

## Effect of topical application of fluoride gel on artificial secondary caries inhibition

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**Purpose:** To evaluate the effect of topical application of fluoride gel on artificial secondary caries around adhesive restorations.

**Materials and Methods:** Two box-shaped cavities were prepared on both buccal and lingual dentin surfaces of bovine roots. Each cavity was restored with either a non fluoride-releasing adhesive, Clearfil SE Bond/a resin composite, Clearfil AP-X (AP-X), or an experimental fluoride-releasing adhesive, ABF/AP-X. Acidulated phosphate fluoride gel (pH 4.2) was topically applied to the restorations and cavity margins for 0 (not applying fluoride at all), 1, 2 and 4 minutes, immediately before immersion in a decalcifying solution (pH 4.5). Longitudinal sections were then cut in half through each restoration. Depths of outer lesion and thickness of inhibition zone around restorations were determined by a confocal laser scanning microscope. The data were analyzed by one- and two-way ANOVA and Fisher's PLSD test ( $p < 0.05$ ).

**Results:** Inhibition zone was observed only in ABF/AP-X with and without topical application of fluoride gel. Topical applications of fluoride for 2 and 4 minutes significantly decreased the depth of outer lesion in both restoration systems ( $p < 0.05$ ).

**Conclusion:** Inhibition zone formation was observed in ABF, while it was not observed in Clearfil SE Bond. Topical application of acidulated phosphate fluoride gel for over 2 minutes was effective to decrease the depth of the outer lesion in both adhesive systems. (**Int Chin J Dent 2003; 3: 53-61.**)

**Clinical Significance:** Application of a fluoride-releasing dentin bonding system and a fluoride gel may be clinically beneficial for root caries prevention.

**Key Words:** dentin bonding system, fluoride gel, fluoride-release, secondary caries inhibition.

### Introduction

Recent advances in adhesive technology for use with resin composite restorative systems have resulted in routine usage of resin-based restorations in clinical dentistry. It has been extensively reported that dentin adhesive systems bond very well to dentin.<sup>1</sup> However, secondary caries is still one of the main reasons for

the replacement of composite restorations.<sup>2</sup>

The caries process is a dynamic balance between demineralization and remineralization of the hard dental tissues that may eventually result in cavitation.<sup>3</sup> Establishment of caries that is clinically detectable depends on both the acid and the enzymes produced by bacteria within the plaque.<sup>4-6</sup> The products of plaque bacteria, including lactic, acetic, and citric acid, reduce the pH on the tooth surface and demineralize the dental tissues. Fluoride has significant effect in inhibiting caries.<sup>5,7,8</sup> As a result, fluoride-releasing restorative materials have been developed, with the intent to help prevent recurrent caries.<sup>9-12</sup> Many researchers have studied the effect of fluoride-releasing restorative materials. It has been then reported that fluoride-releasing restorative materials were effective to inhibit secondary caries *in vitro*.<sup>13,14</sup> Therefore, fluoride-releasing restorative materials can be expected to inhibit the secondary caries.

As fluoride-releasing adhesives directly contact the cavity wall, fluoride ions released from them easily penetrate and diffuse into the dentin at the cavity wall. Ferracane et al.<sup>15</sup> detected fluoride ion release from a fluoride-releasing adhesive into the hybrid layer created at the resin-dentin interface. The fluoride ions penetrating into the dentin enhance mineralization of the dentin and reduce the demineralization of the dentin.<sup>16</sup> Therefore, the dentin penetrated by fluoride ions offers greater resistance against secondary caries as compared to dentin that has not contacted fluoride. Topical application of a fluoride gel has also been focused upon as a means to prevent primary caries.<sup>17</sup> Therefore, the combination of fluoride-releasing materials and fluoride gel is expected to be more effective in secondary caries control.

