Abstract: Ammonium hexafluorosilicate (AHF) has been applied to arrest caries without discoloration. The purpose of this study was to observe structural and elemental changes of demineralized and AHF applied primary tooth enamel. Enamel from the labial surface of 20 primary canines was divided into an unground side and ground side at the center of the tooth, and demineralized with 35% phosphoric acid for 6 min. The teeth were divided into 4 groups according to a 3-min application of AHF and 1 week of soaking in artificial saliva, as follows: group A (neither AHF nor saliva), group B (only saliva), group C (only AHF), and group D (AHF and saliva), and then subdivided according to whether the enamel was ground or unground. Specimens were analyzed with scanning electron microscopy (SEM) and energy dispersive X-ray spectrometry (EDS). The data were statistically analyzed using ANOVA and Fisher’s PLSD test at α = 0.05. In groups A and B, prism structures were seen, however, in groups C and D, enamel surfaces were covered with spherical particles. Ca/P ratio was significantly higher in groups C and D than in groups A and B. There was no significant difference between ground and unground enamel in the content of any element. The values for F, Na, Mg and Si persents and Ca/P ratio were significantly higher for the enamel surface than for points 10-30 µm beneath the surface. Results of this study suggest the possibility that AHF treatment arrests caries, although further study will be required to confirm this result.

Keywords: ammonium hexafluorosilicate; primary tooth enamel; EDS analysis; SEM observation.

Introduction

Silver diamine fluoride (SDF; Ag(NH$_3$)$_2$F) has been used to arrest caries since 1969 (1). Arresting caries treatment (ACT) of primary teeth with SDF is useful for managing caries in uncooperative young children and disabled persons. Annual application of 38% SDF (44,800 ppm F) to carious primary anterior teeth of Chinese preschool children was significantly more effective in arresting caries and preventing new caries formation than 3 monthly applications of sodium fluoride varnish (22,600 ppm F) (2,3). Similarly, biannual application of 38% SDF was clinically effective in arresting caries and preventing new caries over a 3-year period in Cuban school children aged 6-15 years (4).

Although ACT is an acceptable alternative when
restorative treatment is not an option (5,6), adverse psychological issues associated with blackening of anterior teeth have been reported with SDF use (7). Treatment of carious dentin with SDF results in formation of sparingly water-soluble, yellowish-silver phosphate that turns black on exposure to sunlight or reducing agents (8).

In the prevention of dental caries and treatment of dentin hypersensitivity, ammonium hexafluorosilicate (AHF; (NH₄)₂SiF₆) has been used to overcome tooth discoloration caused by SDF treatment (9). AHF increases the acid resistance of enamel and dentin to almost the same extent as SDF (10). In addition, AHF contains Si, which increases apatite formation (11). Immediately after AHF treatment, patent dentin tubules within simulated hypersensitive teeth were completely occluded with a silica-calcium phosphate precipitate to a depth of 20 µm. The same treatment also induced apatite precipitation on the dentin surface after dentin disks were immersed in artificial saliva (10). Thus, AHF treatment might arrest active caries in primary teeth, without tooth discoloration.

In a preliminary study (12), AHF treatment did not cause visible discoloration on primary teeth. After AHF application, enamel prisms and dental tubules of partially demineralized primary teeth were covered with precipitates. Energy dispersive X-ray analysis (EDS) showed that F% and Ca/P ratio were significantly higher on dentin surfaces after AHF application, in the absence of artificial saliva immersion. However, AHF application had no effect on enamel. It was hypothesized that AHF was dissolved by artificial saliva. As compared with dentin, the demineralized enamel surface was smooth, and the capacity of enamel as a reservoir for calcium fluoride and calcium fluoride-like minerals after AHF treatment might be low (12). Therefore, to produce a rougher enamel surface, and thus an effective reservoir, a longer etching time was used in the present study for both unground and ground primary tooth enamel.

The objective of this study was to examine the effects of AHF on the structure and elemental contents of artificially demineralized primary tooth enamel.

