

## Original Article

# The influence of ammonium fluoride solutions with different pH conditions on human intact enamel —morphological study and energy dispersive X-ray microanalysis—

OHASHI TAMIE, HIROSE AKIKO, ISHIZU ETSUKO,  
IWATA SACHIKO, KAWANO SATOSHI<sup>1)</sup> and ISOZAKI ATSUNORI

*The purpose of this study was to investigate influence of  $\text{NH}_4\text{F}$  solutions with different pH levels on human intact enamel. Some kinds of  $\text{NH}_4\text{F}$  solutions (900 ppm  $\text{F}^-$ , pH 2 - pH 7) and freshly extracted human premolars were prepared to examine. We treated enamel blocks in the  $\text{NH}_4\text{F}$  solutions at 37°C for 5 minutes, and washed half of the enamel blocks with artificial saliva for 24 hours. Using scanning electron microscopy (SEM) and an energy dispersive X-ray microanalyzer (EDX), we studied morphological changes and Ca, P, and F concentrations on the enamel surfaces after  $\text{NH}_4\text{F}$  treatment with or without washing in the artificial saliva.*

*The enamel reacted differently depending on the pH of each solution. In scanning electron microscopic observation, some spherical crystals, they were considered  $\text{CaF}_2$ , were seen on the enamel surface of the pH 2 - pH 6 groups after treatment with the fluoride solutions. Demineralization of the enamel surface was observed in the pH 2 and pH 3 groups after washing with artificial saliva. The spherical crystals remained, and confirmed higher F concentrations by the EDX in the pH 2 and pH 4 groups after washing with artificial saliva. It was difficult to detect the spherical crystals after washing with artificial saliva in the pH 5 - pH 7 groups treated with at slightly acidic and neutral solutions. The EDX analysis confirmed higher fluoride concentrations in the pH 3, pH 5 and pH 6 groups than in the control group, suggesting that  $\text{NH}_4\text{F}$  solutions at pH 5 - pH 6 are effective in preventing caries.*

*When the  $\text{NH}_4\text{F}$  solution at pH 4 was used, there were large amounts of  $\text{CaF}_2$  deposition and remaining  $\text{CaF}_2$  even after washing with artificial saliva. This solution enabled the highest fluoride uptake onto the enamel surface.*

Key words: Ammonium fluoride solution, pH, Artificial saliva, Morphological study,  
Energy dispersive X-ray (EDX) microanalysis

## Introduction

Acidulated phosphate fluoride (APF), sodium fluoride (NaF), sodium monofluorophosphate (MFP), and stannous fluoride ( $\text{SnF}_2$ ) solutions are used in the form of topical fluoride applications, fluoride mouth rinses, or fluoride toothpastes for prevention of dental caries. A cariostatic effect of phosphoric acid-acidified ammonium fluoride solution on artificial caries lesion formation was reported previously. We investigated the effect of ammonium fluoride ( $\text{NH}_4\text{F}$ ), which has been suggested as a new preventive medicine for caries, and compared its effect with that of APF and  $\text{NaF}^{1-5)}$ . The use of  $\text{NH}_4\text{F}$  results in a more significant uptake of fluoride, higher acid resistance, and higher amounts of  $\text{CaF}_2$  formation on the enamel surfaces compared with the use of APF in a topical application solution at the same

pH and the same fluoride concentration<sup>2)</sup>. We previously reported that a  $\text{NH}_4\text{F}$  solution of pH 5.0 with 100ppm  $\text{F}^-$  or 900ppm  $\text{F}^-$  which approximated the intraoral condition after using a mouth rinsing solution. We showed that the use of  $\text{NH}_4\text{F}$  resulted in a greater uptake of fluoride onto the enamel surfaces than the use of NaF under the same conditions, and that the use of  $\text{NH}_4\text{F}$  resulted in enamel with the same degree of acid resistance as the use of NaF. We suggest that an  $\text{NH}_4\text{F}$  solution can prevent caries to the same or greater degree than fluoride solutions that are currently in clinical use<sup>3)</sup>.

It is well known that fluoride uptake and acid resistance of the enamel are influenced by pH conditions. Therefore, the aforementioned research studies examined the effect of  $\text{NH}_4\text{F}$  solutions of different pH levels on intact enamel. We subsequently reported on the influence of  $\text{NH}_4\text{F}$  solu-

Department of Community Oral Health, Division of Oral Infections and Health Sciences

<sup>1)</sup>Department of Endodontics, Oral Functional Science and Rehabilitation

Asahi University School of Dentistry  
Hozumi 1851, Mizuho, Gifu 501-0926, Japan  
(Accepted September 8, 2014)

tions with different pH levels on human intact enamel. We used  $\text{NH}_4\text{F}$  solutions (900ppm  $\text{F}^-$ , pH 2 – pH 7) in the chemical analysis. These results suggested that were taken up fluoride into the enamel layers and acquired acid resistance<sup>6)</sup> in the all pH groups.

