

# Prevention of Artificial Caries: Effect of Bonding Agent, Resin Composite and Topical Fluoride Application

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## Clinical Relevance

The combination of a fluoride-containing bonding agent and a fluoride-containing resin composite provided for the best inhibition of secondary caries.

## SUMMARY

**This study investigated the effect of fluoride containing resin composites and bonding agents, as well as the topical fluoride (F) application on the**

**inhibition of artificial caries progression by using a pH-cycling model with alternating demineralizing (pH:4.5) and remineralizing (pH:7.0) solutions.**

Two bonding systems (F-containing bonding system [Reactmer Bond: RB] and non-F containing bonding system [Clearfil SE Bond: SE]), two resin composites, (F-containing [Reactmer Paste: RP] and non-F containing [Clearfil AP-X: AP]) were used. A combination of each bonding agent and a resin composite, RB+RP, RB+AP, SE+RP and SE+AP, was placed in 2 x 3 x 1.5-mm cavities on root dentin of extracted molars (n=96). Specimens were subjected to pH-cycling for 6 or 12 weeks. Half of all specimens were immersed in 0.05% NaF solution for 1 minute once a day as a topical F application. After the pH cycling period, a microradiograph of each specimen was taken, and the outer lesion depth of the artificial caries was measured by means of image analyzing software. The depths of the outer lesions at different periods were analyzed by one-way ANOVA and Sheffe's test at  $p=0.05$ .

The combination that received F treatment showed reduced lesion depth compared to the same combination without F application. Except

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**for the F application group of 12 weeks, there was no significant difference in lesion depth among each bonding and composite combination ( $p>0.05$ ). At week 12 with the F application, RB+RP showed the shallowest lesion compared to the other combinations ( $p<0.05$ ).**

**The results indicated that the F application reduced the progression of artificial caries. Moreover, the combination of fluoride containing bonding agent and restorative material was the most effective for the inhibition of artificial caries progression based on the 12-week experimental period with topical F application.**

## INTRODUCTION

The effect of fluoride on caries prevention and enamel reinforcement against caries has been previously reported (Øgaard, 1990). Fluoride has been widely used in various forms, including water fluoridation (Driks, 1974; Brunelle & Carlos, 1990), fluoride mouth rinse (Stephen, 1994), topical gels (Ekstrand & others, 1981) and fluoride dentifrice (DePaola & others, 1993). In dental materials, it is a well-known fact that fluoride is released from glass ionomer cements (Swift, Bailey & Hansen, 1990; Tam, Chan & Yim, 1997; Attin & others, 1999; Gao & Smales, 2001). Although fluoride released from glass ionomer cements has claimed to be the potential reason for the prevention of secondary caries, the inhibition of secondary caries around resin composites containing fluoride was not shown when being exposed to the risk of caries challenge (Nagamine & others, 1997; Pereira, Inokoshi, & Tagami, 1998; Okuda & others, 2003).

Recently, various fluoride-containing restorative materials have become available on the market for the prevention of secondary caries, and a bonding system and a resin composite can be applied to numerous cavities. Fluoride-containing bonding systems (Han & others, 2002; Hicks & others, 2002; Itota & others, 2002) or fluoride-containing resin composites (Attar & Onen, 2002) serve to reduce secondary caries. In clinical situations, fluoride-containing restorative materials also prevent secondary caries (Hatibovic-Kofman & Koch, 1991; Mjör, 1996; Donly & others, 1999; Çehreli & Altay, 2000). Moreover, a new type of resin-based restorative material that employs pre-reacted glass ionomer (PRG) technology has been marketed as "Giomers" (Roberts & others, 1999). These materials incorporate fillers that are produced from the complete or partial reaction of ion-leachable glasses with polyalkenoic acids and are expected to reduce secondary caries due to fluoride release from the material and to the property of fluoride recharge. However, how fluoride-containing bonding or resin composites affect carious dentin is unclear. When a resin composite is applied into a cavity, a bonding system is employed, then a resin composite is filled.

However, the relationship between the combination of resin composite, bonding agent and caries inhibition has not been well recognized (Itota & others, 2002).

Furthermore, the use of fluoride-containing material associated with topical fluoride application has been more efficient (Bynum & Donly, 1999; Okuyama, Komatsu & Sano, 1999). The combined topical fluoride application (simulating fluoride mouth rinse) helped to inhibit the progression of *in vitro* caries when either fluoride containing or non-containing materials were used.