**Materials and Methods**

**Experimental teeth**
Healthy maxillary primary canines that were extracted to expedite eruption of succedaneous teeth, or for orthodontic reasons, were used as substrates for the present study. Informed consent for tooth collection was obtained from parents and patients, according to the regulations of Nagasaki University Dental School (Permission No. 116). The teeth were fixed in physiologic saline within 10 min after extraction.

**AHF solution and artificial saliva**
AHF (Kanto Chemical Co. Inc., Tokyo, Japan) was used without further purification and dissolved in double-distilled water to produce a 0.476 mol/L solution (pH 3.4) (9). Artificial saliva, which has an electrolyte composition similar to that of human saliva, was prepared from 1.09 mmol/L CaCl₂, 0.68 mmol/L KH₂PO₄, 30 mmol/L KCl, and 2.6 mmol/L NaF. The artificial saliva was buffered to pH 7.0 with 50 mmol/L N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) (13).

**Scanning electron microscopy (SEM) and energy dispersive X-ray spectrometry (EDS) analysis**
Twenty primary canines were used. The labial surface of each tooth were divided into an unground side and a ground side at the center of the tooth. On the ground side, surface enamel was ground with a water-cooled air turbine, using a diamond point (ISO #016, Shofu Inc., Kyoto, Japan). The dental pulp was removed, and each tooth was ultrasonically cleaned in distilled water. The pulp chamber of each tooth was then filled with white-colored wax (Shofu). The unground and ground labial enamel surfaces were both acid-etched with 35% phosphoric acid gel (K-etchant; Kuraray Medical Inc., Tokyo, Japan) for 3 min, ultrasonically cleaned in distilled water, and dried with an air syringe. These procedures were repeated twice. Thus, the total etching time was 6 min.

The teeth were first divided into 4 groups, based on the method of AHF and/or artificial saliva treatment, as follows: group A (neither AHF nor artificial saliva application—control), group B (soaking in artificial saliva only), group C (AHF application only) and group D (AHF application and soaking in artificial saliva). Then, each group was divided into two subgroups: the ground enamel (a) and unground enamel (b) groups. Thus, eight subgroups of Aa, Ab, Ba, Bb, Ca,Cb, Da, and Db were prepared. In the AHF application groups (groups C and D), AHF solution was rubbed on the demineralized enamel surfaces with a cotton swab for 3 min. In the artificial saliva groups (groups B and D), each tooth was soaked for 7 days in a glass bottle filled with 10 mL of artificial saliva at 37°C. The artificial saliva was changed every 24 h. In group C, the AHF solution was applied, and the teeth were kept at 100% relative humidity for 7 days. In the artificial saliva groups, the teeth were cleaned in distilled water for 10 s and dried.

After AHF application and/or artificial saliva immersion, the teeth were fixed in 0.1 mol/L cacodylate...
The data were statistically analyzed using two-way analysis of variance (ANOVA) to examine the effects of treatment (ie, groups A-D and the eight subgroups), location, and the interaction of these two factors on enamel remineralization. In addition, three-way analysis of variance was used to examine effects on elemental distribution associated with 1) unground vs ground enamel, 2) treatment method, and 3) location. Post-hoc comparisons were performed using Fisher’s PLSD test. Statistical significance for all tests was pre-set at α = 0.05.

Results

The results of SEM/EDS analysis are shown in Tables 1, 2, and 3.

Table 1 shows the elemental content of enamel at different measuring points in all subgroups. In subgroups Ba and Da, F% at point 1 was significantly higher than at the other measuring points. In group D, F% in subgroup Db was significantly higher than that in subgroup Da at all measuring points except point 1, at which there was no significant difference in F% between subgroups. In
subgroup Da, Ca% at point 1 was significantly lower than at the other measuring points. In group D, the Ca% of subgroup Da was significantly higher than that of subgroup Db at all measuring points. In subgroups Cb and Db, P% at point 1 was significantly lower than at the other measuring points. In subgroups Ab and Cb, Na% at point 1 was significantly higher than at the other measuring points. In subgroups Ab, Ba, Bb, Cb, and Da, Mg% at point 1 was significantly higher than at the other measuring points. In subgroup Cb, Si% at point 1 was significantly higher than at the other measuring points. In group D, the Ca/P ratio of subgroup Da was significantly higher than that of subgroup Db at all measuring points except point 1. In group D, there was no significant difference in Ca/P.

