Original Paper

Effect of the concentration of water in an MDP-based all-in-one adhesive on the efficacy of smear layer removal and on dentin bonding performance

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ABSTRACT

The effects of the water concentration in an experimental 10-methacryloyloxydecyl dihydrogen phosphate (MDP)-based all-in-one (EX) adhesive were examined on the ability of MDP to remove the smear layer from the ground dentin surface and on the dentin bonding performance.

Four types of EX adhesives were prepared by varying the amount of water (46.6, 93.2, 149.8 and 208.1 mg/g), but the MDP concentration was kept a constant at 49.9 mg/g. Scanning electron microscopy and bond strength measurements were performed on the dentin surface demineralized by each EX adhesive.

Increased amount of water in the EX adhesive increased the ability of MDP to remove the smear layer. However, the solubilization of the smear layer into the EX adhesive decreased the dentin bond strength. The water concentration in the EX adhesive affected the efficacies of smear layer removal and dentin bonding performance more strongly than the pH value of the EX adhesive.

INTRODUCTION

One-step self-etch adhesive systems, often called all-in-one adhesive systems, have been widely accepted by dentists since they have a noticeably high bonding performance and a reliable clinical performance[1,2]. However, many studies have reported that the actual bonding performance attained by all-in-one adhesive systems differs among products[3-7]. This may be due to the demineralization capacity of an acidic monomer being strongly affected by the type of acidic monomers utilized in all-in-one adhesives, the same with two-step self-etch adhesives, so called self-etching primer [8-16].

Meerbeek and coworkers[8,11,14-16] have established the adhesion and decalcification concept. Thus, the 10-methacryloyloxydecyl dihydrogen phosphate (MDP)-based self-etching primer releases both ions of calcium and phosphate through the demineralization of hydroxyapaptite by MDP, and a calcium ion released into the self-etching primer rapidly yields a calcium salt of MDP, and remained unreacted-calcium ion with MDP lately yields di-calcium phosphate dihydride (DCPD) with a crystalline phase.

Fujita et al.[12] previously confirmed a production of a calcium salt of MDP (MDP-Ca salt) at the demineralized enamel and dentin surfaces as a precipitate, after both enamel and dentin surfaces demineralized with an MDP-based self-etching primer (Clearfil MEGA Bond Primer) were rinsed with ethanol. However, precipitates of MDP-Ca salt were removable, since the precipitates were rinsed off from the demineralized enamel and dentin surfaces by water.

Generally, all-in-one adhesives are diluted with a solvent, such as ethanol or acetone, to prevent the phase separation of water from the resin monomer components[8,15,17]. To our knowledge, no published articles have specifically focused on the effect of the concentration of water in ethanol or acetone aqueous solution on the bonding performance of all-in-one adhesives, although the solvent limits the ionization of the acidic group in the acidic monomer in contrast to water.

In this study, the effect of the amount of water added in the experimental MDP-based all-in-one (EX) adhesive was examined on the ability of MDP to demineralize and remove the smear layer and plugs from the ground dentin surface using a series of four types of EX adhesives containing different amounts of water. The effect of the removal of the smear layer from a ground dentin surface on the bonding performance of the EX adhesive to the dentin was then characterized. The null hypotheses tested were that: 1) the amount of water added in the EX adhesive has no effect on the ability of MDP to demineralize and remove the smear layer and plugs from the ground dentin surface, and 2) the removal of the smear layer and plugs from the ground dentin surface through their demineralization has no effect on the dentin bonding performance.

MATERIALS AND METHODS

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

Preparation of the experimental MDP-based all-in-one adhesives

Four types of EX adhesives containing different amounts of water were designed. The components and compositions of the EX adhesive were described in the previous studies[18,19]. 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 Co, Wakayama, Japan) and 9.4 g 4-methacryloyloxyethyl trimellitic anhydride (4-META, purity= 97.0%). One mass% of camphorquinone and dimethylamino benzoic acid ethyl ester, and 2,000 ppm of 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 four types of EX adhesives was then prepared by diluting 39.66 g of the filled resin with 80.5 g of acetone aqueous solutions with different mass ratios of water to acetone (water/acetone [g/g] = 5.6/74.9, 11.2/69.3, 18.0/62.5 and 25.0/55.5). The quantities of water employed in the EX adhesive were 46.6, 93.2, 149.8 and 208.1 mg/g, respectively, but in contrast, the quantity of MDP in each EX adhesive was kept a constant at 49.9 mg/g.