This study investigated the effect of fluoride containing resin composites and bonding agents and topical fluoride application on the inhibition of artificial caries progression. The hypothesis was that resin restorative materials that contain fluoride promote shallower artificial lesion depth as compared to materials that do not contain fluoride, and the topical fluoride application was enhanced to the secondary caries inhibition on a fluoride-containing material.

## METHODS AND MATERIALS

### Specimen Preparation

The restorative materials used in this study were a bonding agent containing PRG filler (fluoride-containing bonding agent: F-bonding), Reactmer Bond (RB; Shofu, Kyoto, Japan), a non-fluoride-containing bonding agent (nF-bonding), Clearfil SE Bond (SE; Kuraray, Osaka, Japan), a resin composite containing PRG filler (F-composite), Reactmer Paste (RP; Shofu, Kyoto, Japan), a non-fluoride-containing resin composite (nF-composite) and Clearfil AP-X (AP; Kuraray) (Table 1).

One of two bonding systems (RB and SE) and one of two resin composites (RP and AP) were combined and employed in this study to search for the effect of F-composite, F-bonding or both materials on secondary caries. Therefore, four combined samples were tested: F-bonding and F-composite (RB+RP), F-bonding and nF-composite (RB+AP), nF-bonding and F-composite (SE+RP) and nF-bonding and nF-composite (SE+AP).

Ninety-six extracted human molars and premolars that were stored in 4°C isotonic saline with 0.5% thymol within four weeks after extraction were used. Ultrasonic scaling was performed to clean debris from the root surface. The root dentin surface was ground flat with SiC papers (grits #400 and #600). One-hundred-and-twenty cavities (2 mm wide x 3 mm long x 1.5 mm deep) were prepared using a diamond point (M411) mounted in a high-speed handpiece with copious water-coolant. The cavities were finished with a steel fissure bur (#557) in a low speed handpiece. A combination of each material filled the cavities as recommended by each manufacturer. All specimens were stored in an incubator with 100% humidity at 37°C for one hour after the preparations were restored. The restorations

Table 1: List of Materials Used for This Study		
Material (Code)	Type	Composition
<b>Bonding System</b>		
Reactmer Bond (RB)	Bonding agent contained PRG filler (F-bonding)	Bond A: Fluoroaluminosilicate glass, F-PRG, Water, Acetone Bond B: UDMA, HEMA, 4-AET, 4-AETA
Clearfil SE Bond (SE)	Non fluoride-containing Bonding (nF-bonding)	Primer: MDP, HEMA, Water Bond: MDP, Bis-GMA, HEMA, Silanated colloidal silica
<b>Resin Composite</b>		
Reactmer Paste (RP)	Resin composite contained PRG filler (F-composite)	F-PRG, Glass filler, UDMA, HEMA
Clearfil AP-X (AP)	Non fluoride-containing resin composite (nF-composite)	Bis-GMA, TEGDMA, Barium glass filler
PRG: Pre-reacted glass-ionomer F-PRG: Full reaction type PRG UDMA: Urethane dimethacrylate HEMA: 2-hydroxyethyl methacrylate 4-AET: 4-acryloxyethyltrimellitic acid		
4-AETA: 4-acryloxyethyltrimellitate anhydride MDP: 10-methacryloyloxydecyl dihydrogen phosphat Bis-GMA: Bisphenol A diglycidylmethacrylate TEGDMA: Triethyleneglycol dimethacrylate		

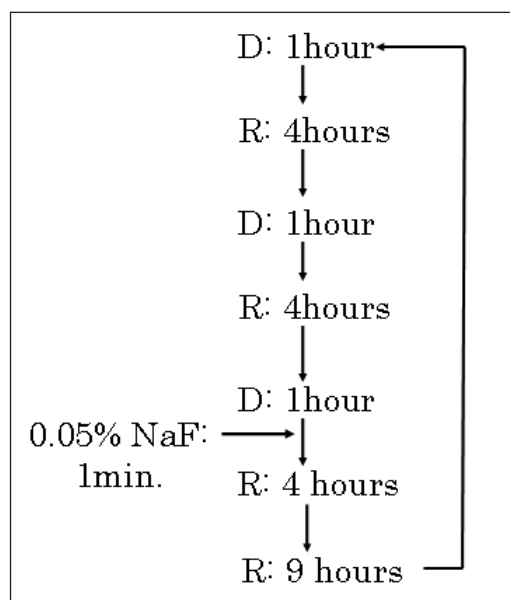


Figure 1. Procedure for caries progression test. D: Samples were immersed in demineralizing solution. R: Samples were immersed in remineralizing solution.

were polished with carborundum points under running water to expose the cavity margins. A thin layer of acid-resistant varnish was painted on the dentin surface, leaving a 1 mm x 1 mm area around the restoration.