In our present study, *in vitro* experiments were performed on intact human enamel surfaces to examine the effect of  $\text{NH}_4\text{F}$  solutions. We studied morphological changes on the enamel surfaces after  $\text{NH}_4\text{F}$  treatment with or without washing in the artificial saliva. The changes were examined using scanning electron microscopy (SEM), elemental analysis (Ca, P, F mapping and point analysis) and quantitative analysis by point analysis using an energy dispersive X-ray microanalyzer (EDX).

## Materials and Methods

Twenty one freshly extracted human permanent teeth (lower premolars) were used. Each tooth was carefully examined to confirm that it did not have caries or a crack. Subsequently, the enamel surface was lightly polished with a sandpaper cone and a Robinson brush with pumice. Then it was divided longitudinally into two so that the buccolingual surfaces could be used as the experimental surfaces, and two enamel blocks were prepared. The blocks were ultrasonically cleaned and dentin was covered with paraffin wax.

The enamel blocks were randomly divided into 7 groups. One of the seven groups was established as the control group and treated with purified water for 5 minutes at 37°C. In the remaining six groups, the enamel blocks were treated with an  $\text{NH}_4\text{F}$  solution (calculated concentration, 900ppm  $\text{F}^-$ ), ranging from pH 2 to pH 7 (pH 2, pH 3, pH 4, pH 5, pH 6 and pH 7 groups), for 5 minutes at 37°C. The pH of each fluoride solution was adjusted using  $\text{H}_3\text{PO}_4$  or  $(\text{NH}_4)_2\text{HPO}_4$ . The pH was determined by pH test paper, and the fluoride concentration was measured using the fluoride ion electrode method.

Enamel blocks of all groups were randomly subdivided into 2 groups ( $n = 3$ ). One subgroup of the enamel blocks was washed with 100ml of artificial saliva at pH 7.3 for 24 hours at 37°C. Artificial saliva was prepared in accordance with Birkeland's prescription<sup>7)</sup>. The other subgroups were washed with purified water after  $\text{NH}_4\text{F}$  treatment.

After the blocks were treated with a  $\text{NH}_4\text{F}$  solution (Tr group as follows) or were treated with a  $\text{NH}_4\text{F}$  solution and washed with artificial saliva (Sa group as follows), after washing artificial saliva, the enamel blocks were washed with purified water. The each specimen was dehydrated with a series of alcohol solutions and coated with gold-palladium for 3 minutes at DC400V and 15mA using an ion sputter coater (E-1030, HITACHI, Tokyo). We studied morphological changes on the enamel surfaces using scanning electron microscopy (SEM as follows), elemental analysis (Ca, P, F mapping and point analysis) and quantitative analysis by point analysis using an energy dispersive X-ray microanalyzer (EDX as follows).

### 1. SEM observation of enamel surfaces

In the Tr group and Sa group, we observed the enamel surface with an accelerating voltage of 25kV and a magnification of  $\times 30,000$  using a field-emission scanning electron

microscope (S-4500, HITACHI, Tokyo).

### 2. Elemental analysis and quantitative analysis of enamel surfaces using EDX

Elemental analysis (Ca, P, F mapping and point analysis) and quantitative analysis by point analysis were performed using an energy dispersive EDX (EMAX-7000, HORIBA, Kyoto), and X-ray detection was performed using a high-purity Si detector (Super Xerophy S-817-X 1, HORIBA, Kyoto). The accelerating voltage was 30kV and the characteristic X-ray energy values were 0-5.12keV. F and Ca mapping and F and Ca composite mapping analysis were performed for the Tr group and Sa group. Elemental analysis of F, Ca, and P and quantitative analysis (spectrum analysis) by point analysis were performed. In the point analysis, the F, Ca, and P concentrations were measured 5 times. Calculations were performed based on the peak intensities obtained from the spectra of the reference materials calcium fluoride ( $\text{CaF}_2$ ) and fluorapatite (FAP).

### 3. Statistical analyses

Data were entered into the SPSS software (SPSS for Windows ver. 9.0.1J, SPSS Inc., Chicago, IL) for analysis. The Bonferroni multiple comparison procedure was used to assess the differences in the mean values of Ca, P and F concentrations among pH groups and the control group in the Tr group and Sa group ( $p < 0.05$ ). The t-test was used to compare the Tr group and Sa group in the same pH group or in the control group ( $p < 0.05$ ).

