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Ammonium hexafluorosilicate increased acid resistance of bovine enamel and dentine

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Although diamine silver fluoride (AgF: $(NH_3)_2AgF$) stains teeth black, it is known as a very effective agent to prevent the dental caries progress. In order to find another fluoride that has a similar anticariogenic effect without changing tooth color, we prepared ammonium hexafluorosilicate (SiF: $(NH_4)_2SiF_6$), in which the silver of AgF is replaced with silicon. In this study, the anticariogenic effect of SiF was evaluated using bovine teeth. Fluoride solutions, SiF, AgF, acidulated phosphate fluoride (APF), and sodium fluoride (NaF), were applied to bovine enamel and dentine blocks, and the depth of demineralization was measured after exposure to a demineralizing solution for 24 h. Also, fluoride was applied to a simulated dentine caries specimen to evaluate the caries progress-preventing ability. For the dentine specimens, mineral loss (ΔZ) was also measured with microradiography. We found that SiF treated enamel showed better acid resistance than specimens treated with NaF or APF. AgF treated enamel also showed similar acid resistance, but was stained black. SiF and AgF treated caries-affected dentine showed reduced demineralization when exposed to a demineralization solution for 24 h. Mineral loss (ΔZ) was reduced to 85% and 75%, respectively. Although the acid resistance of the SiF treated teeth was inferior to that of the AgF treated teeth, we consider that SiF has good potential as anticariogenic agent, since it increased acid resistance without changing tooth color.

1. Introduction

Fluoride has been widely used in professional treatment at dental clinics and in home care in forms such as fluoride solution, gel, dentifrice, etc. It has been noted that preventing the progress of caries is important in dental clinics in addition to preventing caries occurrence, since dentine caries proceeds rapidly. Among the fluoride solutions, diamine silver fluoride [AgF: $(NH_3)_2AgF$] (Saforide®, Beebland Medico Dental Inc., Osaka, Japan) is widely used for preventing the progress of dental caries in Japan [1–3]. Recently, it has also been used in China, and it was reported that AgF treatment was more effective in hardening or arresting dentine caries in primary teeth than 5% NaF varnish [4]. However, AgF cannot be applied to permanent teeth since teeth treated with AgF are stained black due to silver sulfide deposition. To solve this drawback, we prepared ammonium hexafluorosilicate [SiF: $(NH_4)_2SiF_6$] [5]. We employed silicon instead of silver, since silicon does not change tooth color and is known to induce apatite formation, which may be useful in a remineralization process. When synthetic hydroxyapatite powder was immersed in SiF solution, we found that the crystallinity of hydroxyapatite powder significantly increased. Also, we found that a larger amount of fluorapatite was formed after SiF treatment, when compared with sodium fluoride (NaF), acidulated phosphate fluoride (APF) or AgF treatment. These findings indicate that SiF treatment has a potential ability for the prevention of dental caries.

However, no study has been performed for the evaluation of SiF as an anticariogenic agent using teeth. The aim of this study was, therefore, to evaluate the anticariogenic ability of SiF using bovine enamel and dentine.

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TABLE I Fluoride solutions used in this study

Solution	Code	Formula	Concentration (mol/L)	pH
Diamine silver fluoride	AgF	$(NH_3)_2AgF$	2.36	10.1
Ammonium hexafluoro-silicate	SiF	$(NH_4)_2SiF_6$	0.476	3.4
Acidulated phosphate fluoride	APF	$NaF + H_3PO_4$	0.476	5.0
Neutral sodium fluoride	NaF	NaF	0.476	7.2

2. Materials and methods

2.1. Preparation of fluoride solution

SiF was prepared as described previously [5]. In brief, SiF (Kanto Chemical Co., Inc., Tokyo, Japan) was used without further purification and dissolved using double-distilled water to 0.476 mol/L, so that the concentration was consistent with those of NaF and APF. AgF (Saforide®, Beebland Medico Dental Inc., Osaka, Japan) was obtained from the commercial sources shown, and used without further purification. Even though the concentration of AgF (2.36 mol/L) was higher than that of the other fluorides, no dilution was attempted in the present study, to maintain the concentration recommended by the manufacturer. NaF solution was prepared from reagent grade chemical (Kanto Chemical Co, Inc., Tokyo, Japan), and the concentration was 0.476 mol/L. APF was obtained from commercial sources (Floden A, Sunstar Inc., Osaka, Japan). The fluoride solutions used in this study are shown in Table I, with their concentrations and pH.