### In Vitro Caries Progression Test

The procedures for the *in vitro* caries progression test are shown in Figure 1. The demineralizing and remineralizing solutions modified by Okuyama and others (1998), Okuyama, Komatsu and Sano (1999) and Mukai, Lagerweij and ten Cate (2001) were used as follows. The demineralizing solution contained 3.0mM  $\text{CaCl}_2$ , 1.8mM  $\text{KH}_2\text{PO}_4$  and 0.2M Lactic acid. This solution was adjusted with 1M NaOH to a pH of 4.5. The

remineralizing solution contained 3.0mM  $\text{CaCl}_2$ , 1.8mM  $\text{KH}_2\text{PO}_4$  and 0.02M HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid). This solution was adjusted with 1M NaOH to a pH of 7.0. The caries progression test that employed the pH cycling was performed for 6 or 12 weeks. The pH cycling was carried out on a schedule of three cycles of one-hour demineralization and 4-hour remineralization, followed by 9-hour remineralization storage. In combination with NaF rinse, each sample was immersed in 0.05% NaF (225 ppmF) solution for 1 minute after the third demineralization period as a simulation of topical fluoride mouth rinse once a day (Stephen, 1994).

### Evaluation of Caries Progression

After the caries progression test, the acid-resistant varnish was removed, and the specimens were fixed by immersion in 10% formic aldehyde for three days. After fixation, the specimens were dehydrated in ascending grades of ethanol—70% for 6 hours, 80% for 6 hours, 90% for 12 hours, 99% for 12 hours and 100% for 24 hours, then embedded in epoxy resin (Specifix 20; Struers A/S, Ballerup, Denmark). The specimens were sectioned by means of a low speed diamond saw (Isomet; Buehler, Lake Bluff, IL, USA), then ground with #1,000 and #3,000 grinding stones to create  $150 \pm 20\text{-}\mu\text{m}$  thick specimens. All sections were subjected to contact micro-radiographs (CMRs). CMRs were produced on a high-resolution film (Kodak SO-343; Eastman Kodak Company, Rochester, NY, USA) by 10-minute exposure to radiation from a SOFRON SRO-M50 x-ray generator (Soken, Tokyo, Japan). The image data of all CMRs was taken with an image scanner (GT-9800F; Seiko Epson Co, Suwa, Japan) and was observed on a display connected to a personal computer (Dell Latitude C500 installed Windows 2000; Dell Inc, Round Rock, TX, USA). Measurement of the outer lesion depth was per-

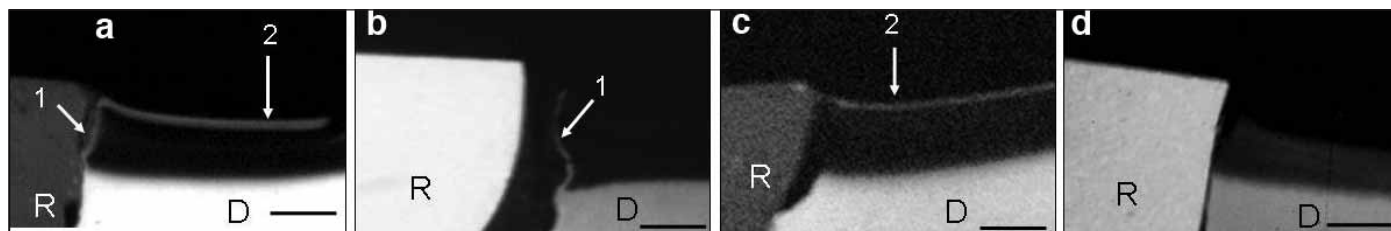


Figure 2. Microradiograph of specimens after caries progression test without fluoride application (a: RR, b: RA, c: SR, D: SA), R: Restorative material, D: Dentin, Arrow 1: Radiopaque layer adjacent to restoration. Arrow 2: The radiopaque layer on the superficial lesion. Bar: 200  $\mu$ m.

