Original

Effect of fluoride mouthrinse and fluoride concentration on bonding of a one-step self-etch adhesive to bovine root dentin

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Abstract: This study investigated the effect of fluoride mouthrinses on bonding of a one-step self-etch adhesive to bovine root dentin. Application of a NaF solution (0, 450, 900, or 9,000 ppm) to bovine root dentin surfaces was performed for 30 s (immediate) or before bonding of an all-in-one adhesive. Micro-tensile bond strength (μ TBS) testing and scanning electron microscopic (SEM) observation of the acid-base resistance zone (ABRZ) were performed. μ TBS values and calculated ABRZ areas were analyzed by using two-way ANOVA and the *t*-test with Bonferroni correction. The significance level was set at $P = 0.05$ ($n = 30$). Fracture mode was analyzed with the Mann-Whitney *U* test with Bonferroni correction. The significance level set at $P = 0.00178$ ($n = 30$). μ TBS did not significantly differ between the control, 450 ppm F, and 900 ppm F specimens ($P > 0.05$) but was significantly lower in 9,000 ppm F specimens ($P < 0.05$). SEM showed a significant increase in acid

resistance at the adhesive-dentin interface, including the ABRZ, after fluoride application.

Keywords: fluoride mouthrinse; bovine root dentin; one-step self-etching adhesive; μ TBS; ABRZ.

Introduction

Because of the rapidly increasing number of older people and important improvements in oral health outcomes, an increasing number of elders are retaining a substantial number of teeth for their entire life (1). Challenges in the treatment of root caries include moisture control, limited adhesion to dentin as the sole adhesive substrate, and lack of retention form in often shallow, saucer-shaped root lesions (2). To manage these complex circumstances and limit damage associated with root caries, multiple approaches and aggressive preventive strategies are required (1,3).

Fluoride ion penetration into dentin generally enhances remineralization and increases acid resistance of dentin against demineralization (4-7). Fluoride helps prevent caries in older people, and some evidence indicates that higher fluoride concentrations and use of multiple methods of fluoride delivery increase the benefit (1). A daily oral rinse with 15 mL of 0.2% neutral NaF mouthrinse was significantly better than a 0.12% chlorhexidine solution or placebo mouthrinse in improving caries

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Table 1 Materials used in this study

	Brand name	Code	Manufacturer	Composition
fluoride mouthrinse	Miranol granules 11%	-	Bee Brand Medico Dental, Osaka, Japan	sodium fluoride, <i>D</i> -mannitol, xylitol, macrogol 6000, sodium dihydrogen phosphate dihydrate, cetylpyridiniumchloride, ethylparahydroxybenzoate, propyl-parahydroxybenzoate, hydroxypropylcellulose, cinnamon oil, <i>L</i> -menthol, perfume
adhesive system	Clearfil SE ONE	SEO	Kuraray Noritake Dental, Tokyo, Japan	MDP, Bis-GMA, HEMA, hydrophobic DMA, sodium fluoride, silanated colloidal silica, accelerators, initiator, CQ, ethanol, water (pH 2.3)

Bis-GMA: bisphenol-A glycidyl methacrylate; CQ: camphorquinone; DET: N, N-diethanol *p*-toluidine; DMA: dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloxdecyl dihydrogen phosphate

resistance among elders receiving long-term care (8).

The concept of minimal intervention dictates that intact tooth structures are preferable to restoration placement. Non-carious cervical lesions (NCCLs), which are characterized by loss of hard tissue at the cemento-enamel junction in the absence of caries, are common in dental practice (9,10). Patients with progressive NCCLs will require direct resin composite restorations without aggressive surface preparation, because of the absence of bacterial infection. However, the treatment approach for NCCLs is controversial (11). The dentin surface of NCCLs can often be simply cleaned by brushing with a fluoride-containing toothpaste or, in practice, a prophylaxis paste, thus exposing the dentin surface to fluoride via toothpastes and mouthrinses over a long period. This process creates a sclerotic surface that is resistant to demineralization.