A new evaluation method using a confocal laser scanning microscopy (CLSM) was developed to evaluate *in vitro* secondary caries formation and inhibition.<sup>18</sup> Specimen preparation for CLSM is simple, and the specimen can be observed under environmental conditions. The aim of this study was to evaluate the effect of topical application of fluoride gel on artificial secondary caries inhibition around resin composite restorations with non fluoride- or fluoride-releasing dentin bonding systems using CLSM.

## Materials and Methods

The materials used in this study are summarized in Table 1. The dentin bonding systems used were non fluoride-releasing dentin adhesive system, Clearfil SE Bond (SE, Kuraray Medical, Tokyo, Japan) and an experimental fluoride-releasing adhesive system, ABF (Kuraray Medical). Both systems are two-step self-etching primer systems. The resin composite was Clearfil AP-X (Kuraray Medical). An acidulated phosphate fluoride gel, 2.2% APF (pH 4.2) was prepared at Department of Pharmacy in the dental hospital, Tokyo Medical and Dental University.

Fig. 1 shows the method of specimen preparations. Sixteen bovine incisors from 20-24 month old cattle were used in this study. The cementum was removed using a carborundum point (HP #20) to expose root dentin. The roots were obtained by separating them from the crown segments at the cementum-enamel junction with a low-speed diamond saw under water spray coolant. The pulp tissue was left *in situ* to prevent dehydration of the root dentin during the restorative procedure, and the cut surface and root apex were sealed with utility wax, and coated with two layers of nail varnish. Two box-shaped cavities

**Table 1.** Materials used.

Material	Components	Batch No.
<u>Adhesive system</u>		
Clearfil SE Bond*	Primer: MDP, HEMA, Water, PI, Accelerators, CA Bond: MDP, HEMA, MFM, PI, Accelerators, CA, Microfiller	011151
ABF (Experimental)*	Primer: MDPB, MDP, HEMA, MFM, PI, Water Bond: MDP, HEMA, MFM, PI, Microfiller, NaF	991130
<u>Resin composite</u>		
Clearfil AP-X*	Bis-GMA, TEGDMA, Barium glass filler, PI, Accelerators	00686A
<u>Fluoride gel</u>		
	2.2% NaF, Phosphoric acid, Distilled water Sodium carboxy methyl cellulose, Glycerin (pH 4.2)	

\*Manufacturer; Kuraray Medical, Tokyo, Japan.

MDP; 10-methacryloyloxydecyl dihydrogen phosphate, HEMA; 2-hydroxyethyl methacrylate, PI; photoinitiator, CA; catalyst; MFM; multi-functional methacrylate, MDPB; methacryloyloxydodecylpyridinium bromide, Bis-GMA; bisphenol A-diglycidyl methacrylate, TEGDMA; triethyleneglycol dimethacrylate.

(approximately 3 mm long, 2 mm wide, 1.5 mm deep) were prepared on buccal and lingual dentin surfaces of the roots of bovine incisors, using a diamond bur (ISO #106) mounted in high-speed turbine with air-water coolant. The distance between the two cavities prepared on the each buccal or lingual surface was about 4 mm. The cavity margins were finished with a straight fissure steel bur (ISO #010) in a slow speed handpiece under copious water spray, to produce a cavosurface angle as close as possible to 90°. Eight cavities were prepared for each group. Each cavity was restored with ABF/AP-X or SE/AP-X according to the manufacturer's instructions and stored in water for 1 week at 37°C,<sup>12</sup> then polished flat with polishing disks (Rainbow Polishing Kit, Shofu, Kyoto, Japan) under running water to expose the cavity margins. The margins were checked with a light stereomicroscope at 10 x magnification to discard any specimens with cracks evident at the margins. The teeth were coated with nail varnish except for the restorations and a 1 mm-window around cavity margins. Fluoride gel was applied to the restorations and cavity margins for 0, 1, 2, and 4 minutes, immediately before immersion in a decalcifying solution (100 mL) that was adopted from Ten Cate et al.<sup>19</sup> for 3.5 days at 37°C. The acid buffer solution contained 2.2 mmol/L CaCl<sub>2</sub>, 2.2 mmol/L NaH<sub>2</sub>PO<sub>4</sub> and 50 mmol/L acetic acid adjusted to pH value of 4.5 with NaOH.

