Effect of Experimental Fluoride-releasing Tooth Separator on Acid Resistance of Human Enamel in vitro

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This study aimed to investigate the fluoride-releasing ability of an experimental tooth separator consisting of polyurethane elastomer with tin fluoride and its effect on the acid resistance of human enamel. The tooth separator was set around an enamel slab and stored in de-ionized water for 10 days. The daily concentration of fluoride in the de-ionized water was measured. Then the enamel surface was artificially decalcified by a lactic acid buffer solution (pH 4.5) for 96 hours. The mineral density at the surface layer of the enamel was measured to evaluate the acid resistance. The fluoride release increased with the amount of fluoride in the separator, but decreased with the immersion time. Both the enamel area contacting with the separator and its surrounding area showed lower mineral loss and lesion depth compared with the controls (P<0.05). It is suggested that the experimental tooth separator would release enough fluoride and improve the acid resistance of the enamel surface layer.

Key words: Fluoride-release, Polyurethane, Tooth separator

INTRODUCTION

Enamel demineralization associated with fixed orthodontic treatment is an extensively rapid process caused by a high and continuous cariogenic challenge in the plaque developed around brackets and underneath ill-fitting bands. After removing appliances, so-called white spots and surfacial or interproximal caries are serious problems. Gorelick et al. reported that 50% of patients experience an increase in white spots after a full term of orthodontic treatment. Not only is plaque removal very difficult with complex appliances on teeth, but the appliances themselves are easy to enhance bacterial formation. So, the routine preventive measures such as oral hygiene instruction, mechanical removal of plaque, chemical agents, and topical fluoride application are more difficult to remove plaque. Moreover, these methods are often dependent on patient cooperation. They have only a limited effect on the reduction of decalcification resulting from fixed orthodontic therapy. An ideal preventive measure would be one that could operate independently of patient cooperation. Fluoride-releasing materials can release fluoride automatically in a patient’s cavity compensating for the deficiency that orthodontic patients have difficulty maintaining oral hygiene. Fluoride-releasing sealants, glass ionomer cements and composite resins have been used in orthodontic patients to prevent demineralization for
many years. However, some long-term clinical trials have been ineffective in reducing tooth demineralization\textsuperscript{7-11}. In the 1990's, the fluoride-releasing elastomeric chains and modules, Fluor-I-chain and Fluor-I-ties, were developed. Some studies on their characteristics were reported. The elastomers released enough fluoride, decreased the number of salivary streptococcus mutans significantly, and hardened the enamel at the 20\textmu m depth\textsuperscript{12,13}. However, Fluor-I-chain was unable to deliver a force within the optimal range for tooth movement after one week\textsuperscript{14}. In 2000 Banks et al.\textsuperscript{15} undertook a prospective controlled clinical trial on the fluoride-releasing elastomeric modules (Fluor-I-Ties) and chains (Fluor-I-Chain). They reported that fluoride-releasing elastomers appeared to provide a clinically worthwhile reduction in enamel decalcification during fixed appliance therapy. In the same year Itoh\textsuperscript{16} reported that two kinds of polyurethane elastomers (E580 and E880) showed excellent mechanical strength and dispersed inorganic fluoride compounds (NaF 1.25 wt\% and SnF\textsubscript{2} 1.25-5.0 wt\%) as fluoride release sources. They reported the experimental elastomeric sheets showed suitable mechanical strength and fluoride release for elastomeric devices such as teeth separators and ligature ties. However, the efficiency of the acid resistance of human enamel was not clear in the previous studies. To clarify the effect of the fluoride-releasing experimental elastomer on the acid resistance the number of samples in the present study, namely the measured places, were increased. So, two kinds of tin fluoride (2.5 and 5.0 wt\%) were chosen at random. Since the elastomer containing sodium fluoride (NaF) shows marked swelling in water\textsuperscript{16} it was not used. In the present study we prepared two kinds of experimental tooth separators that contained 2.5 or 5.0 wt\% tin fluoride (SnF\textsubscript{2}) and investigated the effect of fluoride in the rubbers on the acid resistance of human enamel \textit{in vitro}.

