reacted MDP with calcium (MDP-Ca salt) produced by demineralization of enamel and dentin apatite using NMR technique (mg/g).

#: The amount of unreacted MDP with calcium was obtained by subtracting the amount of reacted MDP with calcium of the enamel or dentin apatite from the quantity of MDP employed (mg/g).

Table 2 The effect of amounts of MDP-Ca salt produced on the types of fracture mode (category: [0, 1, 2, 3][*]), before and after thermocycling.

		Enamel			Dentin		
	Amount of MDP**	Amount of MDP-Ca salt[#]	Category		Amount of MDP-Ca salt[#]	Category	
Code	(mg/g)	(mg/g)	Before[##]	After[†]	(mg/g)[c]	Before[##]	After[†]
EX-0	0	–	[0/11/4/0]	[0/13/2/0]	–	[0/10/5/0]	[0/11/4/0]
EX-3	25.6	14.5 (0.6)	[0/7/8/0]	[0/9/6/0]	16.2 (2.5)	[0/2/13/0]	[0/9/6/0]
EX-6	49.9	35.4 (0.4)	[0/0/15/0]	[0/4/11/0]	37.2 (0.8)	[0/2/13/0]	[0/6/9/0]
EX-10	80.5	45.6 (2.2)	[0/2/13/0]	[0/6/9/0]	57.9 (2.0)	[0/4/11/0]	[0/8/7/0]
EX-15	116.1	78.3 (6.1)	[0/5/10/0]	[0/8/7/0]	96.4 (2.9)	[0/8/7/0]	[0/12/3/0]

( ): SD

*: The four categories [0, 1, 2, 3]: category 0: no adhesive remained on the enamel or dentin surface; category 1: less than half of the adhesive remained on the enamel or dentin surface; category 2: more than half of the adhesive remained on the enamel or dentin surface; and category 3: all of the adhesive remained on the enamel or dentin surface.

**: The quantity of MDP employed in 1.000 g of the respective adhesive.

[#]: The amount of MDP-Ca salt (reacted MDP with calcium) produced by demineralization of enamel and dentin apatite using NMR technique.

##: Before thermocycling.

[†]: After thermocycling.

–: Not identified.

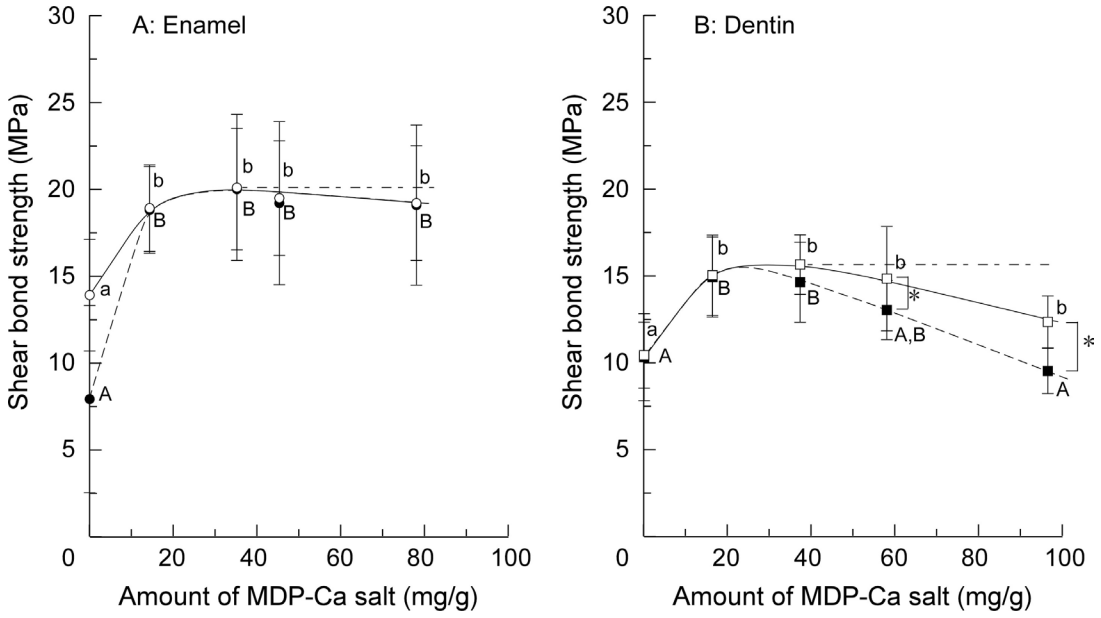
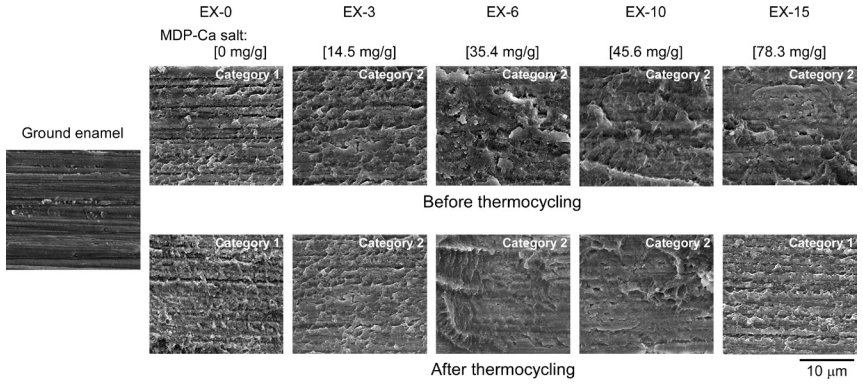


Figure 1 Effect of the quantity of MDP-Ca salt produced on (A) the enamel and (B) the dentin bond strength before and after 30,000× thermocycling.