The corresponding four types of EX adhesives are termed EX-1, EX-2, EX-3 and EX-4, and their pH values were 0.87, 1.42, 1.62 and 1.68, respectively.

Evaluation of the demineralization capacity of MDP utilized in the EX adhesive

The facial enamel surfaces of 12 bovine teeth were ground with 100-grit silicon carbide paper under a stream of water. After cutting the lingual surface of each bovine tooth with a low speed diamond saw (Isomet, Buehler, Lake Bluff, IL, US), flat dentin disks were prepared. To expose the mid-coronal dentin surface, the facial dentin surfaces were then ground with 600-grit silicon carbide paper under running water. After being air-dried with a high-pressure airflow for 5 sec, the ground dentin surface was demineralized with each EX adhesive for 20 sec, immediately thereafter the demineralized dentin samples were placed in

50 mL acetone and allowed to stand for 5 min to remove the resin monomer components employed in the EX adhesive from the dentin surface. The dentin samples were then gently dehydrated by 50, 70, 80, 90 and 100 vol% ethanol solutions, immersed in tert-butyl alcohol and then freeze-dried under vacuum (FDU-1200, EYELA, Tokyo, Japan). The freeze-dried dentin samples were mounted on aluminum stubs and were sputter-coated with a gold-palladium alloy. Scanning electron microscope (SEM, S-2150, Hitachi, Tokyo, Japan) observations of each sample were then performed at numerous magnifications at 10 kV.

As a control, the SEM observations were performed before and after the ground dentin surface was rinsed with an ultrasonic cleaner. Two dentin samples were prepared for the SEM analysis in each experimental group.

Preparation of bonded specimens for the adhesion test

To obtain the mid-coronal dentin surface, the facial enamel surfaces of 128 bovine teeth were ground with a sequence of 100- and 600-grit silicon carbide papers under a stream of water. Thereafter, the ground dentin surface was air-dried for 5 sec, and 80-μm-thick double-faced tape with a circular hole (internal diameter= ϕ3.2 mm, Nichiban, Tokyo, Japan) was then placed on the ground dentin surface. The ground dentin surface inside the circular hole was demineralized by each EX adhesive for 20 sec and was then air-dried with a high-pressure airflow for 10 sec. Light irradiation was applied to the EX adhesive for 10 sec using a Light Curing Unit (Mini LED Ⅲ, Satelec, Viry-Châtillon, France). A 1-mm-thick silicone ring mold with a circular hole (internal diameter= ϕ3.2 mm) was mounted on the double-faced tape. The hole was then immediately filled with a resin composite (Clearfil AP-X, Kuraray Noritake, Tokyo, Japan) and irradiated with light for 20 sec. After the mold and tape were removed, the bonded specimens were immersed in water at 37ºC. The number of specimens was 32 in each experimental group.

Measurement of macro-shear bond strength

After being stored in 37ºC water for 1 day, the 32 bonded specimens prepared for each experimental group were divided into two experimental groups: the before and after thermocycling groups. The 16 bonded specimens in the after thermocycling group were cycled between 5°C and 55°C in water baths for 30,000 cycles. The dwell time in each water bath was 1 min, and the transfer time was 7 sec.

The macro-shear bond strengths of each EX adhesive to the dentin were measured using a universal testing machine (TG-5KN, Minebea Co, Nagano, Japan). The crosshead speed was 1.0 mm/min. The number of specimens in the before and after thermocycling groups was 16 in each group.

Determination of the fracture type of bonded specimens

After the adhesion test, 16 fractured dentin samples for each experimental group were dried at 20°C in a desiccator. The dried dentin samples were then mounted on aluminum stubs and were sputter-coated with a gold-palladium alloy. Thereafter, SEM observations of each sample were performed at numerous magnifications at 10 kV to classify the fracture type. Four categories were scored as follows: Category 0: no adhesive remained on the dentin surface; Category 1: less than half of the adhesive remained on the dentin surface; Category 2: more than half of the adhesive remained on the dentin surface; and Category 3: the cohesive failure of the adhesive and/or dentin.

Statistical analysis

The effect of the amount of water added in the EX adhesive on the dentin bond strength, before or after thermocycling, was analyzed using one-way analysis of variance (ANOVA) and Tukey multiple comparison tests. The effect of thermocycling on the dentin bond strength of each EX adhesive was analyzed using a t-test. The level of statistical significance was set at 0.05.