Dental adhesive systems have been remarkably simplified and improved. All-in-one self-etch adhesive systems are now widely used by clinicians for direct composite restoration placement (12), because of their straightforward application procedures. In addition, incorporation of fluoride-containing particles in adhesive systems, which inhibits recurrent caries, has become increasingly popular.

Ultrastructural studies of the adhesive-dentin interface after acid-base challenge have investigated the degradative mechanisms of recurrent caries at restoration margins. Scanning electron microscopy (SEM) and transmission electron microscopy revealed formation of an "acid-base resistant zone" (ABRZ) beneath the hybrid layer, when dentin was treated with a self-etch adhesive system (13,14). The morphology of the ABRZ is influenced by the composition of the adhesive system used, especially when fluoride is released from the adhesive. However, there is no information on the morphological effects of applying a fluoride solution to dentin surfaces before bonding. Therefore, this study investigated the

effect of treatment with different concentrations of fluoride mouthrinse on bonding of a one-step self-etch adhesive to bovine root dentin. Treatment effects were assessed by using the microtensile bond strength (μ TBS) test and SEM observation of the ABRZ formation.

Materials and Methods

The composition of the fluoride mouthrinse and adhesive system used in this study are shown in Table 1. The 450 ppm F (everyday use; pH 5.3) and 900 ppm F (once weekly; pH 5.4) NaF solutions were prepared by mixing one package (1.8 g) of the mouthrinse kit (MG, MIRANOL Granules 11%, Bee Brand Medico Dental, Osaka, Japan) with 200 mL and 100 mL distilled water, respectively. The 9,000 ppm F solution was prepared by adding NaF (Wako Pure Chemical, Osaka, Japan) to the 900 ppm F solution, to adjust the concentration of the solution to 9,000 ppm (pH 6.5).

A one-step self-etch adhesive, Clearfil SE ONE (SEO, Kuraray Noritake Dental, Tokyo, Japan), was used in this study. The SEO adhesive includes the functional monomer 10-methacryloxyl dihydrogen phosphate (MDP) and specially treated NaF particles (12,14,15).

μ TBS testing

A schematic outline of the μ TBS test procedure is presented in Fig. 1. The roots of bovine teeth were perpendicularly divided into two parts with a low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) under water cooling. Each root segment was embedded in an epoxy resin (Epoxicure Resin, Buehler), and the root dentin surface was ground with # 600-grit SiC paper to produce a standardized smear layer. The specimens were then divided into two main groups according to storage period. In the immediate group, dentin surfaces were treated with 100 μ L of 450-, 900-, or 9,000-ppm F solutions for 30 s, rinsed with distilled water, and dried gently. In the 1-month group, dentin

Fig. 1 Schematic illustration of the method used to prepare specimens for μ TBS measurement.

surfaces were treated, as described below, with one of the three fluoride mouthrinses for 1 month before bonding. According to the manufacturer's instructions, treatment with the 450-ppm F solution was performed for 30 s every day, whereas treatments with the 900- and 9,000-ppm F solutions were conducted for 30 s on a weekly basis over a period of 1 month, i.e., for four exposures. All specimens were stored in artificial saliva (1.0 mM CaCl_2 , 3.0 mM KH_2PO_4 , 100 mM acetate, 100 mM, NaCl, 0.02% NaN_2 ; pH 6.3) (3,16,17) at 37°C between fluoride treatments.

The specimens were then bonded with the SEO adhesive, in accordance with the manufacturer's instructions. A halogen light-curing unit (Optilux 501; Demetrom, Danbury, CT, USA) was used for light curing of the adhesive. A resin composite (Clearfil AP-X; shade A2, Kuraray Noritake Dental) was placed in two increments (2-mm-thick each) and light-cured for 40 s each. After 24-h storage in 37°C distilled water, the bonded specimens were sectioned perpendicularly into serial slabs at the resin-dentin interface. Each slab was further sectioned into 1.0×1.0 -mm resin-dentin beams. The specimens were then fixed with an adhesive (Model Repair; Dentsply-Sankin, Ohtawara, Japan) and stressed in tension at a crosshead speed of 1 mm/min by using a universal testing device (EZ-test; Shimadzu, Kyoto, Japan).

