Fluoride release and microhardness of one-step adhesives in two immersion solutions

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Purpose: To measure the amount of fluoride-release and microhardness of fluoride-releasing one-step adhesives; Xeno CF Bond, Reactmer Bond, and One-Up Bond F in two different solutions.
Materials and Methods: For fluoride-release measurement, five samples for each group were stored in deionized water or demineralizing solution for one day. A resin-modified glass ionomer cement, Fuji II LC (Improved), was used as a control. Fluoride ion release was analyzed with a fluoride ion-specific electrode. For microhardness measurement, the specimens prepared in the same manner as described above were stored in two different solutions or dry condition for one day. After dividing into halves, the microhardness of the specimen was measured using a nanoindentation tester.
Results: Amount of fluoride-release and microhardness of the adhesives were significantly different according to the immersion solutions and the materials. The adhesives demonstrated significantly less amounts of fluoride-release than that of Fuji II LC (Improved). Microhardness of the specimens immersed in solutions were significantly higher than those stored in dry condition for all the adhesives.
Conclusion: The amount of fluoride-release was significantly less than that of the resin-modified glass ionomer cement. Microhardness of the adhesives increased after 1-day storage in the media probably due to subsequent acid-base reaction and cross-linking of polymers. **(Int Chin J Dent 2004; 4: 1-7.)**

Clinical Significance: Amount of fluoride-release and microhardness of the fluoride-containing adhesives were material and immersion solution dependent. The adhesives demonstrated the capacity for fluoride-release, although the amount of fluoride-release was significantly less than that of the resin-modified glass ionomer cement.
Key Words: fluoride-release, microhardness, one-step adhesives.

Introduction

Recurrent or secondary caries has been one of the main reasons for the failure and replacement of restorations. As an attempt to prevent recurrent caries, fluoride (F)-containing restorative materials have been developed.[1] Examples of these materials are amalgam,[2] glass ionomer cements,[3] resin-modified glass ionomer cements,[4,5] compomers,[6,7] and resin composites.[8] These materials release fluoride in different amounts,[9] and the cariostatic effect of fluoride on enamel and root dentin caries has been demonstrated in several studies in vitro[10,11] and in vivo.[12]

Dentin adhesives for direct composite restorations have been widely accepted. Recently, fluoride has been incorporated to dentin bonding systems. Consequently, F-containing dentin adhesives would bond effectively to tooth structure, contribute to reduction of microleakage,[13] and possibly prevent recurrent caries due to F-release.[14] Although there are several reports on measurement of F-release from F-releasing restorative materials,[6,9] studies evaluating F-release and a change of microhardness from adhesive systems

are lacking. The purpose of this study was to measure the amount of F-release and microhardness of three F-releasing one-step adhesives in two different solutions.

Materials and Methods

Specimen Preparation

The materials, manufacturers and batch numbers used in this study are listed in Table 1. Three adhesives, Xeno CF Bond (XB, Dentsply-Sankin, Tokyo, Japan), Reactmer Bond (RB, Shofu, Kyoto, Japan), One-Up Bond F (OB, Tokuyama Dental, Tokyo, Japan), were evaluated. A resin-modified glass ionomer cement, Fuji II LC (Improved) (F II LC, GC, Tokyo, Japan) was used as a control in measurement of fluoride-release.

Table 1. Materials used in this study.

Material (Code)	Batch		Composition	Manufacturer
Xeno CF Bond (XB)	060911 060911	Universal Catalyst	HEMA, Ethanol, Water Pyrophosphate ester, UDMA, PEM-F, Microfiller, Catalyst	Dentsply-Sankin Tokyo, Japan
Reactmer Bond (RB)	099900 099900	Bonding A Bonding B	Aluminum fluorosilicate glass, F-PRG filler, Water, Initiator 4-AET, 4-AETA, UDMA, Initiator, etc	Shofu Inc. Kyoto, Japan
One-Up Bond F (OB)	001 501	Bonding A Bonding B	Phosphoric monomer, MAC-10, Catalyst, Monomer HEMA, Water, Aluminum fluorosilicate glass, Catalyst	Tokuyama Dental Tokyo, Japan
Fuji II LC (Improved) (F IILC)	050491 310391	Powder Liquid	Fluoro-aluminosilicate glass, Shade pigments Acrylic-maleic acid copolymer, HEMA, Water, etc	GC Corp. Tokyo, Japan

HEMA, 2-hydroxyethyl methacrylate; UDMA, urethane dimethacrylate; PEM-F, Penta cyclophosphazene fluoride; F-PRG filler, Full reaction type of pre-reacted-glass ionomer filler; 4-AET, 4-acryloxyethyltrimellitate, 4-AETA, 4-acryloxyethyl trimellitate anhydride; MAC-10, 11-methacryloxy-1,1-undecane dicarboxylic acid.

The materials were placed in a vinyl mold, through which a 3 mm x 0.36 mm hole (diameter x height) was made. The materials were mixed in accordance with the manufacturer's instruction prior to application. Following this, the mold was covered with a plastic matrix strip, pressed flat with a glass slide and light-cured for 20 s per site on the top and the bottom sides. Each specimen was placed in a bottle containing 8 mL of either deionized water (DW) or demineralizing solution (DS; pH 4.5) at 37°C.