The specimens were removed from the decalcifying solution and thoroughly rinsed in running water. Longitudinal sections were then cut through each restoration by means of a water-cooled diamond saw microtome (Leitz 1600 Microtome, Ernst Leitz, Wetzlar, Germany) to form 2 halves. The cut surfaces of the specimens were polished to high gloss with abrasive discs and diamond pastes successively from 6 µm to 0.25 µm grit size (DP-Paste, Struers, Denmark). The polished surfaces were cleaned in ultrasonic bath

and then observed using the confocal laser scanning microscope (CLSM, 1LM15W, Lasertec, Yokohama, Japan) at 500 x magnification (20 x objective lens). The CLSM is a video rate instrument, achieving a frame time of 33 ms by the use of an acousto-optic deflector for high speed scanning in one axis. This allows rapid three-dimensional assessment of samples without damage from prolonged drying or exposure to laser illumination. The instrument was initially developed for non-contact, high-resolution inspection of surfaces such as integrated circuits. The images appear as surface reflection images of the sectioned sample achieved without the use of an immersion medium.

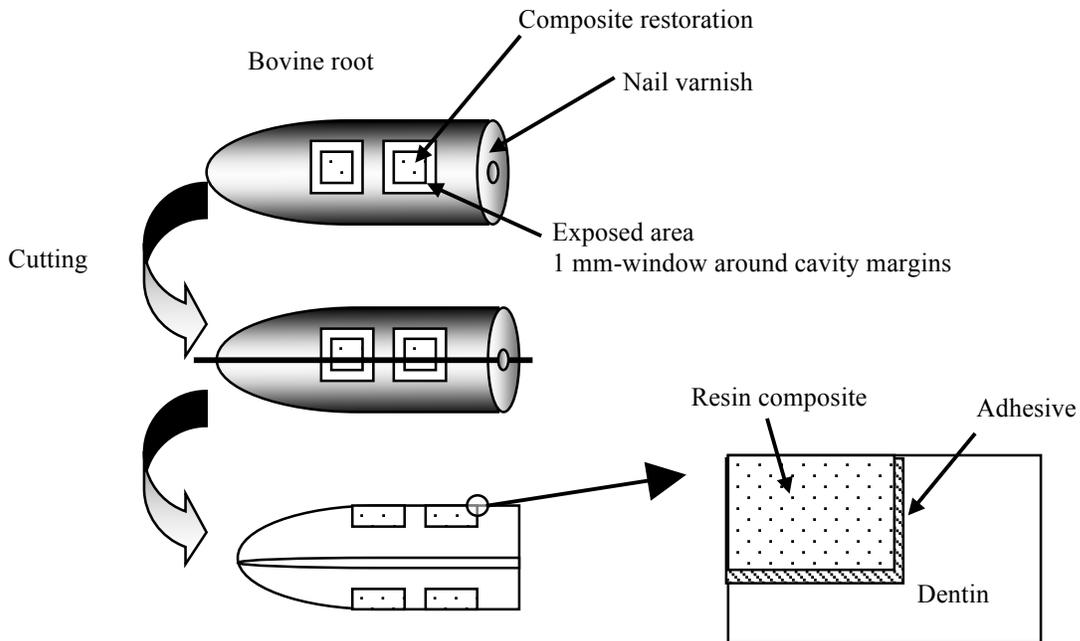


Fig. 1. Sample preparation.