2.2. Evaluation of fluoride solutions in prevention of enamel and dentine caries

Freshly extracted bovine mandibular incisors were used immediately. The tooth was sectioned horizontally below the cementoenamel junction using a low-speed water-cooled diamond saw (Buehler Ltd., Evanston, IL, USA). The roots were sectioned perpendicularly into two almost equal parts (buccal and lingual parts), and then each root surface (buccal and lingual surfaces) was polished to remove cementum. The crown was embedded into a self-curing acrylic resin (Tray resin®, Shofu Inc., Kyoto, Japan), leaving only the labial site exposed. Also, the root was embedded into resin, leaving the buccal or lingual site exposed. The exposed site of the enamel or dentine was polished to a #1500 SiC grade using an automatic polisher (ECOMET3, Buehler, IL, USA), to obtain a flat mirror surface. The polished enamel and dentine surfaces were divided into several small portions using paraffin wax, as shown in the flow chart (Fig. 1). Fluoride solution (AgF, SiF, APF or NaF) was applied to a section enclosed by wax with a cotton swab for 3 min. After the fluoride solution was wiped away with a cotton swab, the specimens were washed gently with distilled water for 1 min. A section that received no fluoride treatment was used as a control.

2.3. Evaluation of anti-caries ability

Enamel blocks and dentine blocks prepared in the above procedure were immersed in 100 mL demineralization solution. The demineralization solution was 0.1 mol/L lactic acid solution containing 6 wt% carboxymethylcellulose (CMC), and the pH of the solution was adjusted to 5.0 with KOH [6]. After the specimen was exposed to demineralization solution for 24 h, the specimens were rinsed with distilled water for 1 min, and the paraffin waxes on the specimens were removed using steam. The specimens were washed with distilled water, and then the depths of demineralized enamel and dentine surface were measured by a surface texture measuring instrument (Surfcom 300A, Tokyoseimitsu, Tokyo, Japan). The demineralized depth is the average value of 60 measurements of at least 20 independently treated enamel or dentine blocks.

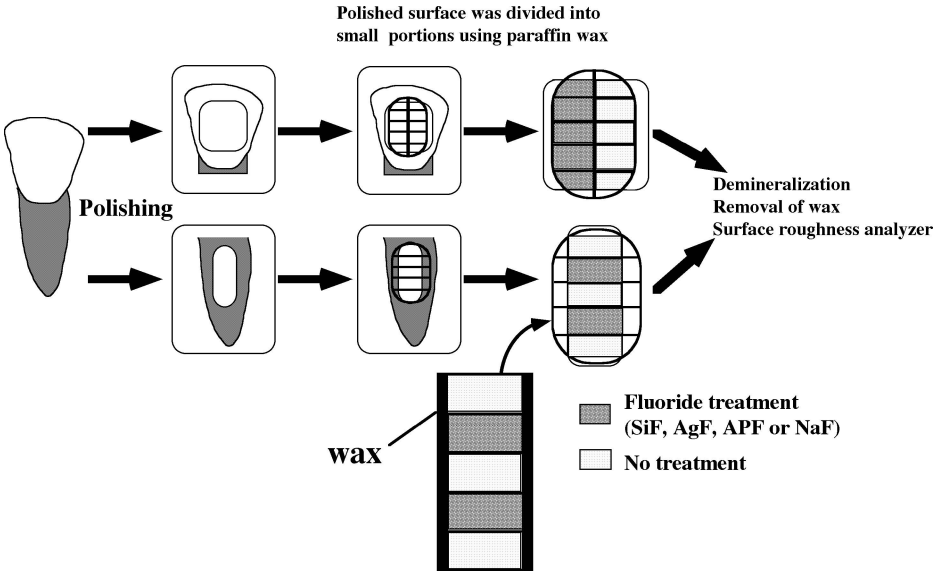

Figure 1 Experimental procedure of preparation of bovine enamel and dentine specimens. Polished bovine enamel and dentine surfaces were divided into several portions using paraffin wax. Each portion received fluoride solution treatment (SiF, AgF, APF or NaF) or no treatment (control).

