Effect of fluoride concentration in adhesives on morphology of acid-base resistant zones

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This study aimed to investigate the effect of fluoride concentration in adhesives on morphology of acid-base resistant zone (ABRZ). Seven experimental adhesives with different concentrations of NaF (0 wt%; F0 to 100 wt%: F100) were prepared based on the formulation of a commercially available adhesive (Clearfil Protect Bond, F100). The resin-dentin interface of the bonded specimen was subjected to demineralizing solution and NaOCl, sectioned, polished and argon-ion etched for SEM observation. Fluoride release from each adhesive was measured using an ion-selective electrode. Fluoride ion release from the adhesive linearly increased with higher NaF concentration. The ABRZ area increased significantly with higher NaF concentration except for F0, F10, and F20 ($p < 0.05$). F100 showed the largest ABRZ, where a slope of acid-resistant dentin was clearly observed at the bottom of the ABRZ. The concentration of NaF in the two-step self-etching adhesive resin influenced the amount of dentin structure remaining after acid-challenge.

Keywords: Fluoride release, Acid-base resistant zone, SEM observation, Self-etching primer system, Dentin bonding

INTRODUCTION

Secondary caries around existing restorations is thought to be caused by a bacterial acid attack initiated from the defective margins of the restoration. Fluoride has been incorporated into adhesive systems to make attempt to inhibit secondary caries around restorations. Some studies reported that fluoride-containing adhesive systems could contribute to the inhibition of secondary caries and recurrent caries¹⁻⁵.

Tsuchiya *et al.* performed SEM observation on dentin-adhesive interfaces and reported the presence of an acid-base resistant zone (ABRZ) beneath the hybrid layer in self-etching adhesive systems after acid-base challenge⁶. Selected area electron diffraction analysis of the zone demonstrated that this zone contained densely arranged apatite crystallites, that had different characteristics from the hybrid layer⁶. Under TEM observation, the crystallites appeared to be enveloped and protected against acid-attack beneath the hybrid-layer of self-etching adhesive systems containing a functional monomer⁷. In particular, the role of chemical interactions between dental apatite and 10-methacryloyloxydecyl dihydrogen phosphate (MDP) in the acid-resistance of dentin has been emphasized in the previous works^{3,7-11}.

Tsujimoto *et al.* reported that morphological features of the interface were influenced by the ingredients of both adhesive material and acidic solution¹². Some researchers reported that the morphological features

of the interface were modified by the fluoride-releasing adhesive systems; Shinohara *et al.* reported that a delta-shaped region of ABRZ was formed from the upper slope to the end of outer lesion when the fluoride-releasing two-step self-etching adhesive system, Clearfil Protect Bond (F100; Kuraray Noritake Dental, Tokyo, Japan) was used⁴. Iida *et al.* reported that the morphological features were different between two fluoride-releasing adhesives Fluorobond and Fluorobond II (Shofu, Kyoto, Japan), suggesting the amount of fluoride release from the adhesive systems affected the level of dentin acid-resistance at the interface¹. Ichikawa *et al.* reported that the interface created at the dentin-adhesive interface by a fluoride-releasing adhesive was more stable than that of a similar fluoride-free adhesive after long-term durability test of the ABRZ¹³. However, there is no quantitative approach in previous reports and the effect of fluoride concentration released from the adhesive on the morphological features of the interface after acid-attack was still unclear.

According to the previous reports, it can be inferred that there should be a minimum threshold for fluoride release to affect the acid-resistance of interfacial dentin. Therefore, the aim of this study was to evaluate the effect of fluoride content in the formulation of two-step self-etching adhesives on the ABRZ by measuring fluoride release amount from adhesives and analyzing the area of ABRZ quantitatively. Seven experimental adhesives with different concentration of sodium fluoride were prepared and tested. The null hypothesis of this study was that amount of fluoride release from the adhesives

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would not influence the amount of dentin reinforced against acid attack at the interface.

MATERIALS AND METHODS

Materials used in this study

Composition of the experimental adhesive materials used in this study was listed in Table 1. Each experimental two-step self-etch adhesive system used the same non-fluoride containing primer from F100, and the difference among their bonding agents was only in the fluoride concentration, which was in proportion to that of the F100 bonding agent (0, 10, 20, 50, 75, 90, and 100 wt%, respectively). In this manner, seven materials with

different concentrations of NaF (F0, F10, F20, F50, F75, F90, and F100) were used.