Fifteen specimens were used for the adhesion test in each adhesive group. The white and black symbols show the bond strengths before and after thermocycling, respectively. Error bars show the SDs.

The different small character shows a significant difference in the enamel bond strengths or the dentin bond strengths obtained before thermocycling, and the different large character shows a significant difference in the enamel bond strengths or the dentin bond strengths obtained after thermocycling ($p<0.05$).

Asterisk denotes a significant difference in the bond strength before and after thermocycling ($p<0.05$).



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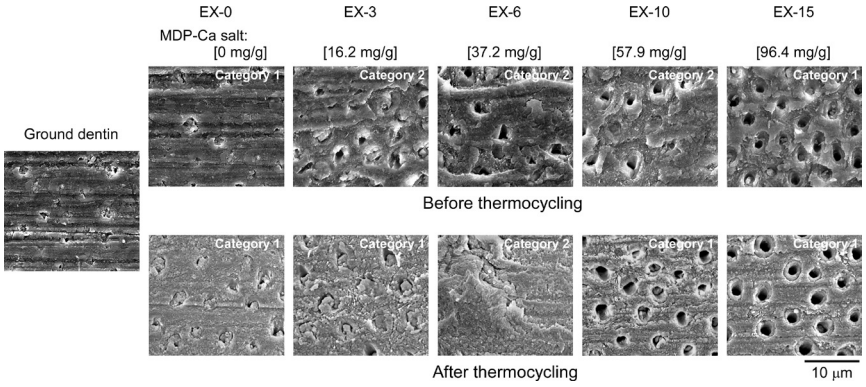

B

Figure 2 Typical SEM images showing the effect of amounts of MDP-Ca salt on (A) the fractured enamel and (B) the fractured dentin surfaces before and after 30,000× thermocycling.

Both middle left images show the ground enamel or dentin surface. The both upper five SEM images show the fractured enamel or dentin surfaces obtained before thermocycling, and the both lower five SEM images show the fractured enamel or dentin surfaces after thermocycling.

The remaining columns of five SEM images show the fractured enamel or dentin surfaces bonded with EX-0 adhesive, EX-3 adhesive, EX-6 adhesive, EX-10 adhesive, and EX-15 adhesive. The category in each image denotes the type of fracture mode.

2A: When the amount of MDP-Ca salt produced reached 78.3 mg/g, scratches caused by the grinding with the silicon carbide paper were seen more clearly in the fractured enamel surface after thermocycling than before thermocycling.

2B: Increases in the amount of MDP-Ca salt produced led to drastic changes in the morphology of the fractured dentin surface during the thermocycling process. The production of MDP-Ca salt more than 37.2 mg/g led to the appearance of a large number of wide-open dentinal tubules, since the resin tags were peeled off from the dentinal tubules. When the amount of MDP-Ca salt produced was increased to 57.9 mg/g, the peritubular dentinal collagen fibers appeared at the circumference inside the dentinal tubules, and scratches were seen in adhesive surface fractured from the intertubular dentin surface after thermocycling more clearly than that before thermocycling.