2.4. Evaluation of caries progress-preventing ability

In addition to the anti-caries ability, it was also desirable that the fluoride could prevent the progress of caries. To evaluate the caries progress-preventing ability, simulated caries-affected teeth were prepared by demineralization. In other words, the enamel or dentine was exposed to demineralization solution for 24 h. Then, fluoride solution (SiF or AgF) was applied for 3 min with a cotton swab. After the remaining fluoride solution was wiped away with a cotton swab, the specimens were washed gently with distilled water for 1 min. Again, the specimens were immersed demineralization solution for 24 h. The depths of the demineralized enamel and dentine surfaces were measured by the surface texture measuring instrument.

2.5. Mineral loss volume (ΔZ) evaluation

Measurement of the depth of demineralization is effective for the evaluation of the anti-caries ability or caries-preventing ability in enamel. However, demineralization depth does not correlate well with the demineralized amount when fluoride is used against dentine, since collagen remains even after demineralization process. Therefore, microradiography was used for the evaluation of demineralization.

After the demineralization, dentine specimens were embedded into Technovit 7200 VLC (Kulzer, Wehrheim, Germany). Then, planoparallel sections of approximately 400 μm thickness were cut from the dentine specimens using a water-cooled diamond coated saw (Buehler Ltd., Evanston, IL). These sections were ground planoparallelwise on a wet 800-grit abrasive paper to a thickness of about 100 μm. They were microradiographed together with a reference aluminum step wedge using Cu-K_α radiation (Softex CMR-2, Softex, Osaka, Japan) generated at 7 kV and 3 mA for 20 min. The films were developed, fixed, and rinsed under standardized conditions. The microradiograms were then evaluated using a light microscope (AX80, Olympus, Tokyo, Japan). The images (100 magnifications) were transferred to a personal computer and analyzed with NIH image (*ver* 1.62), and mineral loss volume (ΔZ) per unit area was calculated as an index of demineralization.

2.6. Energy dispersive X-ray microanalysis

Dentine specimens were also analyzed with energy dispersive X-ray microanalysis (EDXA). The specimens were mounted on carbon holders and carbon-coated. An EDXA apparatus attached to a transmission electron microscope (H-500; Hitachi Co., Tokyo, Japan) was used to line scan from the surface of the specimen to a depth of approximately 100 μm. The carbon-coated specimens were analyzed with an accelerating voltage of 10 kV, a spot size of 100 nm, and a counting time of 100 s. The line scanning was performed at least five times per specimen to avoid clacks and artifacts.

2.7. Statistical analysis

For the statistical analysis, one-way factorial ANOVA and Fisher's PLSD method, used as a post-hoc test, were performed using the program "Stat View 4.02" (Abacus Concepts Inc., Berkeley, CA). p values <0.05 were considered to indicate significant differences.

3. Results

Table II summarizes the demineralized depth of the bovine enamel that was treated with fluoride solution and exposed to demineralization solution for 24 h. The depth of demineralization for enamel and dentine that received no fluoride treatment was 98.6 ± 40.0 μm and 35.1 ± 5.0 μm, respectively. The depth of demineralization after fluoride treatment is shown as a percentage ratio against the adjacent non-treated tooth to minimize the difference based on the individual tooth. Therefore, a smaller value indicates better acid resistance. In enamel, significantly ($p < 0.05$) smaller values were obtained when the enamel surface was treated with SiF ($75.9 \pm 15.8\%$) (Mean $\pm$ SD) or AgF ($76.1 \pm 14.8\%$). On the other hand, no significant difference was observed when the enamel surface was treated with NaF ($98.4 \pm 49.3\%$). The value was significantly ($p < 0.05$) larger when the enamel was treated with APF ($128.9 \pm 23.2\%$). Demineralization results for dentine showed similar results, except with APF. In other words, dentine treated with SiF ($85.2 \pm 3.9\%$) and AgF ($75.5 \pm 4.6\%$) showed a significantly ($p < 0.05$) smaller value than non-treated dentine. In dentine, AgF treatment produced a significantly ($p < 0.05$) smaller value than the SiF treatment. APF treatment and NaF treatment on dentine showed no remarkable difference against a non-treated specimen.