RESULTS

Effect of the concentration of water in EX adhesives on the efficacy of MDP to demineralize and remove the smear layer from the ground dentin surface

Fig. 1 shows typical SEM images of the ground dentin surface, before and after being demineralized with EX adhesives containing different amounts of water. The ability of MDP depended more on the amount of water added in the EX adhesive than on the pH value to demineralize and remove the smear layer and plugs from the ground dentin surface during the 20-sec application of the EX adhesive to the ground dentin surface. In particular, increasing the amount of water from 46.6 to 93.2 mg/g triggered a change in the calcium site of the ground dentin surface that MDP demineralizes from the smear layer to the underlying dentin, since scratches and outlines of the dentinal tubules appeared over the demineralized dentin surface (Fig. 1d,1D). Further increases in the amount of water led not only to the development of a gap between the peritubular dentin and the smear plug but also to the partial removal of the smear plug from the dentinal tubule (Fig. 1e,1E and 1f,1F). However, the intertubular dentin surfaces demineralized by MDP showed no clear morphological differences among EX-2, -3 and -4 adhesives.

In contrast, when the EX-1 adhesive containing water of 46.6 mg/g was applied (Fig. 1c,1C), the demineralized dentin surface showed a similar surface morphology to the dentin surface ground by a 600-grit silicon carbide paper (Fig. 1a).

However, no evidence on the MDP-Ca salt produced by the demineralization of the smear layer, plugs and underlying dentin were detected on the demineralized dentin surface (Fig. 2), in contrast to the previous study[12)]. This is because the spherical precipitates deposited on the demineralized dentin surface were colloidal silica particles used as reinforcement for the EX adhesive, since the particle size of precipitates was uniform and was the same as the colloidal silica utilized.

Effect of the concentration of water in the EX adhesive on the dentin bonding performance

Fig. 3 shows the effect of the concentration of water in the EX adhesive on the dentin bonding performance, before and after thermocycling. Before thermocycling (Fig. 3, circles), increasing the amount of water from 46.6 to 93.2 mg/g led to a significant decrease in the mean bond strength from 16.0 to 13.3

MPa (p<0.05). At water concentrations above 93.2 mg/g, the mean bond strength leveled off at approximately 12.5 MPa.

After thermocycling, an increase in the amount of water from 46.6 to 93.2 mg/g drastically decreased the mean bond strength from 17.0 to 15.2 MPa (Fig. 3, squares), although the thermocycling led to increases in the mean bond strength of the EX adhesive by 1–3 MPa, respectively. At water concentrations above 93.2 mg/g, the mean bond strength leveled off at approximately 15 MPa. There was no significant difference in the mean bond strengths observed between before and after thermocycling in each experimental group.

Effect of the concentration of water in the EX adhesive on the fracture type of the bonded specimen

Fig. 4 shows typical SEM images of the fractured dentin surface for each EX adhesive, before (Top) and after (Bottom) thermocycling. The fracture types of specimens bonded by each EX adhesive are summarized in Table 1. Before thermocycling (Fig. 4, Top), increasing the amount of water from 46.6 to 93.2 mg/g altered the surface morphology of the fractured dentin surface, since scratches, thin debris of the fractured adhesive and dentinal tubules opened by the peeling off of the resin tags were seen over the fractured dentin surface (Fig. 4b,4B). When the amount of water reached 208.1 mg/g, the fractured peritubular dentin was seen at the circumference inside the opened dentinal tubules (Fig. 4D, white arrows). Furthermore, water bubbles were observed at the fractured adhesive surface (Fig. 4B, 4C and 4D, black arrows).

Reflecting a morphological change in the fractured dentin surface, increasing the amount of water from 46.6 to 93.2 mg/g decreased the number of specimens scored as category 3 (Table 1). Further increases in the amount of water increased the number of specimens that were scored as categories 2 and/or 1.

A drastic morphological change in the fractured dentin surface was also observed after thermocycling when the amount of water was increased from 46.6 to 93.2 mg/g (Fig. 4, Bottom, 4b,4B). However, thermocycling led to a drastic change in the fracture type of the resin tag to a cohesive failure at the same fracture plane of the intertubular dentin, since fractured resin tags were irregularly seen inside the dentinal tubules (Fig. 4b,4c and 4d, white arrows).