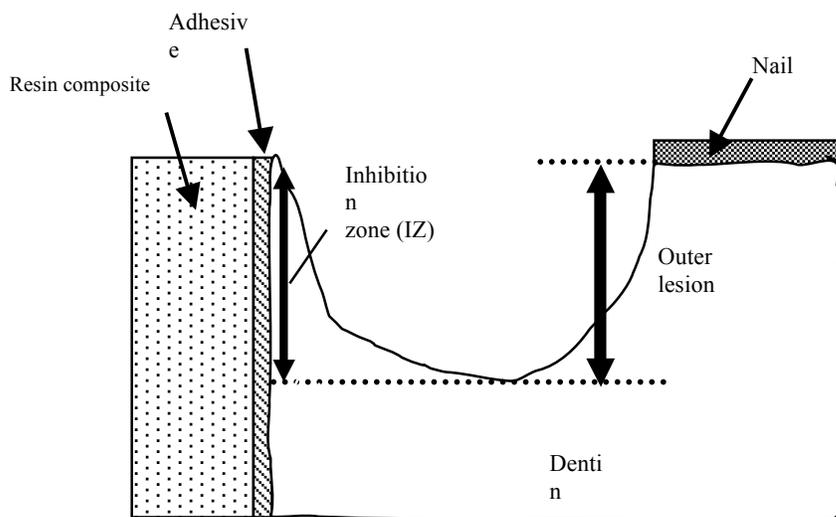
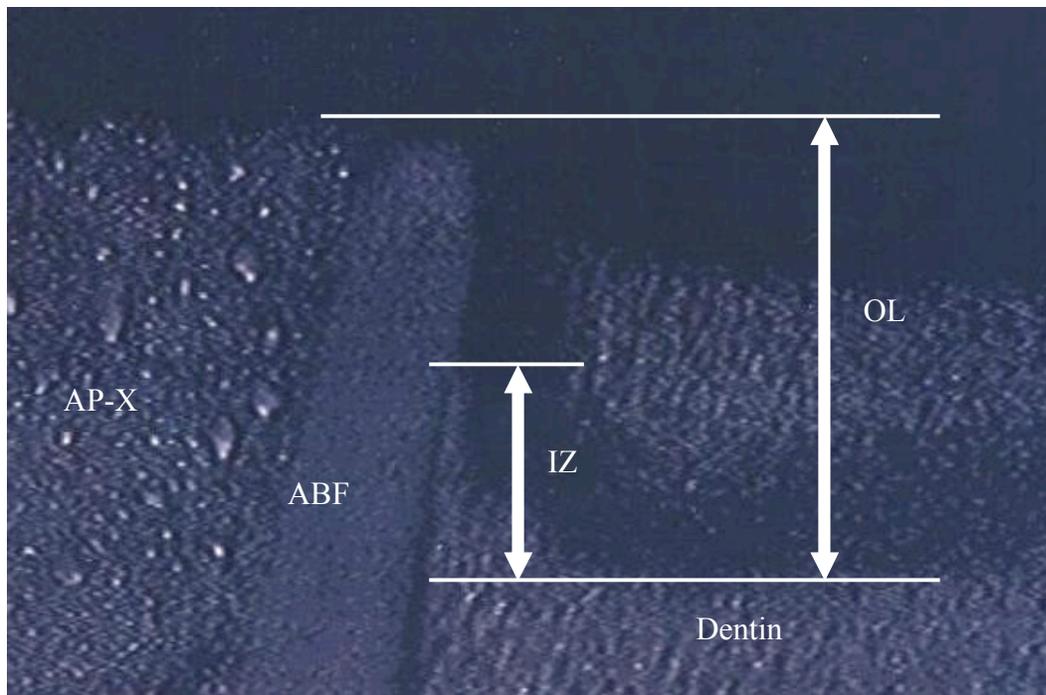


Fig. 2. Measurement of inhibition zone and outer lesion using a CLSM.