**MATERIALS AND METHODS**

**Materials**

Fluoride-releasing polyurethane elastomer sheets were prepared by the following method. The fixed quantities of polyurethane elastomer (E580, Nihon Miractoran, Osaka, Japan) and tin fluoride (G: 10-80\textmu m, Kanto Chem. Co., Tokyo, Japan) were kneaded using a hot kneading machine (160\textdegree C) for 5 minutes and pressed into 0.5 mm-thick sheets at a laboratory in Nihon Miractoran (Atsugi, Japan). The concentrations of tin fluoride in the sheets were 2.5 and 5.0 wt\%, respectively. The o-ring type experimental tooth separator, whose size was similar to ALASIK\textsuperscript{TM} Separator Modules S2 (inner diameter 2.4 mm and outer diameter 4.4 mm, 3M Unitek, USA), were made from the sheet using a hot metal mold (160\textdegree C). The mass of one experimental separator was about 18.0 mg.

**Methods**

**Preparation of samples**

Eight crowns of sound premolar teeth extracted for orthodontic reason were chosen as experimental objects. Each crown was divided into four or five slabs longitudi-
nally using low speed saw (Isomet, Buehler, Lake Bluff, Illinois, USA). Thirty-two enamel slabs, no decalcification, caries, cracks or abrasions under ×4 magnification (Mantis, Vinson Engineering Ltd. UK), were selected and polished with a messeage brush (Shofu Co., Kyoto, Japan) and cool water. Each slab was prepared at approximately similar size using a water-cooled, high-speed handpiece. A 4×2 mm window was designed at the center of the enamel surface and divided into three areas (area A 1.5×2 mm, area B 1×2 mm, area C 1.5×2 mm). Area A was occlusal to area B, area C cervical to area B. Area D was the sound enamel outside the window. Each slab was coated well with acid-resistant varnish except area B and area C. These slabs were divided into two groups at random, group 1 setting with experimental tooth separator with 2.5 wt% SnF$_2$ and group 2 with 5.0 wt% SnF$_2$. The separator was set around the tooth slab, covering area B of the window. The part of the separator that was out with the window was also painted with the same varnish. A schematic drawing of the sample fixed with a tooth separator is shown in Fig. 1. The exposed surface area of a separator was about 6.28 mm$^2$.

**Fluoride ion release**

Each prepared sample was placed in a plastic vial containing 2 ml de-ionized water (DW) and placed in a 37°C incubator for ten days. The DW was changed every 24 hours and preserved in a refrigerator at below 4°C until the measurement day. The concentration of fluoride ion was measured using ion meter and fluoride electrode (720A, Orion Research Inc, Boston, USA).

![Schematic drawing of the sample fixed with an experimental tooth separator.](image)

Fig. 1 Schematic drawing of the sample fixed with an experimental tooth separator.

A: area A where enamel was not affected by fluoride.

B: area B where enamel was contacted by the experimental tooth separator.

C: area C where enamel was near the experimental tooth separator.

D: area D where enamel was sound.
Acid resistance
After 10 days, the samples were taken out of the DW. The tooth separators were taken out of the samples. Then the slabs were immersed into acetone to dissolve the varnish. Having been cleaned and dried, the slabs were coated with the varnish again except the window (4×2 mm) on the enamel surface. Each slab was placed in a plastic container with 20 ml of 0.1 M lactic acid buffer (pH 4.5). The samples were decalcified for 96 hours with stirring in the water bath at 37°C. After artificial demineralization, the slabs were put into acetone to dissolve all the varnish, imbedded with Orthodontic Resin (Dentsply Caulk, DE, U.S.A.), sectioned into about 500 μm-thick slices with Isomet and ground into 100 μm-thick slices with waterproof abrasive paper (Riken Corundum, Kounosu, Japan) and lapping film (Maruto Instrument Co, Tokyo, Japan).

Microradiography (MR)
To determine the lesion depth and mineral content of the demineralized enamel, microradiographs were made of the thin slices together with an aluminum calibration step-wedge on high-resolution photographic film (SO343, Kodak Co. Tokyo, Japan). A monochromatic X-ray generator (SPO-M, Sofron, Tokyo, Japan) was used at 15 kV, 5 mA for 15 minutes. It was 55 mm between the film and the generator.