Specimen preparation for SEM observation

Sample preparation was carried out according to the previous study⁸⁾, as shown in Fig. 1. Twenty-one caries free human molars were used in this study after the individuals' informed consent was obtained according to a protocol approved by the Human Research Ethics Committee, Tokyo Medical and Dental University (No. 725). The teeth were cut vertically to the tooth axis with a low speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) to obtain 1 mm-thick dentin disks. The dentin surfaces were ground with #600-grit silicon

Table 1 Composition of the experimental two-step self-etching adhesive systems used in this study

Adhesive system	Batch No.	Composition	Directions
Primer	0056A	MDP, MDPB, HEMA, hydrophilic dimethacrylate, water, initiator (pH=2.0)	Apply primer for 20 s, gently air blow.
Adhesive		NaF wt%*	
F0	090526	0	
F10	091111	10	
F20	091111	20	
F50	091111	50	
F75	101008	75	
F90	101214	90	
F100*	00092A	100	

*The percentages are relative to the concentration of NaF in original Clearfil Protect Bond.

Bis-GMA: 2,2-bis[4-(2-hydroxy-3-methacryloyloxy-propoxy)-phenyl]propane; CQ: camphorquinone; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxydecyl dihydrogen phosphate; MDPB: 12-methacryloyloxydodecylpyridinium bromide.

Fig. 1 Schematic illustration of specimen preparation procedures.

carbide paper under running water for 20 s. One of the experimental adhesive systems was applied to the dentin surface according to the manufacturer's instructions (Table 1) and light-cured for 10 s with a light curing unit (XL 3000, 3M ESPE, St. Paul, USA). The intensity of the light source was checked with a radiometer prior to the study. An output of 540 mW/cm² was confirmed. A flowable resin composite (Majesty LV shade A2, Kuraray Noritake Dental) was then placed between each pair of the prepared dentin disks and light-cured for 40 s from the top and bottom surfaces to make a dentin disk sandwich⁸. All the specimens were stored in water at 37°C for 24 h. Each specimen was sectioned vertically through the center perpendicular to the dentin surface and embedded in epoxy resin (Epocure Resin, Buehler). Following this, the specimen was stored in 100 mL of a buffered demineralizing solution,

containing 2.2 mmol/L CaCl₂, 2.2 mmol/L NaH₂PO₄ and 50 mmol/L acetic acid adjusted at pH 4.5 for 90 min to create artificial secondary caries¹⁴. The specimens were then immersed in 5% NaOCl for 20 min in an attempt to remove any exposed demineralized dentin collagen fibrils, and rinsed with running water for 30 s. Following this, a 4-META/MMA-TBB resin (Super Bond C&B, Sun Medical, Moriyama, Japan) was applied without acid-etching of the treated surface in order to prevent wear of the edge of the adhesive resin during polishing. After curing of the 4-META/MMA-TBB resin, the specimens were sectioned perpendicular to the dentin-adhesive interface, and reduced to approximately 1-mm thickness with #1200-grit silicon carbide paper. The cross-section was then polished with diamond pastes (Struers A/S, Copenhagen, Denmark) with particle sizes of 6 µm, 3 µm, 1 µm and 0.25 µm, sequentially. The specimens were left in a desiccator for 24 h, and the polished surfaces were etched with an argon-ion beam (EIS-IE, Elionix, Tokyo, Japan) for 6 min to bring the hybrid layer into sharp relief. The operating conditions for the argon-ion beam etching were an accelerating voltage of 1 kV and an ion current density of 0.2 mA/cm², with the ion beam directed perpendicular to the polished surface¹⁵. Number of the adhesive-dentin specimens obtained was as follows: 17(F0), 16(F10), 17(F20), 31(F50), 24(F75), 37(F90) and 25(F100). Specimens treated with each of the experimental adhesives were then gold-sputter coated, and morphological challenges to the dentin-adhesive interface due to the acid-base challenge were observed using an SEM (JSM-5310LV, JEOL, Tokyo, Japan) under ×3,500 magnification. The area of the ABRZ was calculated using region of interest (ROI) mode of the digital image analysis software (SEM Afore, JEOL), according to the schematic presented in Fig. 2. And the SEM images were categorized into three types according to the appearance of interface after acid-base challenge as presented in Fig. 3; butt-joint at the junction of ABRZ and OL with no slope formation (a), a slight slope formation of the joint (b) and a clear, round slope formation at the junction (c).

Fig. 2 Schematic illustration of the area of the ABRZ. ABRZ: acid-base resistant zone, B: bonding, CR: composite resin, D: dentin, HL: hybrid layer, OL: outer lesion. The outlined ABRZ area was measured with ROI mode after SEM observation.

Fig. 3 Representative SEM images of the adhesive-dentin interface after acid-base challenge (magnification ×3,500). An ABRZ was categorized into three types; a: butt joint with no slope formation, b: a slight slope formation of the joint, and c: a clear, round slope formation at the junction. ABRZ: acid-base resistant zone, B: bonding, CR: composite resin, D: dentin, OL: outer lesion.