Fig. 2 shows the measurement method of the inhibition zone and outer lesion. The features evaluated were depth and shape of the lesion, and presence or absence of an inhibition zone adjacent to the cavity wall for each restoration. Since the demineralized tissue may collapse and shrink, a straight line was extended from the top surface of the restorative material, and the depth of the outer lesion was measured from the height to the deepest demineralized front. The height of the inhibition zone was measured from the top of the inhibition zone (the width of approximately 6  $\mu\text{m}$ ) to the deepest demineralized front. Eight cavities from two bovine incisors were used for each group. The data were analyzed by two-way analysis of variance (ANOVA). The two factors analyzed were material, topical application of fluoride gel or no topical application. After that, one-way ANOVA and Fisher's PLSD test at the confidence level of 95% were performed ( $p < 0.05$ ).

## Results

Fig. 3 shows a typical CLSM image of ABF/AP-X restoration. Table 2 shows the depths of outer lesion and the heights of inhibition zone for SE and ABF. For SE, the depths of the outer lesion were approximately 160  $\mu\text{m}$ , when the APF gel was not applied. When acidulated phosphate fluoride gel was topically applied to the restoration for 2 minutes, the depths of the outer lesion significantly decreased to approximately 120  $\mu\text{m}$ . There were no significant differences of the application period between 2 minutes and 4 minutes. An inhibition area was not observed in any group.



**Fig. 3.** A typical CLSM image of ABF/AP-X restoration with topical application of the APF gel for 2 minutes. The depth of the outer lesion was approximately 120  $\mu\text{m}$ . An inhibition area created adjacent to the bonding agent was observed.

**Table 2.** Effect of topical application of fluoride gel on the depths of outer lesion and the heights of inhibition zone for Clearfil SE Bond and ABF.

Topical application time (minutes)	Depth of outer lesion (mm)		Heights of inhibition zone (mm)	
	SE	ABF	SE	ABF
0	159.1 (18.8) <sup>a</sup>	169.6 (4.3) <sup>a</sup>	n.d.	62.4 (8.3) <sup>c</sup>
1	146.1 (10.8)	158.3 (7.2)	n.d.	63.5 (2.4) <sup>c</sup>
2	123.8 (20.0) <sup>b</sup>	115.7 (6.9) <sup>b</sup>	n.d.	62.9 (3.2) <sup>c</sup>
4	113.8 (13.6) <sup>b</sup>	115.2 (19.6) <sup>b</sup>	n.d.	63.7 (10.3) <sup>c</sup>

Values are mean (SD), n=8, n.d.; not determined.

Groups identified by the same superscript letters are not statistically significantly different ( $p>0.05$ ).

For ABF, the depths of the outer lesion were also approximately 170  $\mu\text{m}$  when the APF gel was not applied. However, the depths of the outer lesion significantly decreased when the fluoride gel was topically applied to the restoration. The depths of the outer lesion for 2 minutes and 4 minutes were approximately 120  $\mu\text{m}$ . There were no significant differences of the depth of outer lesion between 2 minutes and 4 minutes. An inhibition area created adjacent to the bonding agent was observed. The heights of the inhibition zone were approximately 60  $\mu\text{m}$  in all the cases.

## Discussion

This study investigated the inhibitory effect of topical application of fluoride gel on artificial secondary caries inhibition around resin composite restorations with non fluoride- or fluoride-releasing dentin bonding systems using a CLSM. Pereira et al.<sup>12</sup> and Okuda et al.<sup>18</sup> focused on the caries-inhibiting effect of restorative materials performed in a chemical-caries inducing test system. Previously, polarized light microscopy (PLM) was used to evaluate outer lesions and inhibition zones. This has been a common method for evaluation of demineralization of dental tissues and inhibition of secondary caries.<sup>11,12,20,21</sup> This method requires extensive specimen preparation procedures, such as thin slicing of the teeth, dehydration with ascending grades of ethanol and immersion and observation in an imbibing medium of quinoline. On the other hand, a CLSM evaluation can be performed under near normal environmental conditions resulting in fewer artifacts. The sample preparation for CLSM is simpler than that for PLM because specimen preparation procedures that produce artifacts are not required. In this study, outer lesions and inhibition zone were positively detected with the CLSM just after simple sectioning and polishing procedures.