The following studies were done only for SiF and AgF treatments, since both SiF and AgF showed much better results than NaF and APF. Table III summarizes the demineralized depth of simulated caries-affected enamel and dentine specimens. Although the demineralized depth of the simulated caries-affected enamel treated with AgF and SiF showed a smaller value than the control specimen, no significant difference was observed due to large standard deviation. Interestingly, SiF and AgF treated dentine showed a

TABLE II Effects of fluoride treatment on demineralized depth when the teeth were exposed to demineralization solution for 24 h after fluoride treatment. Depth was presented as a percentage against a control specimen that received no fluoride treatment

Solution	Enamel	Dentine
AgF	76.1 ± 14.8*	75.5 ± 4.6*
SiF	75.9 ± 15.8*	85.2 ± 3.9*
APF	128.9 ± 23.2*	93.6 ± 7.0
NaF	98.4 ± 49.3	95.5 ± 5.1
Control	100	100

$N = 20$, Mean $\pm$ SD.
The demineralization depth of enamel and dentine that received no fluoride treatment was 98.6 ± 40.0 μm and 35.1 ± 5.0 μm, respectively. This depth was standardized as 100% in this table.
*Significant ($p < 0.05$) difference was observed against the control value.

TABLE III Effects of fluoride treatment on demineralized depth when the simulated caries-affected teeth were exposed to demineralization solution for 24 h after fluoride treatment. Depth was presented as a percentage against a control specimen that received no fluoride treatment

Solution	Enamel	Dentine
AgF	80.2 ± 29.1	78.4 ± 21.1[a]
SiF	91.8 ± 29.4	87.4 ± 20.2[a]
Control	100	100

$N = 20$, Mean ± SD.
The demineralization depth of enamel and dentine that received no fluoride treatment was 200.5 ± 64.8 and 57.8 ± 8.1 μm, respectively. This depth was standardized as 100% in this table.
[a]Significant ($p < 0.05$) difference was observed against the control value.

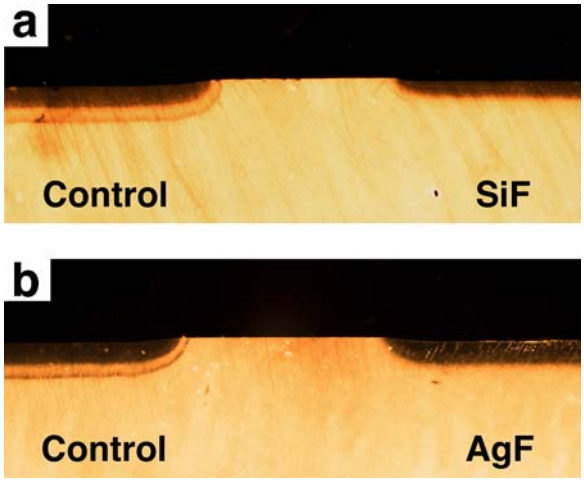

Figure 2 Typical microradiographs of simulated caries-affected dentine that received fluoride treatment followed by exposure to demineralization for 24 h. (a) SiF (b) AgF.

significantly ($p < 0.05$) smaller value than the control specimen.

Fig. 2 may be useful for determining the effect of SiF and AgF treatment on preventing dentine caries progress. As shown, demineralization occurred not only on the surface but also inside the dentine. A clear difference was observed between the SiF or AgF treated areas and the non-treated areas. In other words, a half-demineralized area could be observed between the demineralized area and the sound dentine, in the non-treated areas. In contrast, such area was not observed in the SiF or AgF treated areas. It should be noted that SiF or AgF was applied to the surface of demineralized dentine. Therefore, some of the demineralization occurred during the preparation of simulated caries-affected dentine.