Table 2  
$P$ values for elemental content of enamel: 3-way ANOVA

<table>
<thead>
<tr>
<th>$F$</th>
<th>$Ca$</th>
<th>$O$</th>
<th>$P$</th>
<th>$Na$</th>
<th>$Mg$</th>
<th>$Si$</th>
<th>$Ca/P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P$ value</td>
<td>0.2670</td>
<td>0.2296</td>
<td>0.6624</td>
<td>0.2108</td>
<td>0.1451</td>
<td>0.7152</td>
<td>0.2849</td>
</tr>
</tbody>
</table>

$P$ values for comparison of elemental content between unground and ground enamel

| Group A vs B | <0.0001 | 0.1451 | 0.7152 |
| Group A vs C | 0.0001 | 0.7152 | 0.2849 |
| Group A vs D | <0.0001 | 0.2849 | 0.0001 |

$P$ values for comparison of elemental content between treatment groups

| Group A vs B | 0.1451 | 0.7152 | 0.2849 |
| Group A vs C | 0.0001 | 0.7152 | 0.2849 |
| Group A vs D | <0.0001 | 0.2849 | 0.0001 |

$P$ values for comparison of elemental content between measuring points

| Point 1 vs 2 | <0.0001 | 0.0007 | <0.0001 |
| Point 1 vs 3 | <0.0001 | 0.0007 | <0.0001 |
| Point 1 vs 4 | <0.0001 | 0.0007 | <0.0001 |

Table 3  Mean (SD) values for elemental content of enamel in the four groups (mass %)*

<table>
<thead>
<tr>
<th>Measuring point</th>
<th>Distance from surface (μm)</th>
<th>Group AHF</th>
<th>Artificial saliva</th>
<th>F</th>
<th>Ca</th>
<th>O</th>
<th>P</th>
<th>Na</th>
<th>Mg</th>
<th>Si</th>
<th>Ca/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 0</td>
<td>A − −</td>
<td>0.04(0.04)a,1</td>
<td>30.7(3.3)a,1</td>
<td>46.4(2.9)a,1</td>
<td>14.9(3.8)a,1</td>
<td>0.46(0.28)a,b,1</td>
<td>0.39(0.19)a,b,1</td>
<td>2.14(0.31)a,b,1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 10</td>
<td>B − +</td>
<td>0.16(0.15)a,b,2</td>
<td>43.6(1.2)a,b,2</td>
<td>45.4(3.8)a,b,2</td>
<td>17.6(3.2)a,b,2</td>
<td>0.20(0.15)a,b,2</td>
<td>0.09(0.08)a,b,2</td>
<td>2.18(0.31)a,b,2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 20</td>
<td>C + −</td>
<td>0.49(0.45)a,b,2</td>
<td>44.4(1.6)a,b,2</td>
<td>38.8(1.6)a,b,2</td>
<td>15.3(3.1)a,b,2</td>
<td>0.49(0.23)a,b,2</td>
<td>0.37(0.29)a,b,2</td>
<td>3.07(0.93)b,2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 30</td>
<td>D + +</td>
<td>0.99(1.01)a,b,2</td>
<td>43.1(1.4)a,b,2</td>
<td>37.5(1.7)a,b,2</td>
<td>15.0(3.3)a,b,2</td>
<td>0.32(0.11)b,2</td>
<td>0.04(0.07)a,b,2</td>
<td>2.18(0.31)a,b,2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* data from ground and unground enamel combined

AHF: ammonium hexafluorosilicate

Presence of the same letter indicates that the values for same measuring point do not significantly differ ($P > 0.05$) between groups.

Presence of the same number indicates that the values for same group do not significantly differ ($P > 0.05$) between measuring points.
ratio at point 1 between subgroups.