Mineral density analysis
The radiographic density of the film was measured using the microdensitometer (PDM-7, Konica Medical Co., Tokyo, Japan) at the 2×10 μm slit. Three scans were done at area A, B, C and D, respectively. These values were processed using mineral density analysis software in a computer. The plotted mineral tracings were subse-

![Fig. 2 Schematic distribution of the mineral volume of human enamel after artificial demineralization.](image)

\[ \Delta Z: \text{mineral loss (vol\% μm)}, \ LD: \text{lesion depth (μm)}, \ 89: \text{the value of the mineral content of theoretically sound enamel.} \]

Demineralization condition: 0.1 M lactic acid buffer solution (pH 4.5) at 37°C for 96 hours.
quently assessed for mineral loss ($\Delta Z$, vol% $\mu$m) and lesion depth (LD, $\mu$m), as shown in Fig. 2. The mean value of the sound enamel was caused by several methodological factors like densitometer slit width and curved tooth surface\(^1\). So, the ideal mineral loss of demineralized enamel was the measured value minus the value of mineral loss of the sound enamel ($D_{\Delta z}$). Since area A, B, C and D were in the same enamel slice the value of $D_{\Delta z}$ was equal. The acquired acid resistance rate (AR) was calculated using the following equation:

$$AR = \frac{[(A_{\Delta z}-D_{\Delta z})-(X_{\Delta z}-D_{\Delta z})]/(A_{\Delta z}-D_{\Delta z})]{}/(A_{\Delta z}-X_{\Delta z})/(A_{\Delta z}-D_{\Delta z})}$$

$A_{\Delta z}$: the average mineral loss of the area not affected by fluoride (area A, control)

$X_{\Delta z}$: the average mineral loss of the area affected by fluoride (area, B or C)

$D_{\Delta z}$: the average mineral loss of the sound enamel

From the equation it was found that if the value of $D_{\Delta z}$ was great compared with the value of $A_{\Delta z}$ it had efficiency on the AR, and if small, the efficiency was small.

**Statistical analysis**

The data were statistically analyzed using a paired t test and Tukey one-way analysis of variance. P values at <0.05 were regarded as significance.

**RESULTS**

**Fluoride release**

Since the velocity of fluoride release is related to the surface area of the fluoride-releasing material, the fluoride concentration recorded in ppm unit (Table 1) was converted to $\mu$g/cm$^2$/day unit\(^1^8\). The averages of daily velocity of fluoride release through a part of an experimental separator are presented in Fig. 3. The value of fluoride release speed is different from that of the experimental tooth separator. In the study, the numeral data showed the mean fluoride release velocity was significantly different between the first day and any other day, respectively for groups 1 or 2 (p<0.05). From the second day the daily rate of fluoride release decreased gradually. However the mean fluoride release velocity was significantly different between group 1 and group 2 on the same day (p<0.01). During 10 days for one sample in group 1 the totally released fluoride was 43.2$\mu$g, which was 9.6% of the total fluoride in one tooth separator. For group 2 it was 72.0$\mu$g, 8.0% of the total fluoride.

**Table 1** The daily cumulative F in 2 ml de-ionized water

<table>
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<th>7</th>
<th>8</th>
<th>9</th>
<th>10 day</th>
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<td>4.9</td>
<td>8.4</td>
<td>11.2</td>
<td>13.5</td>
<td>15.4</td>
<td>17.0</td>
<td>18.4</td>
<td>19.6</td>
<td>20.7</td>
<td>21.6</td>
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<td>(2.3)</td>
<td>(3.7)</td>
<td>(4.5)</td>
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<td>(5.8)</td>
<td>(6.1)</td>
<td>(6.4)</td>
<td>(6.6)</td>
<td>(6.9)</td>
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<td>Group 2</td>
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<td></td>
<td>8.4</td>
<td>14.0</td>
<td>18.3</td>
<td>21.8</td>
<td>24.5</td>
<td>27.0</td>
<td>29.2</td>
<td>31.2</td>
<td>33.1</td>
<td>34.7</td>
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<td></td>
<td>(2.5)</td>
<td>(3.6)</td>
<td>(5.4)</td>
<td>(5.9)</td>
<td>(6.2)</td>
<td>(6.5)</td>
<td>(6.6)</td>
<td>(6.8)</td>
<td>(6.9)</td>
<td>(7.0)</td>
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group 1: the samples set with 2.5 wt% SnF$_2$ experimental tooth separators \(^{()}\): SD

group 2: the samples set with 5.0 wt% SnF$_2$ experimental tooth separators
In this experiment, because only a part of the surface of the tooth separator was exposed in de-ionized water, the other covered by varnish, fluoride would be gradually leached from the smaller area. So, the sharp decline in fluoride release rate was not apparent. However, the rate of fluoride release was significantly different between groups 1 and 2 on the same day (p<0.01).

