Dental Materials Journal 24(2) : 172−177, 2005

Formation of Inhibition Layers with A Newly Developed Fluoride-releasing All-in-one Adhesive

Yoshiharu MUKAI[1,2], Kiyoshi TOMIYAMA[1], Toru SHIIYA[1], Kazuko KAMIJO[3], Fukue FUJINO[4] and Toshio TERANAKA[1,2]

[1]Department of Dental Medicine, Division of Operative Dentistry and Endodontics, Kanagawa Dental College, 82 Inaoka-cho, Yokosuka, Kanagawa 238-8580, Japan
[2]Institute for Frontier Oral Science, Kanagawa Dental College, 82 Inaoka-cho, Yokosuka, Kanagawa 238-8580, Japan
[3]Department of Dental Sociology, Division of Sociological Approach in Dentistry, Kanagawa Dental College, 82 Inaoka-cho, Yokosuka, Kanagawa 238-8580, Japan
[4]Department of Dental Hygiene, Shonan Junior College, 82 Inaoka-cho, Yokosuka, Kanagawa 238-8580, Japan
Corresponding author, E-mail:mukaiyos@kdcnet.ac.jp

Received December 10, 2004/Accepted March 7, 2005

This study evaluated the capability of a novel fluoride-releasing, all-in-one adhesive system on forming inhibition layer (radio-opaque layer) as compared with other adhesive systems. Dentin surface was treated with Imperva bond (IB), FL-BOND (FB), Reactmer bond (RE), or FL-BOND S-1 (FS) (which is a novel system). Untreated specimens were categorized as non-bonding group (NB). After storing for 10 days in de-ionized water, the specimens were cut into halves perpendicularly to the pulp chamber and immersed in a buffered demineralizing solution for four days. Longitudinal sections were cut and microradiographed. The width of inhibition layers adjacent to the adhesive surface − at a depth of 50 μm under the demineralization surface − was analyzed. Microradiography revealed distinct inhibition layers adjacent to the experimental surfaces of FB, RE, and FS. No inhibition layers were observed in NB and IB. In particular, the width of the inhibition layer of FS (12.5 μm) was significantly greater than those of FB and RE. These results indicated that a newly developed all-in-one adhesive system, FS, may have a superior ability of forming inhibition layers adjacent to cavity walls, and that it may also protect dentin against further demineralization in case of secondary marginal caries.

Key words: Inhibition layers, Fluoride-releasing, All-in-one adhesive

INTRODUCTION

It has been reported that fluoride-releasing restorative materials have anticariogenic effect[1−5] both *in vitro* and *in situ*. According to Mukai *et al.*[6], fluoride-releasing materials prevented dentin from demineralization even after three weeks of exposure to de-ionized water, and the effect of which was enhanced due to fluoride recharging. Many reports have shown radio-opaque layers around various glass ionomers. This radio-opaque layer is formed by fluoride ions released from fluoride-releasing restorations. It serves as an inhibition layer or zone to resist acid attacks during artificial caries formation at the cavity wall[7].

To date, adhesive systems have not been compared in terms of their ability to form inhibition layers. Pereira *et al.*[8] has reported that an adhesive resin system that released fluoride failed to produce an inhibition zone along the cavity wall adjacent to restoration. However, the ability to produce inhibition layer is influenced by the amount of fluoride released. Recently, all-in-one adhesive systems have been developed and commercialized. FL-BOND S-1 is a novel fluoride-releasing, all-in-one adhesive system with a superior adhesive performance[9] and which induces low pulpal response[10]. However, the ability of this system to form inhibition layers has not been studied. Presently, resin composites and bonding materials are the main restorative materials used in practical dentistry. Against this background, it is very important to have a good and precise knowledge of how fluoride-releasing bonding systems form inhibition layers to help prevent secondary caries.

The aim of this study was to compare the capability of a newly developed, all-in-one adhesive "FL-BOND S-1" on forming inhibition layer as compared with other fluoride-releasing adhesives *in vitro*.