The fluoride-releasing dentin adhesive system, ABF, demonstrated the potential for artificial secondary caries inhibition around the restoration. However, the thickness of the inhibition zone is relatively thinner than those created with a conventional glass-ionomer cement (GIC).<sup>18</sup> This result agreed with those of previous studies.<sup>22,23</sup> The source of the fluoride in ABF is by a proprietary treatment of NaF, which is able to penetrate into the dentin adjacent to the restoration through the matrix. The factor that is most likely to explain the differences is not so much the presence of fluoride, but the way in which it can be released.

Torii et al.<sup>22</sup> mentioned that the amount of fluoride released from the material is related not only to the concentration of fluoride in the material, but also to whether it can spread and diffuse out from within the material. The latter is determined by the permeability of the materials since the presence of water in a material facilitates both the inward and outward diffusion of ions. The fluoride source surrounded by resin matrix may have difficulty being in contact with water because the free movement of water is probably limited in the cured resin matrix. This is probably related to the composition and content of the fluoride source and hydrophilic monomers, such as HEMA.

An experimental self-etching primer system of this fluoride-releasing adhesive system is ABF, which includes the antibacterial monomer 12-methacryloyloxydodecylpyridinium bromide (MDPB) against bacteria in human dentinal carious lesions. For the non-fluoride-releasing dentin adhesive system, Clearfil SE Bond, inhibition zones were not observed in all groups. However, Torii et al.<sup>22</sup> noted that a thin and weak radio-opaque zone was micrographically observed using a non-fluoride-releasing dentin adhesive system, Clearfil Linerbond II (Kuraray Medical). They suggested that this fact should be associated with the adhesion of the restoration-to-cavity wall promoted by the adhesive system. The formation of a hybrid layer by the infiltration of adhesive resin into the dentin is an essential mechanism for dentin adhesion.<sup>24,25</sup> Therefore, the hybrid layer may present acid-resistance against the secondary caries formation.

Topical application of acidulated phosphate fluoride gel for over 2 minutes was effective in decreasing the depths of the outer lesion in both ABF and SE. It was reported that topical application of fluoride gel was effective in protection against root-surface caries.<sup>26</sup> Garcia-Godoy et al.<sup>27</sup> demonstrated the ability of a shorter APF application time to affect caries-like lesion depth in enamel. These findings agree with the results of this study. The role of fluoride in preventing root-surface lesions has been shown by *in vitro* studies.<sup>26,27</sup> The mechanisms of protection against root-surface caries depend on the deposition of fluoride within and on the root surfaces. Calcium fluoride and various fluoridated mineral phases are created as surface deposits and are incorporated into the superficial tooth surface. These fluoride-rich deposits enhance root surface caries resistance.<sup>28</sup> Fluoride provides an inhibitory effect against bacteria in plaque and demineralization of root surfaces, while promoting root caries remineralization. This emphasizes the need for fluoride in the prevention of clinically undetected root-surface lesion and benefit of various fluoride modalities in preventing primary root-surface and enamel caries, as well as secondary caries. Within the limitations of this laboratory study, the combination of a fluoride-releasing adhesive system and topical application of fluoride gel may protect secondary caries formation around restorations on root surfaces.

## Conclusion

1. Inhibition zone formation was observed in ABF using a CLSM, while it was not observed in Clearfil SE Bond.
2. Topical application of acidulated phosphate fluoride gel for over 2 minutes was effective to decrease the depth of the outer lesion in both ABF and Clearfil SE Bond.

3. Application of a fluoride-releasing dentin bonding system and a fluoride gel may be clinically beneficial for root caries prevention.

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