<table>
<thead>
<tr>
<th>Area</th>
<th>LD (µm)</th>
<th>ΔZ (vol%·µm)</th>
<th>AR</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>185±37</td>
<td>8819±2876</td>
<td>7875±2067</td>
</tr>
<tr>
<td>B</td>
<td>109±32</td>
<td>3120±1492</td>
<td>2786±1119</td>
</tr>
<tr>
<td>C</td>
<td>144±48</td>
<td>5698±3554</td>
<td>4389±1640</td>
</tr>
<tr>
<td>D</td>
<td>63±23</td>
<td>1777±586</td>
<td>1751±438</td>
</tr>
</tbody>
</table>

LD: lesion depth (µm)  ΔZ: mineral loss (vol%·µm)  AR: acquired acid resistance rate

There is no significant difference between groups 1 and 2 for the same area (p>0.05)

Acid resistance

The mean mineral loss (ΔZ), lesion depth (LD) and acquired acid resistance rate (AR) of different areas of human enamel are presented in Table 2. The number of microradiographical tracings used was 480. In the same group, the values of LD, ΔZ or AR were significantly different (p<0.05) with different areas (areas A, B, C, and D) except for the values of ΔZ between area B and area D. However, in the same area the values of LD, ΔZ or AR showed no significant difference between group 1 and group 2 (p>0.05).
DISCUSSION

The rate of fluoride release from materials in vitro may be influenced by several factors such as surface area, pH, temperature, frequency of analysis, and the storage medium\(^8\). In this experiment only a part of the surface of the tooth separator (6.28 mm\(^2\)) was exposed in the de-ionized water at 37°C. Since the unexposed area was only covered by varnish, not cut-off from the individual separator, fluoride in the varnish-covered area would gradually leach out from the smaller area. So, the rate and pattern of fluoride release from a whole tooth separator would be different. The rates of fluoride release were different between the materials, the fluoride concentrations, the experimental conditions and the units. Since the units of rate were different in previous studies a direct comparison was difficult. Storie et al.\(^{14}\) and Wiltshire et al.\(^{13}\) respectively studied the fluoride release of Fluor-I-Chain and Fluor-I-Ties. The rate was, respectively, 0.22 and 1.30 µg/ml/day for an individual loop on day 1. Creanor et al.\(^{19}\) investigated the fluoride-releasing characteristics of five commercial glass ionomers: Vitrebond, Ketac-Fil, Fuji II LC, Chemfil-Superior and Aquacem. The rate of fluoride release was, respectively, about 366.1, 120.0, 99.8, 70.8, 36.6 µg/cm\(^2\)/day on day 1. Basdra et al.\(^{17}\) studied the fluoride release of two kinds of orthodontic bonding agents: Fluorobond/Concise and Rely-a-Bond. The rate of day 1 was about 40.2 and 15.2 µg/cm\(^2\)/day. In the present study the fluoride-releasing ability of the experimental tooth separator that was greater than 1/2 covered by varnish was superior to some glass ionomers and sealants on the initial day. Although the long-term fluoride release ability of the total experiment elastomeric ring was not studied, the tooth separator showed a promise as tooth separator for about 7 days.

The different concentrations of fluoride released by the materials may be linked to the structure of the glass powder used in the construction of the glass ionomer\(^21\). A glass ionomer with a high concentration of fluoride would be expected to release more fluoride than one with a lower concentration\(^19\). This agrees with the result that the amount of fluoride released from group 2 was greater than that from group 1.