MATERIALS AND METHODS

Preparation of specimens

Ten bovine, lower incisors were used. A diamond-coated wire-sectioning machine (Well type 3142, Walter Ebner, Mannheim, Germany) was used to separate roots from crowns, and each root was cut into halves longitudinally. Experimental surfaces (approx. 5×3 mm^2) were prepared by cutting 1 mm from the buccal and lingual root surfaces and polished with #1,500 waterproof abrasive paper (Sankyo Rikagaku Co. Ltd., Saitama, Japan). Twenty samples were randomly distributed into five experimental groups (n=4). The adhesive materials used in this study were three fluoride-releasing

adhesive systems (FL-BOND, Reactmer bond, FL-BOND S-1, Shofu, Kyoto, Japan) and one non-fluoride releasing system as a control (Imperva bond, Shofu, Kyoto, Japan). FL-BOND S-1 is a novel, all-in-one adhesive system. Specimens untreated with any adhesive system were categorized as non-bonding group (Table 1).

The experimental surfaces − which simulated cavity walls − were treated with respective adhesive system according to manufacturer's instructions. All materials were light-cured (Optilux 400, Demetron, Danbury, CT, USA) for two minutes at above a pressed polystyrene sheet (0.21 mm thick). Following light-curing, the specimens were stored at 100% humidity at 37℃ for six hours. After removing the sheets, all surfaces − except the pulpal side − were coated with acid-resistant nail varnish. The specimens were stored in de-ionized water for 10 days at 37℃. Finally, they were cut into halves across the adhesive surface to furnish a demineralization surface (approx. 10 mm^2), and the pulpal side was varnished (Fig. 1).

Lesion formation

In order to create lesions, each dentin sample was immersed in 20 ml of a solution of 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, and 0.1 M acetic acid. The solution was adjusted to pH 5.0 with solid KOH and a few droplets of aqueous KOH. Demineralization was performed at 37℃ for four days.

Microradiography

Three 300-μm thick sections were cut perpendicularly to the final cut-cum-experimental surface from each specimen using the sectioning machine. The sections were placed on a Perspex holder in a droplet of water and covered with a thin polyester sheet to avoid dentin shrinkage[11]. To correct optical density, a 13-step aluminum wedge − ranging from 0 to 300-μm thickness − was used. The sections were then radiographed on a high-resolution glass film plate (High-Resolution Glass plate, Konica, Tokyo, Japan) with a nickel-filtered Cu-Kα source operating at 25 kV and 15 mA for 20 minutes (PANalytical PW3830, Tokyo, Japan). The radiographic images of the sections and aluminum step wedge were analyzed using

Table 1 Adhesive systems used in this study.

Materials	Abbreviations	Ingredients	Manufacturer
Imperva bond	IB	060354 (Dentin primer) 4-AET, 2-HEMA, TEGDMA, initiator, water 050352 (Bonding agent) 2-HEMA, UDMA, TEGDMA, initiator	Shofu Inc.
FL-BOND (Imperva Fluoro bond)	FB	060384 (Primer A) water, solvent, initiator 060305 (Primer B) 4-AET, 4-AETA, 2-HEMA, UDMA, TEGDMA, initiator 060302 (Bonding agent) F-PRG filler, 2-HEMA, UDMA, TEGDMA, initiator	Shofu Inc.
Reactmer bond	RE	110310 (Bond A) FASG filler, F-PRG filler, solvent, initiator, water 110309 (Bond B) 4-AET, 4-AETA, 2-HEMA, UDMA, solvent, initiator	Shofu Inc.
FL-BOND S-1 (Fluoro bond shake-one)	FS	A-551F-3 (Bond A) S-PRG filler, FASG filler, initiator, water, solvent B-551F-3 (Bond B) 4-AET, 4-AETA, 6-MHPA 2-HEMA, Bis-GMA, initiator, solvent	Shofu Inc.
No treatment	NB		

S-PRG filler	Surface reaction type Pre-Reacted Glass-ionomer filler
F-PRG filler	Full reaction type Pre-Reacted Glass-ionomer filler
FASG filler	Fluoro Aluminosilicate Glass Filler
4-AET	4-Acryloxyethyltrimelltic acid
4-AETA	4-Acryloxyethyltrimelltic anhydride
6-MHPA	6-Methacryloxyhexylphosphonoacetate
2-HEMA	2-Hydroxyethylmethacrylate
UDMA	Urethane di-methacrylate
TEGDMA	Triethyleneglycol di-methacrylate