Table 2 shows the \( P \) values for comparisons of elemental content of enamel (three-way ANOVA). There was no significant difference between ground and unground enamel for any measure of elemental content (mass%). Regarding elemental content among the four treatment groups, F% in group D was significantly higher than in the other groups. Ca% was significantly higher in groups C and D than in groups A and B, but there was no significant difference between groups C and D. There was no significant difference in P% between groups A and B or between groups B and D; however, significant differences were observed between other groups. Si% was significantly higher in group C than in the other groups. Significant differences in Ca/P ratio were found among the four groups, except for the comparison between groups A and B. Regarding elemental content at the four measuring points, the element content of F, O, Na, Mg, and Si percents, and Ca/P ratio at point 1 was significantly higher than at the other measuring points. By contrast, Ca and P percents at point 1 were significantly lower than at the other measuring points.

After finding with no significant difference between ground and unground enamel for any element, we analyzed combined data (i.e., both ground and unground enamel) from the four groups. Table 3 shows the elemental content of enamel (including data from both subgroups) at the different measuring points in each group and among different groups at the different measuring points. In group D, F% at point 1 was significantly higher than at the other groups. In groups C and D, F% at point 1 was significantly higher than at the other measuring points. The P% of all groups at point 1 was significantly lower than at the other measuring points, except for point 3 in group B. In groups A, B, and C, Mg% at point 1 was significantly higher than at the other measuring points. In group D, Mg% did not significantly differ by measuring point. In group C, Si% at point 1 was significantly higher than at the other measuring points. In groups C and D, Ca/P ratio at point 1 was significantly higher than in groups A and B. In groups B and C, Ca/P ratio at point 1 was significantly higher than at the other measuring points, except for Ca/P ratio at point 2 in group C.

Representative SEM images of unground and ground enamel from each group are shown in Fig. 1. In group A, prismatic structures were clearly visible on both unground (Fig. 1-1) and ground enamel (Fig. 1-5). In group B, prismatic structures were visible on unground enamel (Fig. 1-2) but were only barely visible on ground enamel (Fig. 1-6). Regardless of exposure to artificial saliva, AHF-treated enamel surfaces (groups C and D) were covered with spherical particles, and enamel prismatic structures were not visible (Figs. 1-3, 1-4, 1-7, and 1-8). In group C, enamel surfaces were covered with spherical particles and amorphous materials (Figs. 1-3 and 1-7).

Discussion

A previous study showed that human dentin treated with silver fluoride for 3 min became black, due to silver precipitation. In contrast, a 3-min AHF application did not change the color of dentin (14). Discoloration after SDF treatment resulted from silver phosphate precip-
tate that turned black after exposure to sunlight (5). Conversely, AHF-treated enamel and dentin of primary teeth did not show visible discoloration (12). Although long-term color change due to AHF application should be investigated in future studies, evidence indicates that AHF treatment alleviates behavioral problems related to SDF-induced tooth discoloration in children.

An in vivo study reported that SDF is cytotoxic against human gingival fibroblasts (15). For clinical use of SDF, the manufacturer recommends that the patient rinse his/her mouth with water or saline after topical application of SDF, so as to remove excess solution. Additionally, the use of a rubber dam is recommended to avoid accidental contact with gingival and mucous tissue. However, it may be impractical to use rubber dams for SDF treatment in uncooperative (unmanageable) young children.

In this study, remineralization was not seen in demineralized enamel, and evidence of remineralization after prolonged acid etching of demineralized ground enamel was ambiguous. Lesions in demineralized ground and unground enamel could be arrested. However, it is not certain that a 3-min AHF application and/or 7-day immersion in artificial saliva is adequate to arrest demineralized enamel lesions caused by excessive acid-etching. Data on the elemental content of enamel in subgroups show that tooth-to-tooth differences were considerable (Table 1).