The pattern of fluoride release from the experimental tooth separator was characterized by an initial burst of fluoride release during the 1st day, which was greater than 20% of the total fluoride released during 10 days. This fluoride release pattern was similar to the findings of other studies. Wiltshire et al.\(^{12}\) studied the pattern of the fluoride releasing elastomeric ligature ties (Fluor-I-Ties) and found significant differences between the 1st day and the 2nd day. Its decrease rate was about 60%. The pattern of a sharp decline in fluoride release is a basic feature of the fluoride-releasing glass-ionomers developed recently\(^{18,19,21,22}\). Fluoride-releasing composite resin-based sealant (FluoroShield) and fluoride-releasing orthodontic bonding agents such as Rely-a-Bond, Fluorobond and FluoEver OBA also showed sharp declines\(^{20,24,25}\). However, there was no sharp decline in fluoride release in fluoride-containing orthodontic bonding composites\(^{34}\). This may have been because the
amount of released fluoride was small. The amount of fluoride needed to show a significant reduction in enamel demineralization and the condition of its optimal function are unclear. In one study fluoride release in the order of 200 to 300 mg/cm²/month was calculated to completely inhibit enamel demineralization in the presence of plaque. Some studies demonstrated that concentrations of fluoride of less than 0.05 ppm are beneficial for caries reduction. For the fluoride-releasing glass ionomers and the experimental elastomers the high initial bursts of fluoride release within the first few days showed some anti-cariogenicity, which may be attributed to calcium fluoride formation. The long-term low doses of fluoride that is released from glass ionomers may assist in the prevention of decalcification through the formation of fluorapatite. Therefore, provided the clinical use of the experimental elastomers as ligature ties and chains for orthodontic patients the cariostatic effect will be enhanced by prolonging the fluoride-releasing duration from 10 days to 3 or 4 weeks.

Demineralization of enamel was suggested to consist of two processes: (1) dissolution of tooth mineral at the advancing front of the lesion, and (2) diffusion processes in which acid ions are transported from the plaque into the lesion, and soluble tooth mineral ions are transported. Calcium fluoride that was formed on the enamel surface may act as a potential reservoir, slowly releasing fluoride ions acting as a diffusion barrier during acid attacks. During demineralization, dissociation of fluoride ions from calcium fluoride crystals, diffusion into the pores in the enamel and incorporation into fluorapatite (FAP) may have occurred, which enhanced the acid resistance of enamel. A clinical trial showed the stannous fluoride-releasing elastomeric modules (Fluo-I-Ties) and chain (Fluo-I-chain) reduced the enamel decalcification by 49%. The enamel surfaces affected by fluoride from Fluo-I-Ties and Fluo-I-chain were not in direct contact with the elastomers. In the present study, we investigated the acquired acid resistance rate (AR) of two kinds of enamel surface, a separator-contacting area and a separator-surrounding area. The inhibition rate in the separator-surrounding area was appropriately equal to the Banks clinical trial. In the separator-contacting area AR was as high as about 80%. Since fluoride was continuously leached out from the exposed part of the experimental separator like a fountain, and although the mean daily concentration of fluoride of 2 ml de-ionized water was 0.6-14.3 ppm, the fluoride concentration of the local solution between the enamel surface and the experimental separator may be high enough with respect to that of the separator (25,000-50,000 ppm). So, a step in the fluoride concentration may be formed from the contacting area to the surrounding area. It was confirmed by the phenomenon that the more distant it is from area B, the bigger the lesion depth and lesion body (Fig. 4). The fluoride concentration of the solution on the enamel surface surrounding the experimental separator should be lower than that in the solution between the local enamel surface and the experimental separator, and higher than that in the 2 ml de-ionized water. At higher fluoride concentrations, greater calcium fluoride-like depositions would occur. Therefore, the calcium fluoride-like depositions on the local enamel surface contacting the experi-
Fig. 4 Typical contact microradiographs of enamel lesion by decalcification.
At area A, the subsurface decalcification (the gray stripe) was produced apparently because enamel was not affected by fluoride. At area B, the decalcification was little because of the local high concentration of F. At area C, although the decalcification was scattered it became deeper than area B to the end of area C. At area D the sound enamel was not affected by F or by lactic acid.

mental separator (area B) would be higher than that on the enamel surface surrounding the experimental separator (area C). The present results also showed that AR was significantly different between area B and area C. However, in area B or C, AR was not significantly different between group 1 and group 2. This may be because the maximum efficacy of the fluoride concentration was 550-600 ppm. Although AR was not significantly different between group 1 and group 2 in area B or C, the relation between the fluoride concentration of the experimental separator and the acquired acid resistance rate was not clear.

Further research is needed to clarify the relation between the fluoride concentration of the experimental separator and the acquired acid resistance rate to determine the most suitable fluoride concentration in the elastomeric.

CONCLUSION

From the present findings it is suggested that:
1. The experimental elastic separator leached out adequate fluoride during the experimental period.
2. The acid resistances of enamel contacting and surrounding the separator can be respectively enhanced about 80% and 50% by the experimental elastic separator.

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