Fig. 3 shows mineral density as a function of depth from the surface. The line located at the lower area indicates the small mineral density at that depth, whereas the line located at the upper area indicates the high mineral density at that depth. Figs. 3 (a) and (b) correspond to Fig. 2(a), which shows the SiF treated area and its control. Figs. 3 (c) and (d) correspond to Fig. 2(b), which shows the AgF treated area and its control. Mineral density measurement confirmed that SiF and AgF treatment reduced mineral loss. Mineral loss (ΔZ) is summarized in Table IV. Again, this ΔZ value includes

TABLE IV The mean mineral loss values, ΔZ, after SiF or AgF treatment

Solution	ΔZ
AgF	7238.2 ± 192.4*
SiF	8097.9 ± 189.1*
Control	9537.3 ± 216.7

$N = 10$. Mean ± SD.
*Significant ($p < 0.05$) difference was observed against the control value.

initial mineral loss from the preparation of simulated caries-affected dentine. Therefore, it is needed to subtract the value that corresponds to the ΔZ value of simulated caries-affected dentine from the total ΔZ to discuss quantitatively. Although quantitative comparison is difficult, it is clear that AgF and SiF have a good ability to prevent caries progress in dentine. The ΔZ value of the AgF treated lesions was less than those treated with SiF.

Fig. 4 shows the results of the EDXA analysis of simulated caries-affected dentine (a) that received no fluoride treatment, (b) was treated with SiF for 3 min, or (c) was treated with AgF for 3 min, and was then exposed to demineralization solution for 24 h. As shown, Si was detected with calcium phosphate. In contrast, Ag was detected on the surface of calcium phosphate.

4. Discussion

The results obtained in the present study clearly demonstrate that SiF is effective for preventing both dental caries and dental caries progress. Although the anti-caries abilities of NaF and APF has been proven and they are widely used in dental clinics, we found no significant caries-preventing effect by APF or NaF in the present study, as shown in Table II. It is obvious that our results do not indicate that APF and NaF have no anti-caries ability, but our results indicated that APF and NaF are not effective for preventing the dissolution of enamel in an acidic environment at an early stage. It is known that loosely bound fluoride or CaF_2-like fluoride is formed on the surface of enamel when enamel is exposed to concentrated fluoride solution. Unfortunately, loosely bound fluoride works more on the fluoride reservoir, rather than having an anti-caries effect, and it washes away easily in the oral environment [7–9]. Low-concentration fluoride released from loosely bound fluoride forms firmly bound fluoride or fluoridated apatite, and fluoridated apatite contributes to the increase in acid resistance of teeth [10]. Since we exposed NaF treated enamel to demineralizing solution immediately after treatment, the amount of firmly bound fluoride may have been negligible. The situation may be more complex in the case of APF. APF dissolves enamel since it is an acidic solution (Table I). An acidic solution leads to the formation of firmly bound fluoride. However, the initial demineralization due to its acidity may be greater than the reduction of the demineralization in the demineralization solution, under the conditions employed in the present study. It has been reported that SiF treatment forms a larger amount of