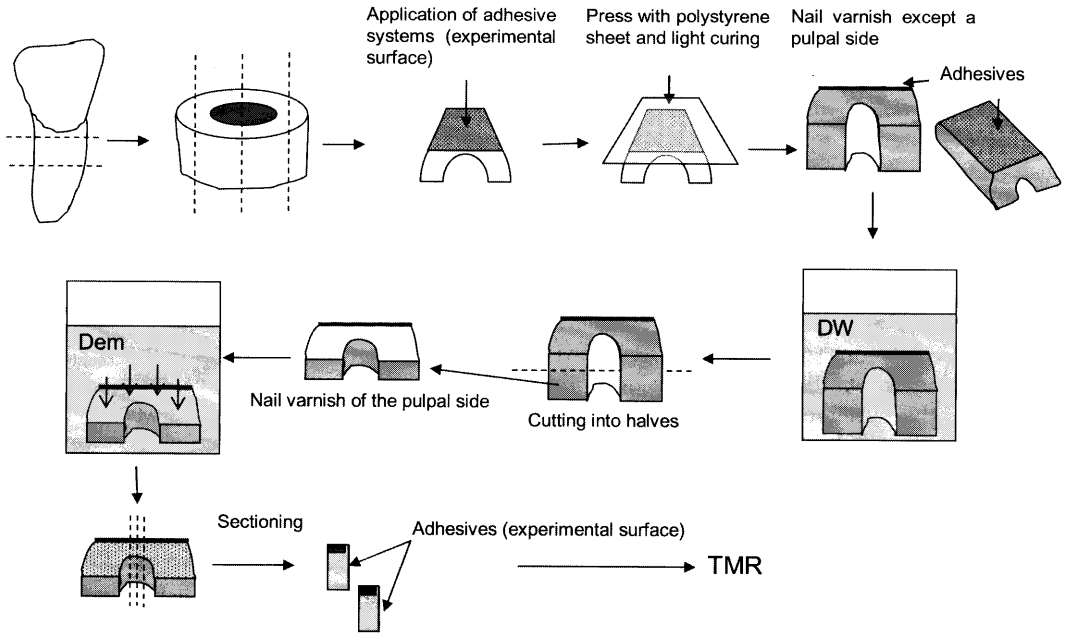

Fig. 1 Schematic drawing of a prepared dentin section. Lesions and inhibition layers on TMR films are analyzed at the corner of each section.

a microscope - video camera - microcomputer setup and software (TMR 2001, Inspektor, Amsterdam, the Netherlands)[12] at ×200 magnification. The layer exhibiting the same density as normal dentin, and adjacent to the adhesive-applied surface, was defined as the inhibition layer. To evaluate the inhibitory effect of each adhesive system, thickness of the inhibition layer at a depth of 50 μm under the demineralization surface was measured and the values were averaged for each sample[13,14]. Each thickness measurement was calculated based on difference in values of coordinate points on a TMR image.

Statistical analysis

Differences among the groups were tested for significance at $p<0.05$ level by ANOVA followed by Duncan's multiple range test using SPSS version 10.1.

RESULTS

Fig. 2 shows representative microradiographs of each group. All groups had lesions. The inhibition layers were shown as a radio-opaque layer beneath the adhesive-applied surfaces. RE and FS revealed distinct bonding layers. Wall lesions along the adhesive surface of IB and NB extended beyond the length of the outer lesions. NB showed no inhibition layers beneath the experimental surface. IB, FB, RE, and FS showed inhibition layers along the adhesive surface. The IB width was significantly narrower (1.0 μm) than that of the fluoride-releasing adhesives (FB: 7.5 μm, RE: 7.8 μm, FS: 12.5 μm) ($p<0.05$). Further, the width of FS was significantly greater than other adhesives ($p<0.05$) (Table 2).