The demineralizing solution was formulated according to van Dijk et al.[15] and ten Cate et al.[16] composition: 2.2 mmol/L $CaCl_2$, 2.2 mmol/L NaH_2PO_4, and 50 mmol/L acetic acid adjusted to pH value of 4.5 with NaOH.

Measurement of Fluoride Release

After storage in each solution, specimens were rinsed with 1 mL of DW or DS, which was added to 8 mL of storage solution. The specimens were then placed in a new solution of either DW or DS. One milliliter

of total ions strengths adjustment buffer (TISAB) was added to the 8 mL of old storage solution and 1 mL of rinse solution, making a total volume of 10 mL, which was measured for concentration of fluoride ions. The F-ion release was measured with an ion-specific electrode (combination electrode fluoride 960900; Orion Research, Boston, MA, USA) and calculated in parts per million (ppm). Theoretically, the limit of F-ion measurement is 0.02 ppm with this ion-specific electrode.

Ten disks were prepared for each material and divided into two groups. Means and standard deviations for five samples of each group were calculated. Statistical analysis of the amount of fluoride release from each material was done using one- and two-way analysis of variance (ANOVA) and Fisher's PLSD test at the 95% level of confidence.

Microhardness Measurement

Sample preparation for a nanoindentation testing is similar to that for the F-release measurement. The specimens were stored in either DW or DS for one day as described above. Also, the specimens were kept dry at room temperature for one day, which were used as a dry control. Following this, the disc was carefully cut into two halves at the center of the disc using a safety razor. The specimens were then embedded in epoxy resin (EPON 815, Nisshin EM, Tokyo, Japan). After 24 hours of polymerization of the resin, the surfaces were polished with waterproof silicon carbide papers (grit #600, 800, and 1,000) and diamond pastes (particle size 6, 3, 1, and 0.25 µm). Specimens were set on the stage of the nanoindentation tester (ENT-1100, Elionix, Tokyo, Japan).

The instrument used for this experiment was a depth sensing computer controlled instrument with a three-sided pyramidal diamond probe as previously reported.[17] After setting a sample on the stage of the testing machine, the instrument was programmed to create the position of indentations. Five indentations were performed around the center of the polished surface for each specimen. The load on the indenter was 0.049 N (5 gf). After the performing the indentation, nanohardness were calculated by an attached computer. Indentation tests were carried out on ten specimens for each group. After the tests, the geometry of the indentation marks was confirmed on the attached display.

Fifteen disks were prepared for each material and divided into three groups. Means and standard deviations for five samples of each group were calculated. Statistical analysis of microhardness of one-step bonding agent was done using one- and two-way ANOVAs and Fisher's PLSD test at the 95% level of confidence.

Results

Table 2 shows the amount of fluoride released from FII LC and F-releasing dentin adhesive materials in DW and DS after one day, respectively.

Analysis with two-way ANOVA revealed that the amount of F-release of FII LC and the other F-releasing adhesive materials tested was significantly different in according to the immersion solution (F=170.091, p<0.0001) and the materials (F=38.582, p<0.0001).

As expected, FII LC had the highest amounts of F-release in both DW and DS solutions, and a

significant differences were found when compared to those obtained with dentin adhesive materials (p<0.05).

The tendency of the amount of F-release varied for each adhesive material. For XB, the amount of F-release in DW (0.06 ppm) was higher than that in DS (0.03 ppm) (p<0.05). For RB and OB, there were no significant differences in the amounts of F-release between in DW and in DS (p>0.05).

Table 2. Fluoride release from each material after one day (ppm).

Material	Deionized water	Demineralizing solution
F II LC	0.13 (0.019)	0.34 (0.064)
XB	0.06 (0.009) [A]	0.03 (0.005) [B]
RB	0.02 (0.003) [A, C]	0.03 (0.003) [B, C]
OB	0.03 (0.005) [A, D]	0.03 (0.006) [B, D]

Mean (SD), n=10. No statistically significant differences within columns are shown by superscript letters (p>0.05).

Table 3. Microhardness of one-step bonding agent after one day (MPa).

Material	Dry	Deionized water	Demineralizing solution
RB	292.76 (25.71)	309.80 (14.80) [D]	312.55 (18.06) [D]
OB	223.78 (3.47) [A]	243.06 (4.39) [B, E]	245.92 (6.22) [C, E]
XB	207.55 (2.04) [A]	231.22 (1.33) [B]	250.71 (1.73) [C]

Mean (SD), n=10. No statistically significant differences within columns are shown by superscript letters (p>0.05).

Table 3 indicates the microhardness of the one-step adhesives after 1-day storage in the dry, water and acidic conditions, respectively. Analysis with two-way ANOVA revealed that the microhardness of F-releasing adhesive materials tested was significantly different in according to the storage condition (F=44.561, p<0.0001) and the materials (F=359.094, p<0.0001). The microhardness of Dry groups indicated significantly lower than those of DW and DS in all the adhesives (p<0.05). For RB and OB, there were no significant differences in the microhardness between DW and DS (p>0.05), while the microhardness of DS was significantly higher than that of DW for XB group (p<0.05). The microhardness of RB indicated the highest in the three adhesives for each condition.