Failure mode analysis

After μ TBS testing, the debonded specimens of the dentin side were gold sputter-coated and observed under SEM (JSM-5310LV; JEOL, Tokyo, Japan). Fracture mode was assessed at nine areas in each specimen. In each area, failure mode was recorded as complete adhesive failure at the resin-dentin interface (A), complete cohesive

Fig. 2 Schematic illustration of the method used to prepare specimens for ABRZ.

failure in the resin composite (R), complete cohesive failure in the dentin (D), or complete cohesive failure in the bonding resin (B). The percentages of specimens in these fracture categories were calculated based on the frequency of the fracture mode observed for a total of 270 areas in each experimental group (30 specimens with nine areas each).

SEM observation of ABRZ formation

Samples for SEM observation of ABRZ formation were prepared as described previously (4,5,7,12,18) (Fig. 2). Bovine root dentin blocks were cut parallel to the tooth axis with a low-speed diamond saw (Isomet; Buehler) and embedded in epoxy resin (Epocure Resin; Buehler). The root dentin surfaces were then ground with #600-grit SiC paper under a stream of water. The aforementioned methods of fluoride mouthrinse treatment were applied for 1 day or 1 month. Then, the dentin surface of each block was treated with SEO adhesive in accordance with the manufacturer's instructions. A flowable resin composite (Estelite Flow Quick; Tokuyama Dental, Tokyo, Japan) was then placed between pairs of prepared dentin root samples and light-cured for 40 s from the top and bottom surfaces to create a root dentin-adhesive sandwich (8,19). After the bonded specimens were stored in distilled water for 24 h, each specimen was sectioned perpendicularly to the adhesive-dentin interface with a diamond saw and embedded in epoxy resin. The bonded specimens were subjected to acid-base challenges by using a previously described protocol (7). Each specimen was initially stored in 100 mL of a buffered demineralizing solution (2.2 mol/L CaCl_2 , 2.2 mmol/L NaH_2PO_4 , and 50 mmol/L acetic acid at pH 4.5) for 50 min to create artificial recurrent caries. The specimens were then immersed in 5% NaClO for 20 min, to remove any demineralized dentin

Fig. 3 Schematic illustration of the area of the ABRZ. The defined area, including the ABRZ (red part) and dentin (hatched area), was measured with Image J after SEM observation. B: bonding, CR: composite resin, D: dentin, HL: hybrid layer, OL: outer lesion.

collagen fibrils, and rinsed with running water for 30 s. Then, a 4-META/MMA-TBB resin (Super-Bond C&B; Sun Medical, Moriyama, Japan) was applied, without acid-etching of the treated surface, to prevent wear of the remaining structure during polishing. The specimens were then cut perpendicularly to the adhesive-dentin interface and polished with diamond pastes (Struers A/S, Copenhagen, Denmark) down to a particle size of 0.25 µm. An argon-ion etching device (EIS-IE; Elionix, Tokyo, Japan) was used for the polished surface to bring the adhesive-dentin interface into sharp relief (accelerating voltage, 1 kV; ion current density, 0.2 mA/cm², 30 s). Finally, the specimens were gold sputter-coated, and SEM (S-4500; HITACHI, Ltd. Tokyo, Japan) at $\times 3,500$ magnification was used to observe morphological changes in the adhesive-dentin interface caused by the acid-base challenge.

Image J analysis of the ABRZ area

To compare acid-base resistance at the interface quantitatively, a square frame (15 $\times$ 15 µm) was superimposed on the SEM image, as shown in Fig. 3. The left side of the frame was placed on the hybrid layer at the adhesive interface, and the upper side of the frame was placed on the original surface of the bonded specimens. The defined area including the ABRZ and dentin were measured by using the region of interest (ROI) mode of a digital image analysis software program (Image J; NIH, Bethesda, MA, USA).