DISCUSSION

This study simulated the formation of inhibition layers in wall lesions where resin composite and adhesive system were applied. In a sample preparation, experimental surfaces to be applied with adhesive were created in perpendicular direction to dentinal tubules and stored in de-ionized water for 10 days. This was done to accelerate diffusion of fluoride from adhesive into dentin, as compared with wall lesions in clinical situations where the adhesive surface is created along the tubular orientation. Indeed, our preparation method was useful in facilitating forma-

Table 2 Width of inhibition layer in each group. Values with same subscript are not significantly different at $p>0.05$.

Adhesive system	NB	IB	FB	RE	FS
Width(μm)	$0(0)^a$	$1.0(1.2)^a$	$7.5(2.9)^b$	$7.8(1.5)^b$	$12.5(2.7)^c$

Mean (SD) n=4

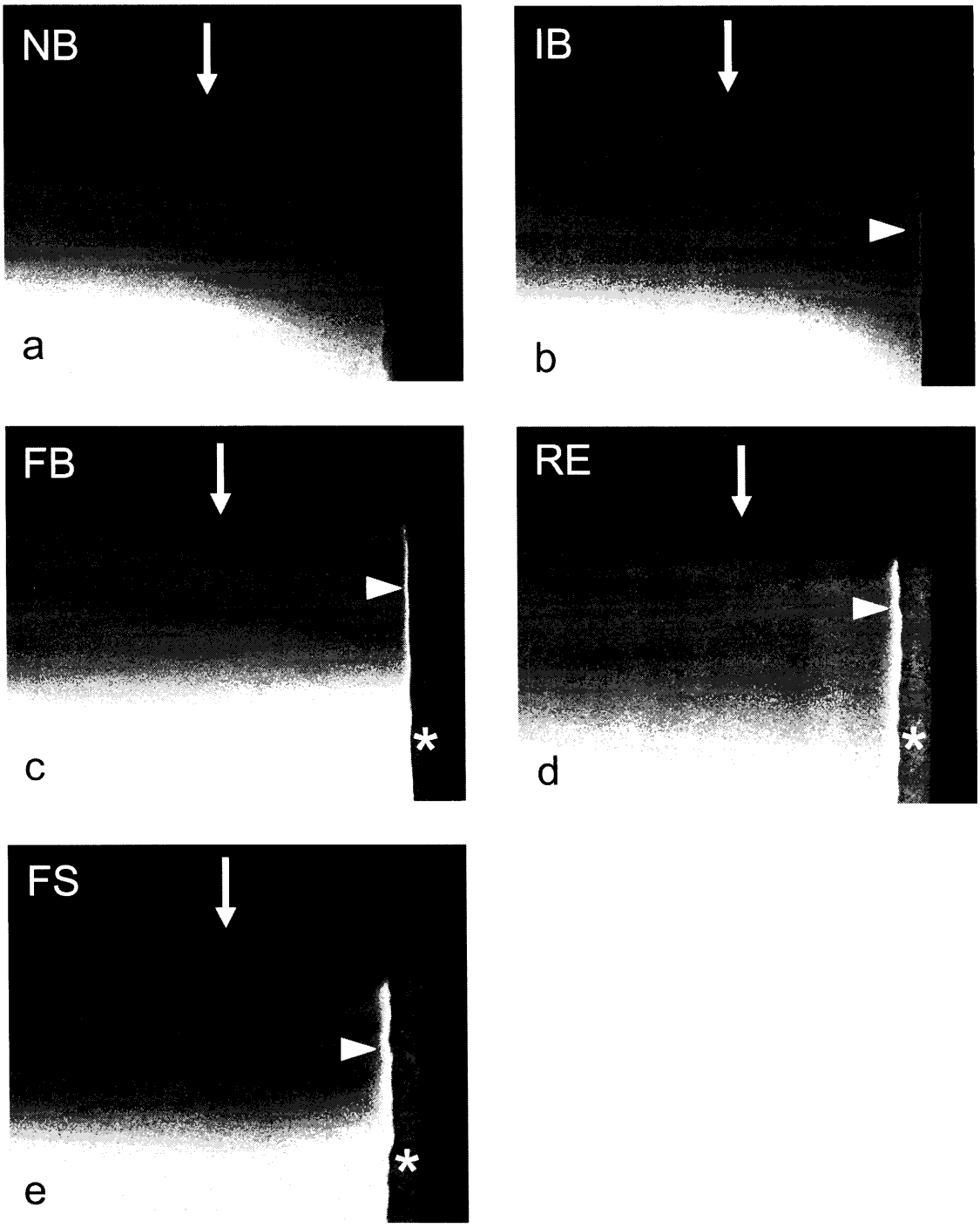

Fig. 2 Typical microradiographs of dentin lesions and inhibition layers formed adjacent to each adhesive (arrow head shows the radio-opaque inhibition layer; asterisk indicates bonding layer; white arrow indicates acid penetration).
a: No treatment (NB);
b: Imperva bond (IB);
c: FL-BOND (FB);
d: Reactmer bond (RE); and
e: FL-BOND S-1 (FS)

tion of inhibition layers within a relatively short timeframe.

It was reported that recently developed bonding systems — that promote good adhesion to dentin — showed penetration of silver ion through nano-sized spaces at the base of hybrid layers (nanoleakage)[15]. By considering the role of fluoride on dentin demineralization[16], it can be said that sufficient diffusion of fluoride from adhesive beyond the nano-sized spaces will promote formation of inhibition layers against demineralization. In this study, demineralization of NB and IB extended beyond the length of the outer lesions. In NB, demineralization from the outer surface and experimental surface grossly affected the progression of the wall lesion. Also, in IB, acid penetration through nano-sized spaces affected the progression of wall lesion. As for FB, RE, and FS adhesives, they contained PRG filler (S-PRG filler or F-PRG filler) as a fluoride-releasing source. These filler particles were created by Pre-Reacted Glass-ionomer (PRG) technology[17,18]. In this technology, glass ionomer phase is formed on glass particles through the reaction of acid-reactive