Fig. 4 Categorization of the morphological features of the SEM images into three types.

The number on each bar indicates the number of specimens observed under SEM for each experimental adhesive. Sloped ABRZ formation (type c) was predominately observed only in F90 and F100.

Table 2 The area of the ABRZ of the experimental adhesives calculated using ROI.

Values represent mean (SD). The same letter means no significant differences at $p=0.05$ level

Adhesive system	Area of the ABRZ (μm^2)
F0	5.3 (1.4) ^a
F10	5.1 (1.2) ^a
F20	6.5 (1.5) ^a
F50	8.6 (2.1) ^b
F75	8.5 (1.8) ^b
F90	10.8 (2.6) ^c
F100	15.1 (3.0) ^d

Measurement of fluoride ions released from the adhesives

Measurement of released fluoride ions was carried out according to the previous studies^{16,17}. Twenty-eight disk specimens (6 mm in diameter and 1 mm in thickness) were prepared from each adhesives ($n=4$ for each concentration) by placing the material into a custom vinyl cylindrical mold. The adhesives were covered with a plastic strip, and light-cured through the strip with the halogen light unit (XL3000). After light curing for 10 s, each specimen was removed from the mold and immersed in 1 mL deionized water (DIRECT-Q3 UV, Merck Millipore, Billerica, MA, USA) in a small air tight polyethylene vial at 37°C. After 24 h, the vials were thoroughly shaken, and the water was removed. In order to analyze of fluoride concentration, standards

were prepared from sodium fluoride solution with concentrations of 0.05, 0.1, 0.5, 1 and 5 ppm to which a total ionic strength adjustor and buffer (TISAB, Orion, Boston, USA) was added in order to obtain a constant background ionic strength. These standard solutions were used to plot the calibration graph. Fluoride release was detected using a fluoride ion selective electrode (8010-10C, 2060A-10T, HORIBA, Kyoto, Japan) connected to an ion analyzer (F-53, HORIBA). Fluoride ion concentration was recorded and converted to the amount of fluoride release per unit volume of each specimen ($\mu\text{g}/\text{mm}^3$).

Statistical analysis

The area of ABRZ calculated was statistically analyzed using one-way ANOVA and multiple comparisons were made using Dunnett's C test at $\alpha=0.05$ level. Mean and standard deviation of 24-h fluoride ion release from the adhesives were plotted against the NaF content of each adhesive and compared using regression analysis. All data were analyzed using the Statistical Package for Medical Science (SPSS ver. 16 for Windows).

RESULTS

SEM observation

Representative SEM images of the adhesive/dentin interface after acid-base challenge are shown in Fig. 3. The outer lesion (OL) observed in each sample was approximately 10 to 15 μm thick, which is the part of demineralized dentin after acidic challenge. A hybrid layer which should be distinguished by argon-ion beam etching could not be clearly identified at the interface for each specimen under $\times 3,500$ magnification. The ABRZ was clearly observed beneath the hybrid layer in all the specimens; however, morphological features of the ABRZ were different among the experimental materials with various NaF contents as presented in Fig. 4. The sloped ABRZ formation (type c) was predominantly observed in the adhesive groups of F90 and F100. In the case of F50 and F75, the slope formation had variations in the specimens (type b and c). The slope was not formed in F0, F10, and F20 (type a).

The areas of the ABRZ of each adhesive calculated using ROI are showed in Table 2, indicating that the area of the ABRZ tended to increase with increasing concentration of NaF in the adhesive. The F100 adhesive created the largest ABRZ among the adhesives ($p<0.05$). However, there were no significant differences in the area of ABRZ among F0, F10, and F20, and between F50 and F75 ($p>0.05$).

Measurement of fluoride ions released from the adhesives

The amount of fluoride ion release from the experimental adhesive resins is summarized in Fig. 5. Fluoride release was detected from each experimental adhesive except for F0. The greatest amount of fluoride release was obtained in F100. According to the linear regression analysis, there was strong positive correlation between concentration of NaF in the composition and amount of

Fig. 5 The amount of fluoride ion release from the experimental adhesives.

There was strong positive correlation between concentration of NaF in the composition and amount of fluoride ion released from the adhesive ($R^2=0.99$).

fluoride ion release from the adhesive ($R^2=0.99$).

DISCUSSION

Dental caries occurs when demineralization dominates in the dynamic local balance of de/remineralization. It is well known that the fluoride ions penetrating into the dentin enhance the mineralization process and increase the resistance of dentin to demineralization^{18,19}. The potential benefits of fluoride release from adhesives have been speculated by several studies; it has been reported that a zone of inhibition is created along the interface between adhesives and dentin, by fluoride ions released from adhesive systems after cariogenic challenge²⁰.