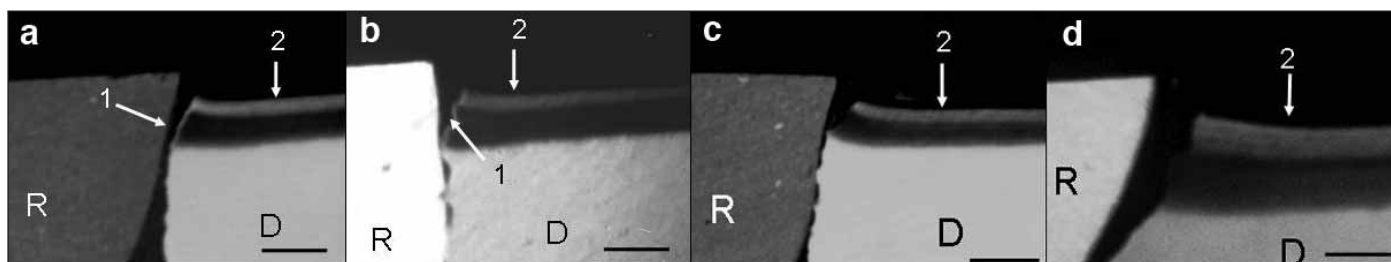


Figure 3. Microradiograph of specimens after caries progression test with fluoride application (a: RR, b: RA, c: SR, D: SA), R: Restorative material, D: Dentin, Arrow 1: Radiopaque layer adjacent to restoration. Arrow 2: The radiopaque layer on the superficial lesion. Bar: 200  $\mu$ m.

Table 2: The Depth of Artificial Caries Lesion on Each Combination ( $\mu$ m)

Combination	6-week		12-week	
	F (-)	F (+)	F (-)	F (+)
RB+RP	274.4 $\pm$ 16.4	244.3 $\pm$ 18.4	376.7 $\pm$ 23.0	298.7 $\pm$ 20.4*
RB+AP	282.1 $\pm$ 22.1	244.8 $\pm$ 23.4	411.1 $\pm$ 18.3	347.2 $\pm$ 18.6
SE+RP	269.0 $\pm$ 13.5	245.1 $\pm$ 4.6	387.0 $\pm$ 15.4	329.1 $\pm$ 17.2
SE+AP	289.2 $\pm$ 9.3	231.0 $\pm$ 17.4	404.5 $\pm$ 23.8	333.0 $\pm$ 15.0

N=6, Mean  $\pm$  SD

\*: the value is significant difference among value on the same column ( $p < 0.05$ )

F(-): Without fluoride application

F(+): With fluoride

formed by means of imaging analysis software (Scion Image; Scion Co, Frederick, MD, USA) installed on a personal computer. All images were then analyzed via the software. To determine outer lesion depth, each section was measured at three equidistant positions (200  $\mu$ m apart) away from the restoration at each side. The six depths per section were then averaged. The depths of outer lesions at each condition (two different periods with or without fluoride exposure) were statistically analyzed by one-way ANOVA, and multiple comparisons were done by Sheffe's test at  $p = 0.05$ .

## RESULTS

### CMR Observation

Figure 2 shows the image of the CMR for each adhesive and resin composite combination (F-bonding and F-composite [RB+RP], F-bonding and nF-composite [RB+AP], nF-bonding and F-composite [SE+RP] and nF-bonding and nF-composite [SE+AP]) after the caries progression test without fluoride application. A radiopaque layer adjacent to the restoration was

observed in RB+RP and RB+AP. RB+RP and SE+RP were provided to the radiopaque layer on the surface of the outer lesion. There was no radiopaque layer in SE+AP (Figure 2d). Figure 3 shows an image of the CMR with fluoride application for each combination after the caries progression test. A superficial radiopaque layer on the outer lesion was shown in all combinations. However, a radiopaque layer adjacent to the restoration was also observed in RB+RP and RB+AP—the sample into which F-bonding was incorporated.

### Outer Lesion Depth

The depths of the outer lesions are presented in Table 2. Except for the fluoride application 12-week group, there was no significant difference among each bonding and resin paste combination ( $p > 0.05$ ). On the 12-week with fluoride application group, the RB+RP (F-bonding and F-composite) combination indicated a shallower lesion than the other combinations ( $p < 0.05$ ).