In a previous study (12), AHF application on enamel did not significantly affect the elemental content of enamel. However, the present results show that Ca/P ratio in groups C and D (i.e., those with AHF application) was significantly higher than in groups A and B (those without AHF application) (Tables 2 and 3). Both unground and ground enamel were more heavily demineralized by a 6-min period of phosphoric acid etching as compared with a 3-min period of phosphoric acid etching, as reported in a previous study (12). The 6-min exposure resulted in clearer and deeper enamel etching patterns (Figs. 1-1 and 1-5), which might explain the absence on three-way ANOVA of any significant difference in elemental content between unground and ground enamel (Table 2). The prismatic structures on highly etched rough enamel surfaces could have more efficiently preserved AHF, thereby yielding a higher Ca/P ratio in the two groups with AHF application. The Ca/P ratio in groups C and D was significantly higher than in groups A and B (Tables 2 and 3). However, the results of three-way ANOVA (Table 2) indicated that the Ca/P ratio of group D (AHF application plus soaking in artificial saliva) was significantly lower than that of group C (AHF application only). One reason for this result is the higher Ca/P ratio in subgroup Cb, which was due to the lower P% in subgroup Cb (Table 1). Some of the mineral components produced by the reaction of AHF with enamel may have been partially dissolved by artificial saliva in group D. The same results were obtained for Si included in AHF; Si% in group C was significantly higher than in the other groups (Tables 2 and 3).

The F content of Group D (AHF application plus soaking in artificial saliva) was significantly higher than Group A (neither AHF nor artificial saliva treatment), Group B (soaking in artificial saliva only) and Group C (AHF application only) (Table 2). Especially in measuring point 1, the F% of Group D was significantly higher than that of the other groups (Table 3). The significantly higher F% in Group D may be attributed to the inclusion of NaF in the artificial saliva, and the presence of F in AHF.

In this study, the amorphous materials deposited over the enamel surface in group C (Figs. 1-3 and 1-7) were not analyzed. However, these materials were not seen in group D (Figs. 1-4 and 1-8). Presumably, the materials were dissolved during the 7-day immersion in artificial saliva. It is possible that AHF was partially or fully dissolved by the artificial saliva, thereby nullifying its anti-caries effect. Because the AHF solution used in the present study was acidic (pH 3.4), repeated AHF application during a short period of time is not recommended. If this in vitro inference is correct, the efficacy of AHF treatment may be diminished in the oral environment. Future studies should evaluate the efficacy of repeated AHF application and longer exposure to artificial saliva.

As shown in Tables 1 and 3, Ca/P ratio was high in all groups, especially at points 1 and 2. This may be attributed to silica-calcium phosphate precipitation by AHF (14), regardless of reaction with artificial saliva (Figs. 1-3, 1-4, 1-7, and 1-8). However, in group A (neither AHF nor artificial saliva treatment) the Ca/P ratios at points 1 and 2 were also high (Tables 1 and 3). As shown in Table 2, the P values for the comparisons of elemental content among all measuring points show that Ca% and P% at measuring point 1 were significantly lower than values at the other measuring points. However, the P value for P% is lower than that for Ca% at point 1, which explains the high Ca/P ratio at point 1. Similar findings with regard to the relationship of Ca% and P% are shown in Tables 1 and 3.

Previous studies (14,16) of permanent tooth dentin reported that precipitates deposited in the dentinal tubules after AHF treatment contained F, Ca, and P, and that the precipitates appeared to be composed of a mixture of fluoridatedapatite and calcium fluoride-like minerals.
Calcium fluoride and calcium fluoride-like minerals act as fluoride reservoirs in the oral environment. As compared with fluorapatite, calcium fluoride deposits are more susceptible to washout and dissolution (17,18). Because it is supersaturated with respect to saliva, fluorapatite is stable and does not dissolve in saliva. Thus, continuous dentin tubule occlusion can be achieved with fluorapatite deposition (18). In addition, silica-containing compounds induce apatite formation from simulated body fluids and artificial saliva (11). If the silica content of dentinal tubular precipitates is increased, further mineralization of the dentin surface may be expected, resulting in a longer duration of tubule occlusion (18). As compared with dentin structure, demineralized rough enamel surfaces may not act efficiently as fluoride reservoirs in the oral environment. Additional components may be required to efficiently retain AHF reaction products in demineralized enamel.

Within the limits of the present study, the null hypothesis that AHF application does not affect the structure and elemental content of artificially demineralized primary tooth enamel is rejected, and AHF application on demineralized primary tooth enamel is partially validated. To investigate the efficacy of AHF in arresting caries for primary enamel, future studies should evaluate the stability of precipitates formed by AHF treatment in the oral environment and the efficacy of AHF in arresting caries treatment in vivo.

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References