fluoride (which contains glass) and poly acid in the presence of water, and the resultant product is classified as "Giomer". FB, RE, and FS adhesives formed inhibition layers of differing characteristics as the release of fluoride and other ions from each adhesive compared with NB and IB. Among these adhesives, the inhibition layer of FS was wider than those of FB, RE and IB. Generally, immersion solution with high fluoride concentration produces a thick surface layer in subsurface lesions[19,20]. However, in inhibition layers, it is unknown whether an identical mechanism supports their formation. Further research is therefore needed to investigate other related factors such as released fluoride content, diffusion profile, and remineralization which follows.

RE and FS contained adhesive-promoting monomers, FASG filler, and water as basic constituents. Therefore, acid-base reaction occurred between adhesive-promoting monomer and FASG filler in the presence of water as a curing reaction, the same reaction mechanism found in glass-ionomer cement[21], in addition to light cure and chemical cure. In fact, these adhesives had two sources from which fluoride and other mineral ions were released. One source was the PRG filler in the bonding layer, and the other source stemmed from fluoride and other mineral ions taken into the bonding layer during acid-base reaction. Difference in the inhibition layers of RE and FS was probably due to the type of PRG filler and adhesive-promoting monomer that they contained[22]. In particular, FS contained phosphoric adhesive-promoting monomer called 6-MPHA, in addition to carboxylic adhesive-promoting monomer such as 4-AETA and 4-AET which were also contained in RE. Since phosphoric adhesive-promoting monomer reacts faster with FASG filler in acid-base reaction, and that it has a higher erosion effect on FASG filler, more fluoride and other mineral ions are therefore trapped inside the bonding layer. This then leads to improved releasing ability of fluoride and other mineral ions from the bonding layer.

CONCLUSION

Based on the observations in this study, we concluded that FS system demonstrated a superior ability in forming a distinct inhibition layer in wall lesions, thus helping to deliver anticariogenic effect against secondary caries in clinical situations.