In addition to surface-treated sodium fluoride which can release fluoride ions from the adhesive, the two-step self-etching primer adhesive system, Clearfil Protect Bond contains an antibacterial primer and a fluoride-releasing adhesive. The primer includes MDP and 12-methacryloyloxydodecylpyridinium bromide (MDPB). MDPB is an antibacterial resin monomer, which is expected to inhibit the growth and accumulation of bacteria²¹. This monomer was also found to possess anti-matrix metalloproteinase (MMP) activities²². The formulation has also resulted in stable bond strength values reported in laboratory studies^{2,23-26}.

Morphological differences in the ABRZ were significant between the fluoride-releasing and fluoride-free adhesive systems^{1,3,4-6,8}. It was reported that the slope formation of the ABRZ was observed only in the use of fluoride-releasing adhesives⁴, and that fluoride containing adhesives created obviously thicker ABRZ than the fluoride-free adhesive at the middle portion of the ABRZ with more densely arranged crystallites legion⁶.

The tendency of fluoride release from the adhesive is material dependent^{1,27} generally, the amount of the released fluoride ions from an adhesive is affected by initial fluoride content, thickness, degree of polymerization of the adhesives and time²⁷. In this study, qualitative analysis of the ABRZ with different fluoride concentrations of adhesives was carried out by classification of the ABRZ in SEM images. Moreover, quantitative assessment of the ABRZ area was performed by using the ROI mode of the digital image analysis software. To minimize the technical error in the analysis, only one operator, well-trained, analyzed the whole SEM images in the present study. There was a significant difference in the ABRZ area between F20 and F50 and between F75 and F90, respectively. Therefore, the threshold of fluoride concentration in the adhesive to influence the morphology of the ABRZ may exist between F20 and F50. Approximately 0.07 µg/mm³ of fluoride ion was released from the F50 adhesive, which appears to be the minimum concentration to provide the morphological influence of the ABRZ after acid-base challenge in the present study. It should be noted that the specimen preparation and test conditions for fluoride ion measurement were different from those of the morphological examination of the adhesive applied onto dentin.

Few studies have quantified the depth and the amount of fluoride penetrated into dentin in terms of the inward migration of ions from resin-based materials. Using energy dispersive spectroscopy (EDS) and wavelength dispersive spectroscopy (WDS), it was determined that fluoride ions released from the filler particles in the adhesive resin moved through the resin matrix and then down a concentration gradient into the hybrid layer and superficial dentin²⁸. Fluoride ions may migrate from the hybrid layer²⁶, and continue to diffuse into the deeper dentin over time. Han *et al.* used electron probe micro-analyzer (EPMA) to demonstrate that the fluoride density in dentin up to a few µm in depth is increased by the diffusion of pre-reacted glass ionomer fillers in the adhesive system²⁹.

On the mechanism of action of fluoride from adhesives in prevention of dentin demineralization, it has been suggested that the fluoride could enter calcium-phosphate rich spaces created by self-etching adhesives interact to prevent the future demineralization. While transformation of hydroxyapatite to fluoroapatite in as short as 24 h after application of the bonding agent is difficult to be expected, it can be speculated that the uptake of a small fluoride amount into dentin and the presence of these ions during acid challenge shift the local degree of saturation (DS) with respect to fluoridated apatite in favor of formation of the crystals. The DS with respect to hydroxyapatite in the solution favors dissolution, which in turn results in local pH increase and release of calcium and phosphate ions that further contribute to formation of fluoridated apatite in the presence of fluoride.

The self-etching adhesive systems demineralize dentin mildly and partially, leaving hydroxyapatite

crystals in the base of the hybrid layer¹⁰). Such residual apatite crystals may serve as a template for additional chemical reaction with the phosphate-ester functional monomer, MDP. It was reported that MDP adheres to hydroxyapatite readily and intensively¹⁰), forming a less soluble salt. It can be seen that hybridization at the adhesive-dentin interface and the protection of the residual apatite crystals in dentin by the functional monomer is a key to prevent wall lesion along the interface⁷). Moreover, the thick, sloped junction formed in the presence of fluoride should serve as the demineralization resistant front.

The current study demonstrated the presence of a threshold for fluoride concentration of the adhesive to create a remarkable morphological change in the ABRZ area. However, the effect of fluoride concentration in excess of F100 on the morphology was still unclear. Further studies should be carried out to evaluate the relationship between the fluoride release and the area of the ABRZ using different types of the adhesive systems, and to elucidate the mechanism of fluoride action at the interface.

CONCLUSION

The concentration of NaF in the adhesive resin influenced the amount of dentin structure remaining after acid-challenge in the two-step self-etching adhesive system. An adhesive with fluoride release of over 0.07 µg/mm³ in 24 h after curing appeared to improve the resistance of interfacial dentin against demineralization.

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