## DISCUSSION

There are many reports available on the relationship between dental materials and artificial caries *in vitro*. Most of these reports describe the results of specimens immersed in acidic solution only (Tam & others, 1997; Millar, Abiden & Nicholson, 1998; Pereira & others, 1998; Okuda & others, 2003). A pH cycling system that

alternated demineralization and remineralization was carried out in the current research in order to clarify the behavior of materials during remineralization. Since pH cycling leads to the dissolution and re-precipitation of tooth mineral, this phenomenon is basic to caries development and regression (ten Cate, 1993). Some researchers have indicated the effects of fluoride against demineralization use by pH cycling (Herkströter, Witjes & Arends, 1991; Chow, Takagi & Shih, 1992; Nelson & others, 1992; Takagi & others, 1997; van der Reijden & others, 1997). Several studies used pH cycling to examine the effect of caries inhibition as an effect of dental materials (Okuyama & others, 1998, 1999; Konishi & others, 1999). In the oral environment, fluoride released from materials is diluted and washed by saliva. This dilution of fluoride was simulated by pH cycling due to constant changes in de- and remineralizing solution (Okuyama & others, 1998). The pH cycling seemed to be useful for studies

of caries prevention. This study used fluoride application with the intent to simulate the use of daily fluoride mouth-rinse, because it is a well-known fact that fluoride application is effective for caries prevention (Stephen, 1994; Brambilla & others, 1999; Zimmer, Robke & Roulet, 1999). There were reports about the effect of fluoride application on de- and remineralization by means of the cycling system (Chow & others, 1992; Nelson & others, 1992; Takagi & others, 1997); thus, the protocol for this research was to build and modify according to their reports.

Table 3 shows the summary of results of CMR observation and indicates whether there was a radiopaque layer on the lesion in each of the combined samples. When fluoride application was performed, the radiopaque layer was found superficially on the outer lesion. When F-composite was used as a restoration, this layer was also found on the surface of the outer lesion. A radiopaque layer was shown in the area where

it was adjacent to the restoration when using F-bonding. The radiopaque layer was observed on the surface of tooth structure when fluoride application (Arends & others, 1990; Takagi, Liao & Chow, 2001) or fluoride-containing materials (Nagamine & others, 1997; Okuyama & others, 1998; Itota & others, 2001, 2002) were used. The result of the current study is consistent with these reports.

Figure 4 shows hypothetical fluoride shifts between tooth structure and dental material. Fluoride is diffused from fluoride-containing materials to the tooth structure directly or through surrounding oral fluid (saliva). A radiopaque layer adjacent to the restoration may be associated with direct fluoride shift from the material; whereas, the same layer on a

Fluoride Application	Combination	Superficial	Adjacent to Restoration
Without fluoride application	RB+RP	+	+
	RB+AP	-	+
	SE+RP	+	-
	SE+AP	-	-
With fluoride application	RB+RP	+	+
	RB+AP	+	+
	SE+RP	+	-
	SE+AP	+	-

+: Radiopaque layer was present. -: The layer was not present.