The EX-1, -2 and -3 adhesives increased the number of specimens that were scored as categories 3, 2 or 1 during the thermocycling process, respectively (Table 1). In contrast, the EX-4 adhesive increased the number of specimens that were scored as category 0.

DISCUSSION

In this study, 4-META was used for designing an MDP-based 2-hydroxyethyl methacrylate (HEMA)-free all-in-one (EX) adhesives, since 4-MET, which is produced by the hydrolysis of the trimellitic anhydride group in the 4-META, enhances the adhesiveness of the EX adhesive to the dentinal collagen fibers as the same as a HEMA[20)]. However, 4-MET will little yield its calcium salt, even the EX adhesive is exposed to the cut enamel and dentin particles. This was due to MDP yielding a calcium salt more rapidly and preferentially than 4-MET[18)], since an acid dissociation constant (pKa) value of the carboxyl group in the 4-MET was higher than that of the phosphate group in the MDP. In fact, Nishiyama et al.[10,13)] have reported that N-methacryloxy alkyl phosphonic acid (NMAP) forms an acid-base interaction with released calcium ion more preferentially than N-methacryloxy glycine (NMGly), when NMAP-NMGly self-etching primer was applied to enamel and dentin samples. This is because the pKa value of the phosphate group in the NMAP is approximately 60 times greater than that of the carboxyl group in the NMGly[10,13)].

Generally, self-etch adhesives can be classified into four categories: "strong" ($pH<1$), "intermediately strong" ($pH\approx1.5$), "mild" ($pH\approx2$) and "ultra-mild" ($pH\geq2.5$)[8,15,17)]. 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[21,22)]. This is due to the smear layer causing the early neutralization of the acidic monomer utilized in self-etch adhesives[23,24)], since the smear layer consists of the debris of the shattered and cracked dentin apatite and the dentinal collagen fibers[25)].

However, the results obtained showed a different demineralization behavior from the previous studies[21,22)]. This was due to increasing the water concentration in the EX adhesive leading to increases in the ability of MDP to demineralize and remove the smear layer and plugs, even with increasing the pH value of the EX adhesive from 0.87 to 1.68. In particular, increased amount of water from 46.6 to above 93.2 mg/g increased the solubilizability of the smear layer into the EX adhesive. This was due to water not only acting as an ionizing medium for the phosphate group in the MDP but also having a high affinity for the hydrated dentinal components. Therefore, the null hypothesis that the amount of water added in the EX adhesive has no effect on the ability of MDP to demineralize and remove the smear layer from the ground dentin was rejected. These results clearly show that the amount of water added in the EX adhesive affects the demineralization and removal efficacies of the smear layer from the ground dentin surface more strongly than the pH value of the EX adhesive. This was due to the pH value of the EX adhesive being not a specific pH value (just an apparent pH value of the EX adhesive). The observed increases in the pH value of the EX adhesive with increasing the amount of water in an acetone aqueous solution used as a diluent for the resin monomer components are due to a decrease in the apparent dissolution amount of MDP into the water added in the EX adhesive, since MDP is little insoluble in water.

Previously, Nishiyama and coworkers[26-28)] have reported that MDP-Ca salt and DCPD with an amorphous phase are produced during the 30-sec application of EX adhesives to the cut enamel and dentin particles as byproducts. The calcium salt formation of MDP occurs more rapidly and preferentially than the production of DCPD with an amorphous phase[27,28)]. However, the SEM views of the dentin surfaces demineralized by EX adhesives had no evidence on even MDP-Ca salt produced, although EX adhesives containing above 93.2 mg/g of water completely demineralized and removed the smear layer from the ground dentin surface (Fig. 1). The observed results disagreed with the previous study[12)]. This may due to the amount of MDP-Ca salt produced by the demineralization of the smear layer and underlying dentin by EX adhesives being lower than that by Clearfil MEGA Bond Primer. In fact, Fujita et al. have reported that the amount of MDP-Ca salt produced by an MDP-based all-in-one adhesive, so called Clearfil Tri-S Bond is lower than that by Clearfil MEGA Bond Primer [29)]. The other possibility is due to MDP-Ca salt produced being removed from the demineralized dentin surface during the removal process of resin monomer components by acetone and/or during the dehydration process of the demineralized dentin sample by ethanol aqueous solutions containing different amounts of water, respectively. This is due to MDP-Ca salt produced just depositing on the demineralized dentin surface as a removable phase[12)].