Statistical analysis

The Shapiro-Wilk test indicated normal data distributions in all groups. μ TBS values were analyzed with two-way ANOVA and the *t*-test with Bonferroni correction.

Table 2 μ TBS values of SEO to dentin (MPa)

	Immediate	1 month
control	70.4 $\pm$ 10.6 ^{a,A}	60.5 $\pm$ 9.2 ^{d,A}
450 ppm F	71.1 $\pm$ 7.3 ^{b,B}	58.6 $\pm$ 10.1 ^{e,B}
900 ppm F	66.4 $\pm$ 6.6 ^{c,C}	55.2 $\pm$ 8.8 ^C
9,000 ppm F	57.7 $\pm$ 5.5 ^{a,b,c,D}	50.0 $\pm$ 5.1 ^{d,e,D}

Values represent mean $\pm$ SD, *n* = 30 per group.

Within each column, values indicated by the same lowercase letters are significantly different.

Within each row, values indicated by the same capital letters are significantly different.

(two-way ANOVA and *t*-test with Bonferroni correction, *P* < 0.05).

tion. The significance level was set at *P* = 0.05 (*n* = 30). Fracture mode was analyzed with the Mann-Whitney *U* test with the significance level set at *P* = 0.00178 (*n* = 30). The calculated area for each group was analyzed with two-way ANOVA and the *t*-test with Bonferroni correction. The significance level was set at *P* = 0.05. Statistical analyses were performed with SPSS software, ver. 22 (IBM, Chicago, IL, USA).

Results

μ TBS measurement

The μ TBS values of SEO to the dentin are summarized in Table 2. The Shapiro-Wilk test showed a non-normal distribution of μ TBS data in the immediate 900 ppm F group (*P* < 0.05). However, the data were normally distributed according to the guidelines of the SPSS software, in which a skewness value of greater than twice the standard error is defined as a departure from symmetry (Montgomery et al., Applied statistics and probability for engineers, 4th ed., John Wiley & Sons, Inc., 239-240, 2006). μ TBS values were significantly higher in the immediate group than in the 1-month group for all treatment groups (*P* < 0.05, two-way ANOVA and *t*-test with Bonferroni correction). μ TBS did not differ between the 450 ppm F, 900 ppm F, and control groups (*P* > 0.05) in the immediate or 1-month group; however, μ TBS values were significantly lower for the 9,000 ppm F group than for the other groups in the immediate group (*P* < 0.05). In the 1-month group, μ TBS was significantly lower for the 9,000 ppm F specimens than for the other specimens combined (*P* < 0.05).

Fracture mode analysis

The results of fracture mode analysis of debonded specimens after μ TBS testing are summarized in Table 3. Figure 4 shows typical SEM images on the dentin side of debonded specimens from the control (immediate) and 9,000 ppm F (1 month) specimens. The Mann-Whitney *U* test with Bonferroni correction was used for fracture

Table 3 The results of the fracture mode analysis of the debonded area (%)

Mouthrinse	Immediate (A/R/D/B)	1 month (A/R/D/B)
control	22.6/15.2 /24.2/38.0	27.8/6.9 ^b /36.5 /28.8
450 ppm F	23.0/13.3/40.7/23.0	41.8/13.0/29.3/15.9
900 ppm F	12.2 ^a /26.5/17.9/43.4	23.0 /20.3/25.2/31.5
9,000 ppm F	41.5 ^a /21.1/11.1/26.3	35.2/28.5 ^b /13.3/23.0

Values identified with same letters are significantly different. (Mann-Whitney *U* test. $P < 0.00178$). Complete adhesive failure at the resin-dentin interface (A), complete cohesive failure in resin composite (R), complete cohesive failure in dentin (D), complete cohesive failure in bonding resin (B).

Fig. 4 Representative SEM images of the dentin side of debonded specimens.

a. Control-Immediate group/ b. 9,000 ppm F-1-month group

A: adhesive failure at the resin-dentin interface, B: cohesive failure in the bonding resin, R: cohesive failure in the resin composite, D: cohesive failure in dentin.