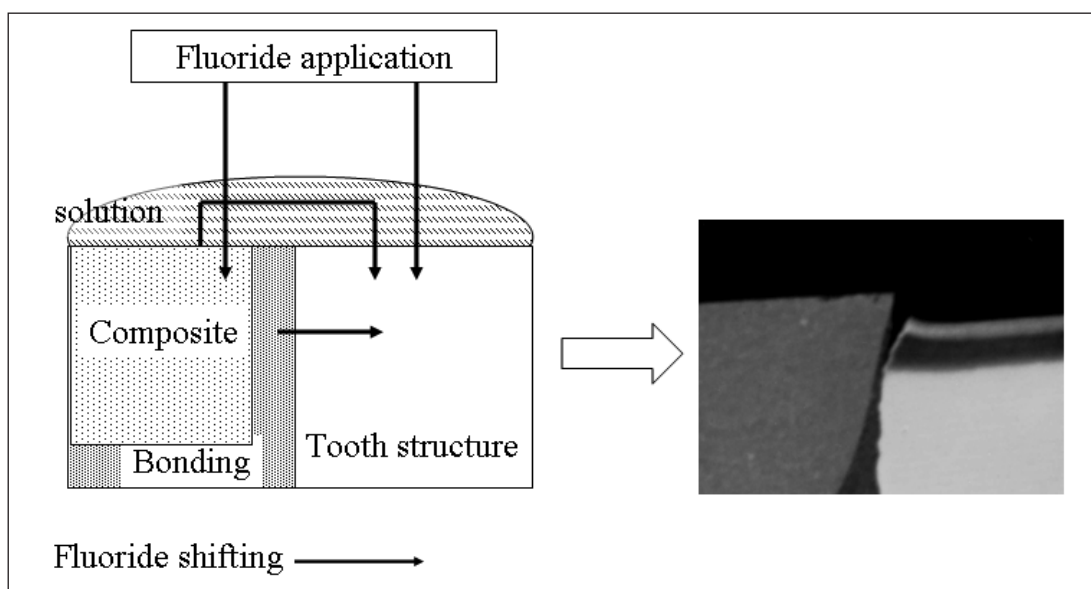


Figure 4. Schematic drawing of the supposition of shifting fluoride around fluoride containing-materials. Arrows indicate shifting fluoride. Fluoride moves material to the tooth structure directly or through a surrounding oral fluid, such as saliva. A radiopaque layer adjacent to a restoration may associate with directly applying fluoride from the material. A radiopaque layer on the superficial outer lesion may be contributed to by fluoride application and released fluoride from the material.

superficial outer lesion may be the result of a topical fluoride application plus fluoride released from the material. That is why the layer was shown on the superficial lesion or adjacent to the restoration area when fluoride-containing material was used.

Some research (Nagamine & others, 1997; Okuyama & others, 1998; Itota & others, 2001, 2002) exhibited the radiopaque layer adjacent to the fluoride-releasing restoration, much like the results of this study. Some reports showed the "caries inhibition layer" in the same area as the above researchers—on the image of a polarizing microscope (Donly & Grandgenett, 1998; Pereira & others, 1998; Hicks & others, 2000; Hicks & Flaitz, 2000) or on a confocal laser scanning microscope (Okuda & others, 2003). The radiopaque layer probably has the role of "caries inhibition layer" on the CMR image. From this viewpoint, F-bonding provided the performance of caries inhibition on the cavity wall. Fluoride release from fluoride-containing materials changes hydroxyapatite to fluoroapatite or hydroxy-fluoroapatite, providing better resistance against demineralization (Shellis & Duckworth, 1994). Therefore, the radiopaque layer could have been formed by fluoride ions released from materials and provided a medium to improve resistance to acid-challenge during artificial caries formation. Though it was unclear just how far caries had progressed along the cavity wall, at least the use of F-bonding helped to reduce its advance compared to F-bonding.

In this study, there was no difference in lesion depth on the outer lesion among each combination except for the 12-week fluoride application group. Consequently, F-composite (composite with PRG filler) or F-bonding could not provide lower outer lesion depth as compared to specimens that used nF-composite or bonding in this study. Moreover, this study showed that the layer did not promote caries inhibition with RB+RE or SE+RP, though the radiopaque layer was present on top of the outer lesions when fluoride-containing resin materials were used. The radiopaque layer may have no relationship to caries lesion depth in this study. In terms of a relationship between the radiopaque layer and caries progression, it is doubtful whether the layer provides resistance to a clinical caries challenge (Itota & others, 2001). The radiopaque layer does not offer protection from the underlying dentin against continued demineralization (Damen, Buijs & ten Cate, 1998).

Minami and others (2001) reported that the degree of remineralization depended on the amount of fluoride released from the material. A radiopaque layer or caries lesion is formed according to the amount of fluoride a material releases, uptakes and re-releases and adheres to tooth structure. However, the combination of RB+RP (F-composite and F-bonding) with fluoride application showed less lesion depth than other resin combinations in a 12-week period. Itota and others (2002) pointed out

that the combination that had a fluoride containing bonding agent and fluoride containing resin composite probably supplied a high concentration of fluoride ion. Moreover, RP, which was contained with F-PRG filler, had a high ability to fluoride recharge and showed a high amount of cumulative fluoride release (Itota & others, 2004a,b). Under the experimental conditions of this study, F-composite and F-bonding combination needed 12 weeks of external fluoride exposure to more effectively reduce caries progression as compared to other combinations. The amount of fluoride uptake and release from F-PRG filler that was included in RB and RP, may have influenced the artificial outer lesion depth in this study. Because a large amount of fluoride might provide low mineral loss (ten Cate, Damen & Buijs, 1998), small caries lesion depth was expressed when fluoride-containing restorative materials were used in the cavity.

In the future, it will be necessary to study the inter-relationship between amounts of fluoride released from different materials, fluoride concentration around a tooth and the inhibition of secondary caries.

## CONCLUSIONS

The combination of fluoride containing bonding agent and fluoride containing restorative material is more effective for the inhibition of artificial caries progression than for other resin combinations in a 12-week period with fluoride application. When fluoride application was not used, a difference in outer lesion depth among resin material combinations was not observed. In addition, there was no relationship between caries lesion depth and the presence of a radiopaque layer on dentin.

*Received 16 December 2004*

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