The removal of the smear layer and plugs from the ground dentin surface and thus the solubilization of MDP-Ca salt produced by the demineralization of the smear layer and plugs into the EX adhesive seemed to decrease the dentin bond strength and change the fracture type of bonded specimens. Therefore, the null hypothesis that the removal of the smear layer from the ground dentin surface has no effect on the dentin bonding performance of the EX adhesive was rejected. The observed alterations in the dentin bond strength and the fracture type with increasing the amount of water added in the EX adhesive before and after thermocycling, were due to a decrease in the quality of the adhesive layer created by the polymerization of EX adhesives. This decrease in the quality of the adhesive layer may correspond to two factors. First, development of water bubbles in the adhesive layer created by EX adhesive[17,30)]. Second, a decrease in the polymerization conversion of the adhesive layer of the EX adhesive, since increases in the dentin bond strengths of EX adhesives are observed during the thermocycling process by 1–3 MPa. This decrease in the polymerization conversion of the adhesive layer of EX adhesives may be caused by MDP-Ca salt produced during the bonding procedure of each EX adhesive to the ground dentin surface, thus the demineralization of the smear layer and plugs[26-28)].

To clarify the effect of byproducts on the dentin bonding performance of EX adhesives containing different amounts of water, future studies are required for quantitation of each dentin reactant residue of EX adhesives. This is because DCPD with an amorphous phase is also produced along with MDP-Ca salt during the bonding procedure of EX adhesives to the ground dentin surafce[26-28)].

CONCLUSION

Increasing the amounts of water added in the EX adhesive from 46.6 to above 93.2 mg/g drastically increased the ability of MDP to demineralize and remove the smear layer and plugs from the ground dentin surface, even with increasing the pH value of the EX adhesive from 0.87 to 1.68. However, the solubilization of the smear layer and plugs into the EX adhesive decreased the dentin bond strength by approximately 3 MPa before thermocycling and by approximately 2–3 MPa after thermocycling. The amount of water included in the EX adhesive affected the efficacies of smear layer removal and dentin bonding performance more strongly than the pH value of the EX adhesive.

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Figure Legends

Figure 1 Typical SEM images of the ground dentin surface, before and after demineralization by EX adhesives containing different amounts of water.

The magnifications of SEM images a-f and C-F are ×2,500-fold and ×5,000-fold, respectively. a: the ground dentin surface, b: the ground dentin surface after ultrasonic cleaning, c&C: the ground dentin surface demineralized by the EX-1 adhesive, d&D: the ground dentin surface demineralized by the EX-2 adhesive, e&E: the ground dentin surface demineralized by the EX-3 adhesive, and f&F: the ground dentin surface demineralized by the EX-4 adhesive.

Figure 2 Typical SEM images of the dentin surface demineralized by EX adhesives containing different amounts of water.

The magnification of SEM images a-d is ×15,000-fold. a: the dentin surface demineralized by the EX-1 adhesive, b: the dentin surface demineralized by the EX-2 adhesive, c: the dentin surface demineralized by the EX-3 adhesive, and d: the dentin surface demineralized by the EX-4 adhesive.

Figure 3 Effect of the concentration of water in the EX adhesive on the mean bond strength to the ground dentin surface, before (circles) and after (squares) thermocycling (30,000 times).

The number of bonded specimens in each experimental group was 16. Error bars show the SDs. The small characters (a and b) indicate a significant difference in the mean bond strength, before thermocycling, respectively ($p < 0.05$).

Figure 4 Typical SEM images of the fractured dentin surface bonded by EX adhesives containing different amounts of water, before (Top) and after (Bottom) thermocycling.

The magnifications of the SEM images in the upper and lower panels are ×100-fold and 1,000-fold, respectively. The category in each image denotes the fracture type of the corresponding sample. a&A: the fractured dentin surface bonded by the EX-1 adhesive, b&B: the fractured dentin surface bonded by the EX-2 adhesive, c&C: the fractured dentin surface bonded by the EX-3 adhesive, and d&D: the fractured dentin surface bonded by the EX-4 adhesive.

FA: Fractured adhesive; FD: Fractured dentin; DS: Dentin surface.