Fig. 5 SEM images of the adhesive-dentin interface after acid-base challenge (magnification $\times 3,500$). ABRZ: acid-base resistant zone, B: bonding, CR: composite resin, D: dentin, OL: outer lesion.

mode analysis. To avoid accumulation of errors from multiple comparisons, the significance level was adjusted for the number of comparisons.

In the immediate group, the proportions of fracture mode A (resin-dentin interface) specimens significantly differed between the 900 ppm F and 9,000 ppm specimens ($P < 0.00178$). In the 1-month group ($P < 0.00178$), the proportions of fracture mode R (resin composite cohesive failure) specimens significantly differed between the control and 9,000 ppm F specimens ($P < 0.00178$). A representative image of a control specimen shows bonding resin cohesive failure (B), which was present in about half the observed area. In contrast, in a 1-month

9,000 ppm F specimen, more than half the area exhibited mode R failure.

SEM observation of the adhesive-dentin interface after acid-base challenge

Representative interfacial morphologies in adhesive and dentin after acid-base challenge are shown in Fig. 5. SEM images show bonding between adhesive and dentin after the acid-base challenge. Outer lesions (OL), which develop because of mineral loss after the acid-base challenge, were observed in all groups. However, OL depth varied, which suggests that acid resistance varies in relation to differences in fluoride application.

Table 4 The area inside of the flame ($15 \times 15 \mu\text{m}^2$) demarcated (μm^2)

Fluoride concentration	Immediate	1 month
control	$50.9 \pm 17.7^{\text{a,A}}$	$50.6 \pm 13.6^{\text{f,B}}$
450 ppm F	$72.3 \pm 16.7^{\text{a,b,C}}$	$88.3 \pm 14.7^{\text{f,g,C}}$
900 ppm F	$77.8 \pm 14.3^{\text{a,c,D}}$	$77.6 \pm 17.8^{\text{f,h,E}}$
9,000 ppm F	$71.0 \pm 14.8^{\text{a,c,F}}$	$110.0 \pm 12.4^{\text{f,g,h,F}}$

Values represent mean $\pm$ SD, $n = 20$ per group.

Within each column, values indicated by the same lowercase letter are significantly different.

Within each row, values indicated by the same capital letters are significantly different.

(Two-way ANOVA and t -test with Bonferroni correction, $P < 0.05$).

In all specimens, the hybrid layer was barely visible at the adhesive-dentin interface. In the immediate group, ABRZ thickness measured at the mid-portion of the OL was about $1.5 \mu\text{m}$ in the control group. A sloped ABRZ formation was seen in all groups, particularly in the 450, 900, and 9,000 ppm F groups. As the concentration of fluoride mouthrinse increased, ABRZ thickness and angle tended to increase. In the 1-month group, ABRZ thickness (approximately $3.0 \mu\text{m}$) was greater in the control group than in the immediate group. Sloped ABRZ formation in the 1-month groups was much larger than that observed in the immediate groups.

Table 4 shows changes in the size of the demarcated area inside the frame ($15 \times 15 \mu\text{m}$). The normality of the data distribution was first analyzed with the Shapiro-Wilk test. Two-way ANOVA and the t -test with Bonferroni correction revealed that the areas in the 1-month group were significantly larger than those in the immediate groups for 450 ppm F and 9,000 ppm F specimens ($P < 0.05$). In the immediate groups, the areas of the 450, 900, and 9,000 ppm F groups were significantly larger than those of the control group ($P < 0.05$). However, no significant difference was observed between these three treatment groups ($P > 0.05$). Among the 1-month groups, the 9,000 ppm F group had the largest area ($P < 0.05$), followed by the 900 and 450 ppm F groups, but there was no significant difference between the two latter groups ($P > 0.05$). The area of control group specimens was smaller than those of specimens in both the immediate and 1-month groups ($P < 0.05$).

Discussion

The bonded specimens were prepared immediately or 1 month after fluoride treatments. Frequent exposure of dental tissues to fluoride solutions is beneficial for arresting caries lesions, as well as for caries prevention (20). Artificial saliva, which is composed of the mineral components of saliva, is likely to closely replicate dentin remineralization in the oral cavity (3,21,22). In the present study, to simulate frequent use of fluoride mouth-

rinses at home, dentin specimens were placed in artificial saliva for 1 month.