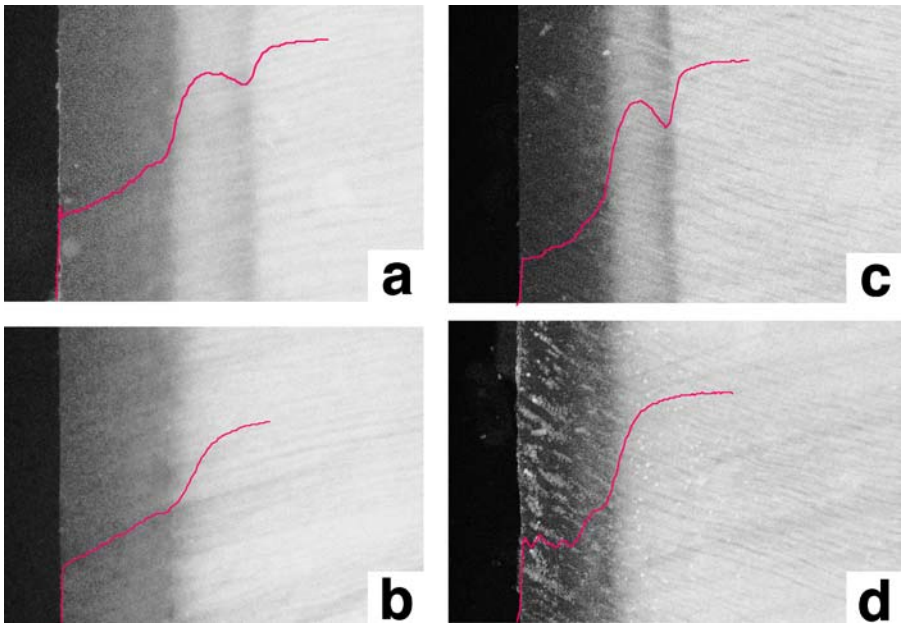

Figure 3 Typical microradiographs of simulated caries-affected dentine that received no treatment or fluoride treatment followed by exposure to demineralization for 24 h. Mineral density was measured using NIH image, and is shown in the image. (a) control image of SiF treated specimen (b) SiF treated specimen (c) control image of AgF treated specimen (d) AgF treated specimen.

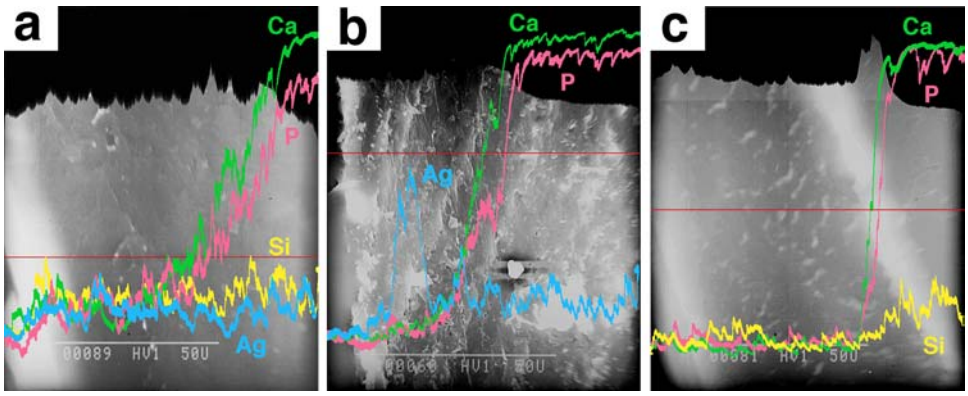

Figure 4 Typical EDXA line scan of simulated caries-affected dentine that received no treatment or fluoride treatment followed by exposure to demineralization for 24 h. (a) no fluoride treatment (b) AgF treatment, (c) SiF treatment. Bar represents 50 μm.

firmly bound fluoride than NaF or APF treatment [5]. This may be the reason why SiF showed a better anti-caries effect than APF and NaF. AgF treatment also reduced the demineralization of enamel. However, the use of AgF to prevent caries is not realistic, since AgF stains teeth black due to sulfonization.

With respect to caries progress prevention, AgF showed a better ability than SiF. Although we found no significant ($p < 0.05$) difference between SiF and AgF treatments due to large standard deviation, AgF produced a shallower demineralized depth, as shown in Table III. The difference was clear when we measured mineral loss in the simulated dental caries region (Figs. 2 and 3, Table IV). AgF showed significantly ($p < 0.05$) lower mineral loss than SiF. In the present study, we focused on mineral loss only when caries-affected dentine was exposed to demineralizing solution. It is known that collagen fixation also plays an important role in preventing caries progress. SiF is expected to show good collagen fixation since silica compounds are used in tanning. AgF also fixes collagen. Although the evaluation of the ability to fix collagen was not the aim of this study, such evaluation should be done based on these results.

Fig. 4 may be interesting for the elucidation of the mechanism of the anti-caries effect of SiF and AgF. In AgF treated dentine, Ag was located on the surface mineral. In other words, Ag covered the surface of the mineral. In contrast, Si was located in the mineral lesion. It was not clarified why Si was located in the mineral lesion. Silica is known to induce apatite precipitation [11–14]. Therefore, SiF may be incorporated into the mineral during the re-precipitation of apatitic mineral. Alternatively, SiF may just penetrate the dentine tubules. Still, this is preferable for the prevention of the progress of dental caries, since the dentine tubules are one of the routes for the rapid progress of dental caries.

5. Conclusion

We found that SiF is effective in preventing both dental caries and dentine caries progress. Unfortunately, the ability of SiF was inferior to that of AgF. However, SiF

does not stain teeth and thus we consider that SiF is a promising agent for preventing dental caries and dental caries progress.

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