Before thermocycling (Top): When the EX-1 adhesive containing 46.6 mg/g of water was applied, the cohesive failure of the adhesive and/or dentin was observed. The addition of water in the EX adhesive more than 46.6 mg/g led to the appearance of scratches, debris of the fractured adhesive, and a large number of opened dentinal tubules where the resin tags had peeled off from the dentinal tubules. In particular, increasing the amount of water to 208.1 mg/g led to appearance of the fractured peritubular dentin at the circumference inside the opened dentinal tubules (white arrows), and of water babbles were seen on the fractured adhesive surface (black arrows).

After thermocycling (Bottom): Similar to before the thermocycling, when the amount of water added in the EX adhesive more than 46.6 mg/g led to the appearance of scratches, debris of the fractured adhesive, and opened dentinal tubules. However, since the fracture type of the resin tag changed from an interfacial failure from the peritubular dentin inside the dentinal tubules to a cohesive failure in the same plane at the intertubular dentin during the thermocycling process, the fractured resin tags are partially seen inside the closed dentinal tubules, as marked by the white arrows.

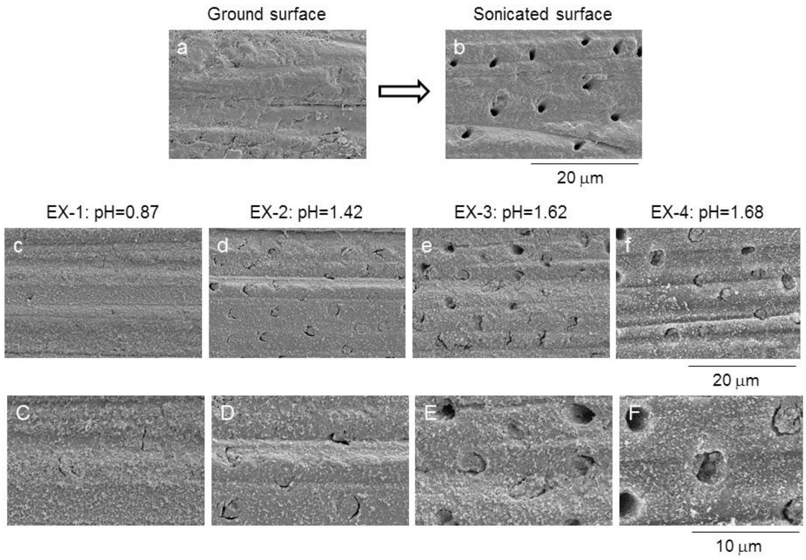

Ground surface
Sonicated surface
a
b
20 μm
EX-1: pH=0.87
EX-2: pH=1.42
EX-3: pH=1.62
EX-4: pH=1.68
c
d
e
f
20 μm
C
D
E
F
10 μm