SEO is a one-step self-etch adhesive system that contains the functional monomer MDP and sodium fluoride. MDP has several important roles: it etches tooth substrates, enhances monomer penetration, and interacts chemically with hydroxyapatite (12,23-25). MDP was reported to have strong chemical bonding potential with hydroxyapatite (26-28). In addition, fluoride release from adhesive systems affected the level of dentin acid resistance at the bonding interface (5,7).

The bond strength of SEO to dentin did not differ between control, 450 ppm F, and 900 ppm F specimens in the immediate or 1-month group. This suggests that regular use of lower-concentration fluoride mouthrinses does not adversely affect dentin bonding performance of the SEO adhesive. However, μTBS values were significantly lower in 9,000 ppm F specimens ($P < 0.05$). High NaF concentrations are believed to increase the acid resistance of dentin (28-30) and, possibly, reduce the effect of the self-etching capability of SEO. μTBS values in the 1-month groups were significantly lower than those in the immediate groups, including the control group (without fluoride treatment) ($P < 0.05$). The storage of teeth in artificial saliva before bonding may have enhanced the mineralization of the smear layer on the ground dentin, thereby making it difficult for the SEO adhesive to remove the smear layer and penetrate the underlying dentin. The lowest μTBS value was obtained in the 9,000 ppm F group after 1 month of storage in artificial saliva, which are the most severe conditions for dentin bonding when using self-etch adhesive.

Fracture mode analysis of debonded specimens showed significantly higher ratios of adhesive failure in 9,000 ppm F specimens in the immediate and 1-month groups, which also highlights the effects of high fluoride concentration on dentin bonding ($P < 0.05$).

A previous study reported that, when a self-etch adhesive was used, ABRZ formed adjacent to the hybrid layer after acid-base challenge (13); however,

ABRZ morphology depended on the adhesive material. Although a previous report found that a funnel-shaped lesion was created below the ABRZ when an all-in-one adhesive system was used (14), this was not seen in the present SEM images.

When a fluoride-containing adhesive was used, a sloped ABRZ formed at the bottom of the ABRZ (6,14). However, the sloped ABRZ seemed to be related to fluoride concentration in the adhesive resin, which had to attain a threshold level to form the sloped ABRZ that occurred with the two-step self-etch adhesive system (7). The current study demonstrated slope formation of ABRZ with the one-step self-etch adhesive of SEO, which contains a specially treated NaF-containing adhesive. In addition, the area of acid resistance of the bonded interface on the dentin side increased with application of the fluoride mouthrinse. Acid resistance of the bonded interface was quantitatively assessed by using the ROI mode of the digital image analysis software (7). In the immediate group, acid-resistant areas treated with 450, 900, and 9,000 ppm F mouthrinses were significantly larger than those that were not treated ($P < 0.05$) (Table 4). However, there was no significant difference in area between 450, 900, and 9,000 ppm F NaF specimens ($P > 0.05$). Thus, the application of 450 ppm NaF was sufficient to increase acid resistance at the interface. This finding is clinically meaningful, because the 450 ppm NaF concentration is the same as that of the daily-use fluoride mouthrinse and did not adversely affect the dentin bonding performance of SEO in this study. The area was significantly larger in the 1-month groups than in the immediate groups ($P < 0.05$). This suggests that repeated fluoride treatments enhance the acid resistance of the adhesive-dentin interface ($P < 0.05$). Future studies should evaluate the effects of different concentrations of fluoride mouthrinses on dentin bonding durability. Fluoride mouthrinses containing 450 and 900 ppm F NaF did not affect μ TBS testing of the all-in-one adhesive. However, a high fluoride concentration had adverse effects on μ TBS. The resistance of interfacial areas, including the ABRZ, was significantly enhanced after fluoride application.

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Conflict of interest

The authors have no commercial interests in the products herein investigated.

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