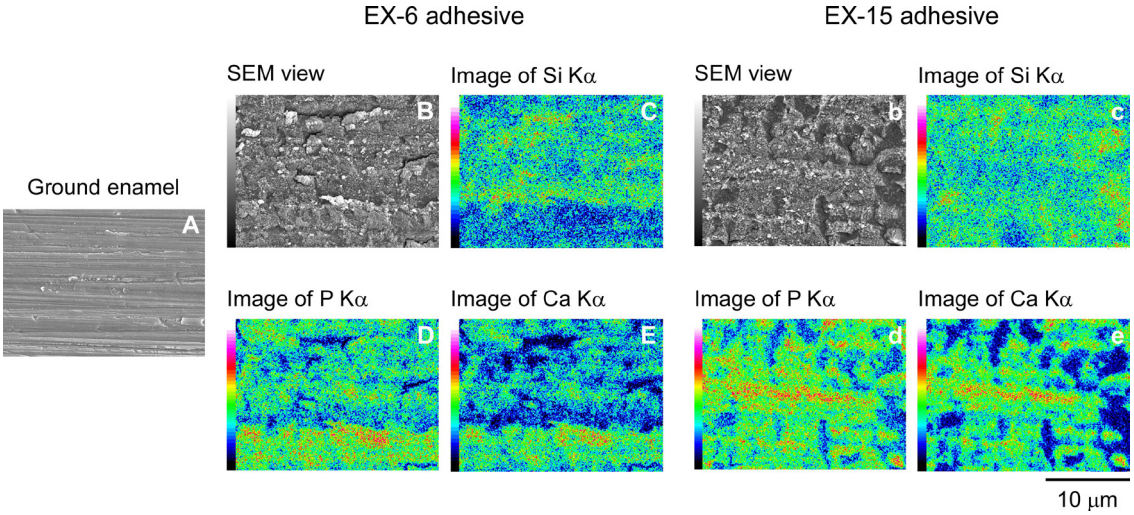

Figure 3 Elemental distributions on EX-6 (C, D, and E) and EX-15 adhesives (c, d, and e) fractured from enamel surfaces after thermocycling.

The image A shows the SEM view of ground enamel surface, both SEM images B and b show the same area of the EX-6 or EX-15 adhesive surface analyzed by EDX, both images C and c show the silicon elemental distribution of the fractured EX-6 or EX-15 adhesive surface, both images D and d show the phosphorus elemental distribution of the fractured EX-6 or EX-15 adhesive surface, and both images E and e show the calcium elemental distribution of the fractured EX-6 or EX-15 adhesive surface.

When the EX-6 adhesive was applied to the enamel (amount of MDP-Ca salt: 35.4 mg/g), the overlapping calcium and phosphorus signals spread in a band on the adhesive surface (D and E). This zone of overlapped signals was attributed to the fractured enamel remaining on the adhesive surface, since the silicon intensity of this region was lower than that of other regions (C). With 78.3 mg/g of MDP-Ca salt (EX-15 adhesive), the overlapped calcium and phosphorus signals were widely distributed on the adhesive surface (c and d), and a part of the overlapping area of both elements provides the same level of the silicon element signal as found on the adhesive surface.

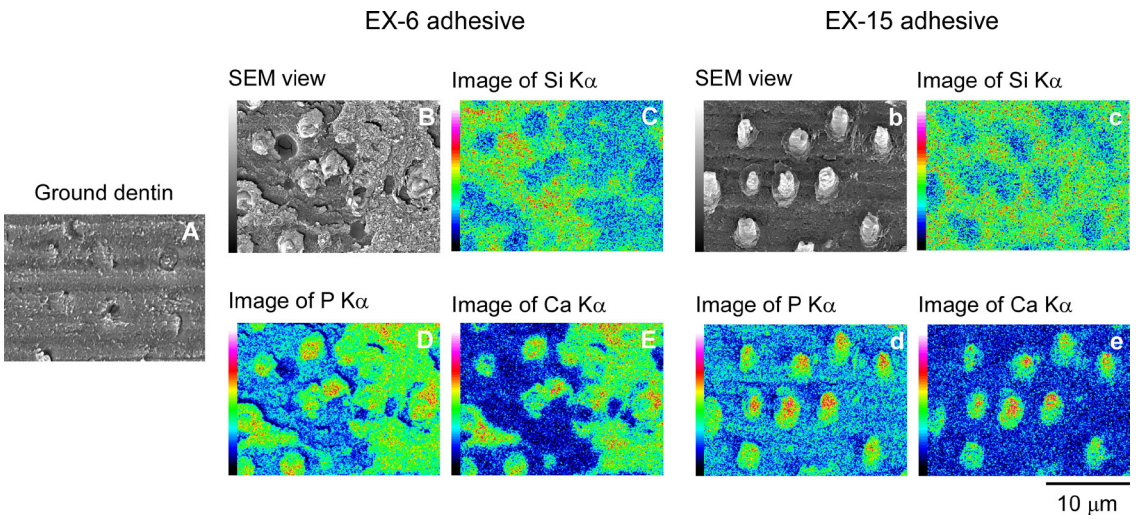

Figure 4 Elemental distributions on the EX-6 (C, D, and E) and EX-15 adhesives (c, d, and e) fractured from a dentin surface after thermocycling.

The image A shows the SEM view of ground dentin surface, both SEM images B and b show the same region of the EX-6 or EX-15 adhesive surface analyzed by EDX, both images C and c show the silicon elemental distribution of the fractured EX-6 or EX-15 adhesive surface, both images D and d show the phosphorus elemental distribution of the fractured EX-6 or EX-15 adhesive surface, and both images E and e show the calcium elemental distribution of the fractured EX-6 or EX-15 adhesive surface.

When the EX-6 adhesive was applied, fractured dentin was observed on the adhesive surface (B), since the silicon intensity of this portion was very low (C). However, when the EX-15 adhesive was applied, scratches, resin tags, and unimpregnated peritubular dentinal collagen fibers were observed on the fractured adhesive surface (b). On the EX-6 and EX-15 adhesives, both calcium and phosphorus element signals were concentrated at the resin tag portions (D and E, d and e). The signal intensities of both calcium and phosphorous elements at the resin tag portion were higher than those at the adhesive surface that had fractured from the intertubular dentin surface.