Figure 1

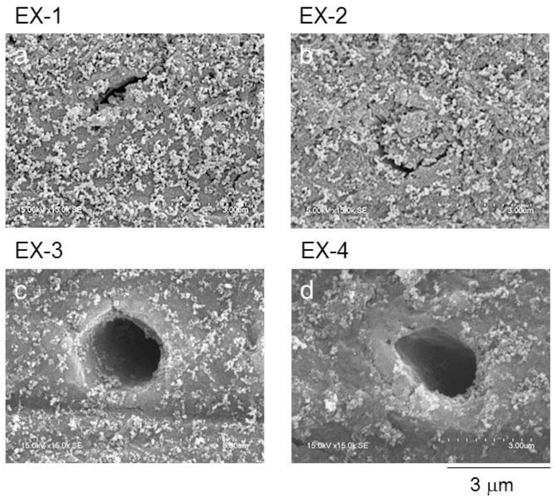
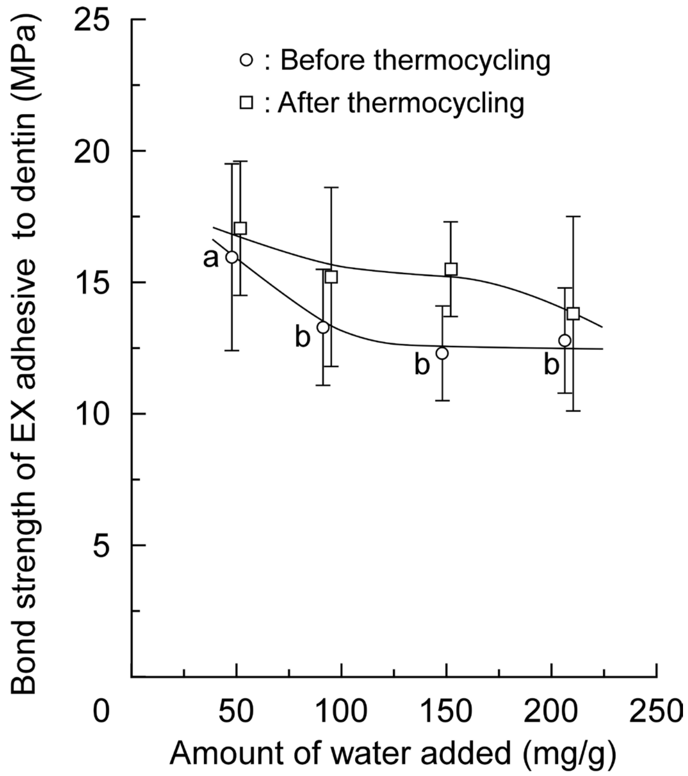
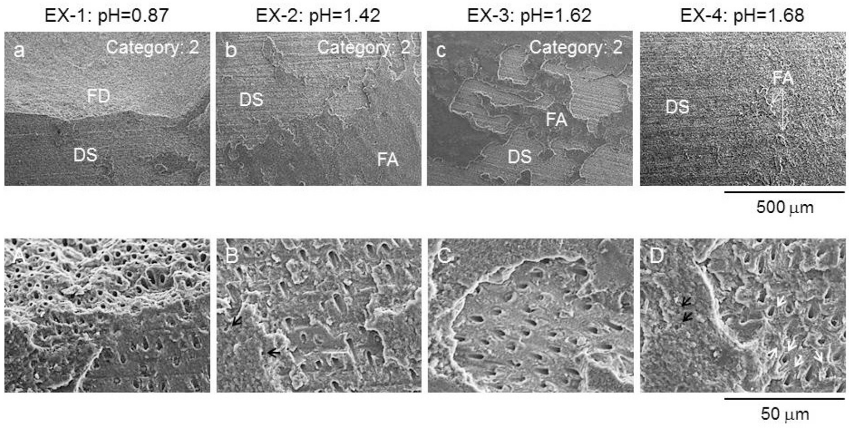


Figure 2

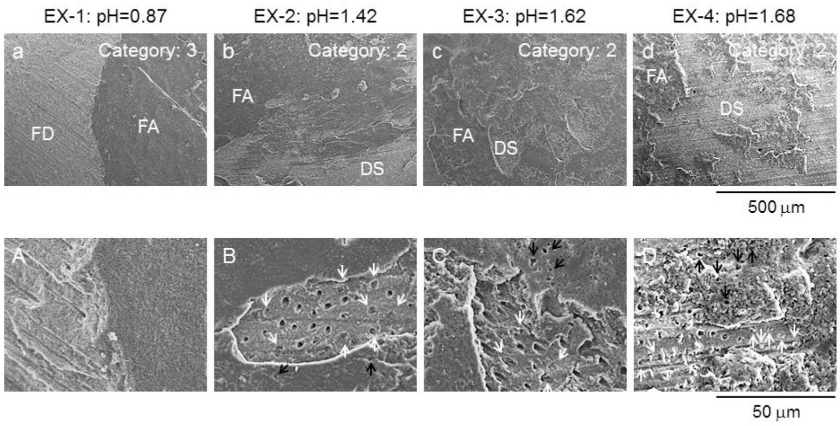


25
20
15
10
5
0
50
100
150
200
250
○ : Before thermocycling
□ : After thermocycling
a
b
b
b
Bond strength of EX adhesive to dentin (MPa)
Amount of water added (mg/g)

Figure 3



Top



Bottom

Figure 4

Table 1 Type of fracture mode (Category: [0/1/2/3]) of the bonded specimens, before and after thermocycling, by varying the amount of water added in the EX adhesive.

Adhesive	Amount of water		Fracture type
(mg/g)	Before	After	
EX-1	46.6	[0/0/10/6]	[0/2/6/8]
EX-2	93.2	[0/3/10/3]	[0/2/12/2]
EX-3	149.8	[0/5/10/1]	[0/5/8/3]
EX-4	208.1	[0/6/9/1]	[3/5/7/1]

[0/1/2/3]: category 0: no adhesive remained on the dentin surface; category 1: less than half of the adhesive remained on the dentin surface; category 2: more than half of the adhesive remained on the dentin surface; and category 3: a cohesive failure of the resin and/or dentin.