REFERENCES

1) Nagai Y, Inaba D, Minami K, Matsuda K. The effects of fluoride-releasing restorative materials on inhibition of secondary caries *in vitro*. Cariology Today 2000; 1: 16-19.
2) ten Cate JM, van Duinen RNB. Hypermineralization of dentinal lesions adjacent to glass-ionomer cement restorations. J Dent Res 1995; 74: 1266-1271.
3) Kotsanos N. An intraoral study of caries induced on enamel in contrast with fluoride-releasing restorative materials. Caries Res 2001; 35: 200-204.
4) Hsu CYS, Donly KJ, Drake DR, Wefel JS. Effects of aged fluoride-containing restorative materials on recurrent root caries. J Dent Res 1998; 77: 418-425.
5) Zaura-Arite E, Exterkate RAM, ten Cate JM. Effects of high fluoride concentrations on bovine dentin demineralization in narrow grooves *in vitro*. Eur J Oral Sci 1990; 107: 455-460.
6) Mukai Y, Tomiyama K, Kamijo K, Shiiya T, Fujino F, Teranaka T, ten Cate JM. Lasting efficacy of fluoride-releasing filling materials against dentin demineralization. Caries Res 2003; 37: 295.
7) Itota T, Nakabo S, Iwai Y, Konishi N, Nagamine M, Torii Y, Yoshiyama M. Efect of adhesives on the inhibition of secondary caries around compomer restorations. Operative Dent 2001; 26: 445-450.
8) Pereira PNR, Inokoshi S, Tagami J. *In vitro* secondary caries inhibition around fluoride releasing materials. J Dent 1998; 26: 505-510.
9) Ogata M, Takada T, Harada N, Nakaiima M, Tagami J. Micro-shear bond strength of a newly developed fluoride releasing all-in-one adhesive. Adhes Dent 2003; 21: 74-81.
10) Seki Y, Mohammed AU, Sasafuchi Y, Shimada Y, Ohtsuki M, Tagami J. Pulpal response of a newly develop one-step bonding system SI-IB551. Adhes Dent 2003; 21: 324-325.
11) van Strijp AJP, Buijs MJ, ten Cate JM. Contact microradiography of dentine under wet conditions to prevent lesion shrinkage. Caries Res 1995; 29: 107-110.
12) Lagerweij MD, de Josselin de Jong E, ten Cate JM. The video camera compared with the densitometer as a scanning device for microradiography. Caries Res 1994; 28: 353-362.

13) Arends J, Christoffersen J, Ruben J, Jongebloed WL. Remineralization of bovine dentine *in vitro* — The influence of the F content in solution on mineral distribution. Caries Res 1989; 23: 309-314.
14) Iijima Y, Ruben JL, Zuidgeest TGM, Arends J. Fluoride and mineral content in hyper-remineralized coronal bovine dentine *in vitro* after an acid challenge. Caries Res 1993; 27: 106-110.
15) Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG, Pashley DH. Nano-leakage within the hybrid layer. Operative Dent 1995; 20: 18-25.
16) ten Cate JM, Buijs MJ, Damen JJM. pH-cycling of enamel and dentin lesions in the presence of low concentration of fluoride. Eur J Oral Sci 1995; 103: 362-367.
17) Ikemura K, Kouro Y, Endo T. Effect of a new pre-reacted glass-polyalkenoate filler in a dental adhesive on bonding durability to teeth under fatigue load cycling. Adhes Dent 1998; 34: 47-57.
18) Ikemura K, Kouro Y, Endo T. A new fluoride-releasing dental adhesive and its bonding durability to teeth under longterm water immersion. Adhes Dent 1998; 34: 85-97.
19) Mukai Y, Lagerweij MD, ten Cate JM. Effect of a solution with high fluoride concentration on remineralization of shallow and deep root surface caries *in vitro*. Caries Res 2001; 35: 317-324.
20) Mukai Y, ten Cate JM. Remineralization of advanced root dentin lesions *in vitro*. Caries Res 2002; 36: 275-280.
21) Hosoda H. Glass ionomer dental cement — The materials and their clinical use, Ishiyaku EuroAmerica Inc.Publishers, St Louis, 1993, pp.16-24.
22) Ikemura K, Tay FR, Kouro Y, Endo T, Yoshiyama M, Miyai H, Pashley DH. Optimizing filler content in an adhesive system containing pre-reacted glass-ionomer fillers. Dent Mater 2003; 19: 137-146.