Discussion

Recently, the materials for one-step restorative adhesives with F-releasing potential have been developed.[18] These systems are simple to handle, bond to dentin and show sustained F-release.[19,20] Bonding agents are being used as bases in restorative dentistry because of their ability to provide efficient cavity sealing to prevent microleakage.[21]

The source of fluoride from F II LC was from the gel layer of fluoro-aluminosilicate glass. However, the sources of fluoride from the F-release dentin adhesive materials were different for each material. For XB, the source of F-release was from F-release methacrylate, PEM-F. For RB, the sources of fluoride are from

both pre-reacted glass ionomer (PRG) filler and aluminum fluorosilicate glass. The PRG filler is specially synthesized by the acid-base reaction of glass-ionomer cement. The acidic monomer of 4-AET in the adhesive is believed to promote release of fluoride from the PRG filler and aluminum fluorosilicate glass. For OB, the source of F-release was from the aluminum fluorosilicate glass.

In order to simulate the clinical conditions, the specimen size of adhesive materials for F-releasing measurement in this study was much smaller and thinner than the previous one for conventional glass ionomer cement, compomer or F-releasing resin composite. In clinical situation, the thickness of the adhesives might be much less than the size used in this study. It was reported that the maximum thickness of the bonding agent used in the resin coating of composite inlays at the cavity walls were between 63 µm and 145 µm.[22] However, the sample size influenced the amount of F-release from that, which was close to the limit of the F-measuring electrode (0.02 ppm). And also, it is very difficult and technique sensitive to make such a thin specimen. Therefore, the sample size was decided as thinner as possible in consideration of both the limitation of the F-measuring devices and the handling difficulties to make the specimens.

Generally, greatest amounts of F-release from F-releasing restorative materials occur in the first 24 hours, especially the first four hours.[9] The amount of fluoride loss may be associated with the setting and maturation reactions of the materials.[3,23] The amount of F-release from the resin-modified glass ionomer cement was significantly greater than those from dentin adhesives.

A greater amount of F-release from FII LC was obtained in DS compared with DW, which was similar to the previous studies by Forss et al.[24] and Forsten,[4] documenting higher amounts of F-release in acidic solutions. It was believed that this greater release of fluoride was caused by the dissolution of the GIC-matrix in the demineralizing solution. Karantakis et al.[9] reported that the drop of pH by use of lactic acid solution also affected the F-release to such a degree for resin-modified glass ionomer cements, compomers, and resin composites.

However, there were no significant differences of released fluoride between DW and DS for RB and OB. Surprisingly, the amount of F-release in DW was significantly higher than that in DS for XB. The source of fluoride from XB is the F-releasing monomer, PEM-F. The release of fluoride may be more stable in an acidic condition than in a basic condition because pKa of PEM-F is around 2.0 (unpublished data). Therefore, XB may release more fluoride in DW than in DS.

Most of the materials used in this study contain a hydrophilic resin monomers like HEMA, which forms poly-HEMA and has strong affinity to water with its hydrophilic hydroxyl group causing F-release to be able to take place.[25] Several investigators have attributed this to the composition and content of the fluoride source and hydrophilic monomers.[26] For dentin adhesive materials, the F-source surrounded by a resin matrix may have difficulty being in contact with deionized water or demineralizing solution.[27] The free movement of deionized water or the solution is probably limited in the cured resin matrix.[28] Differences may also be explained by the mechanism of F-release, as the release partly occurs by diffusion through pores and crack. Therefore, it could be expected that F-release will differ among different materials, as was found in this study. The polymerized resin matrix may be a barrier to release of fluoride ions, but further

study is necessary to understand the mechanism of the F-release from dentin adhesive materials.

Munack et al.[29] reported that surface microhardness of compomers dropped significantly due to wet storage, and the type of the storage media did not influence microhardness significantly. Therefore, it was expected that microhardness of the one-step adhesives would decrease in the media. However, microhardness of the one-step adhesive systems in DS or DW was significantly higher than that of Dry group. Though the reason for the higher values is unclear, the subsequent acid-base reaction and cross-linking of polymers might continue after initial polymerization under water, leading increased microhardness.

Okada et al.[30] reported microhardness of the conventional glass ionomer cement and the compomer increased 40 days storage in distilled water, while that of the resin composite decreased. Urabe et al.[17] reported that microhardness of F-releasing bonding resin and Protect Liner F decreased after 6 months storage in DW and DS. Because of the F-release from the adhesives for a long time, microhardness would decrease. It seems that further long-term study should be done to evaluate the stability of the F-releasing adhesive materials.

Conclusion

The fluoride-releasing dentin adhesive materials used in this study demonstrated the capacity for F-release in both solutions, although the amount of F-release from the materials was significantly less than that of the resin-modified glass ionomer cement. Microhardness of the adhesives increased after 1-day storage in the media probably due to subsequent acid-base reaction and cross-linking of polymers.

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