## Results

### 1. SEM observation of enamel surfaces

In the Tr group, spherical crystals deposition was observed on the enamel surface in the pH 2 to pH 6 groups. In the pH 2 group, the particle diameters were approximately 0.44 $\mu\text{m}$ , larger than those of the pH 3–pH 6 groups had adhered closely. In the pH 3 group, although the spherical crystals had adhered closely, the particle diameters were approximately 0.36 $\mu\text{m}$ , smaller than those in the pH 2 group. In the pH 4 group, although the spherical crystals had adhered closely, the particle diameters were approximately 0.44 $\mu\text{m}$ , smaller than those in the pH 3 group. Furthermore, adhesion of the spherical crystals decreased as the pH rose. The pH 7 group had a surface structure that was similar to that of the control group (Fig. 1).

In the Sa group, the enamel surface was demineralized in the pH 2 and pH 3 groups. In the pH 2 group, a few remaining spherical crystals were observed. In the pH 4 group, there were more remaining spherical crystals than in the other groups. In the pH 5, pH 6 and pH 7 groups, the enamel surface structure was similar to that of the control group (Fig. 2).

### 2. Elemental analysis and quantitative analysis of enamel surfaces using EDX

In the elemental analysis (Ca, F mapping) of the Tr group, F concentrations were higher

at the sites where spherical crystals were observed on the SEM images. The results of F and Ca composite mapping also indicated that spherical crystals were  $\text{CaF}_2$ . In the pH 2–pH 6 groups,  $\text{CaF}_2$  formation was observed on enamel surfaces after treatment with  $\text{NH}_4\text{F}$  solutions, and

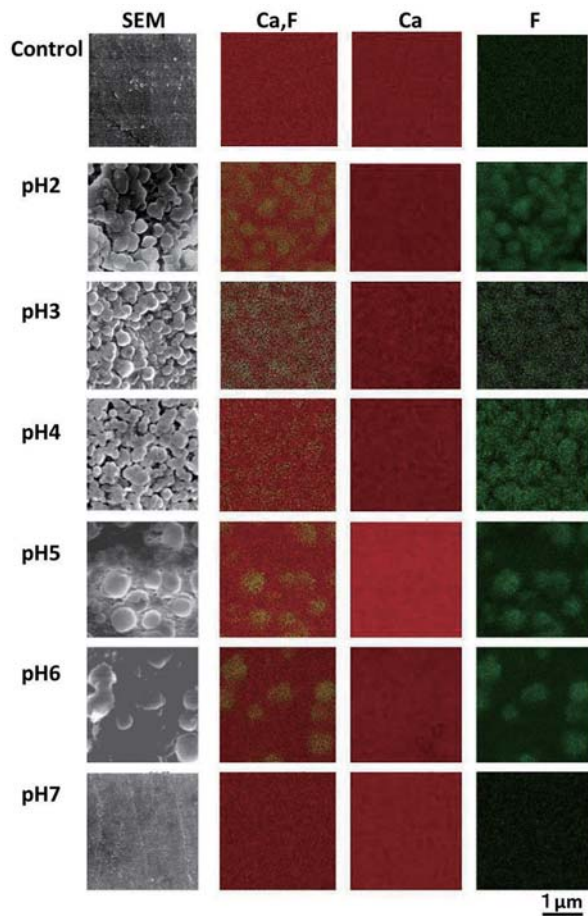


Fig. 1 SEM images and elementary analysis (Ca, F mapping) images using EDX in Tr groups

dense deposition of  $\text{CaF}_2$  was seen in the pH 2–pH 4 groups (Fig. 1). In the spectra for quantitative analysis using EDX, the peak for F was observed in the pH 2–pH 6 groups but not in the pH 7 group or the control (Fig. 3).

When the results of mapping were examined for the Sa group, slightly higher F concentrations were observed in the areas with remaining spherical crystals and in the demineralized areas in the pH 2 group and pH 3 group. In the pH 4 group, the F concentration was clearly higher in the areas with remaining spherical crystals. The spherical crystals were also thought to be  $\text{CaF}_2$  based on F and Ca composite mapping. In the pH 5 group and pH 6 group, spherical crystals were not observed after washing with artificial saliva. In the pH 7 group and the control group, there was no difference between the Tr group and Sa group in mapping analysis (Fig. 2).

In the spectra for quantitative analysis by EDX analysis, the peak for F was observed in the pH 2 group and pH 4 group. This finding is consistent with the finding of spherical crystals remaining in the SEM images (Fig. 3).

The Ca, P, and F concentrations on the enamel surfaces were examined for each group. In the Tr group, the F concentration decreased in the order of the pH 3, pH 2, pH 4, pH 5, pH 6, and pH 7 groups. The F concentrations were higher or tended to be higher in the pH 2–pH 6 groups than in the control group. However, the F concen-

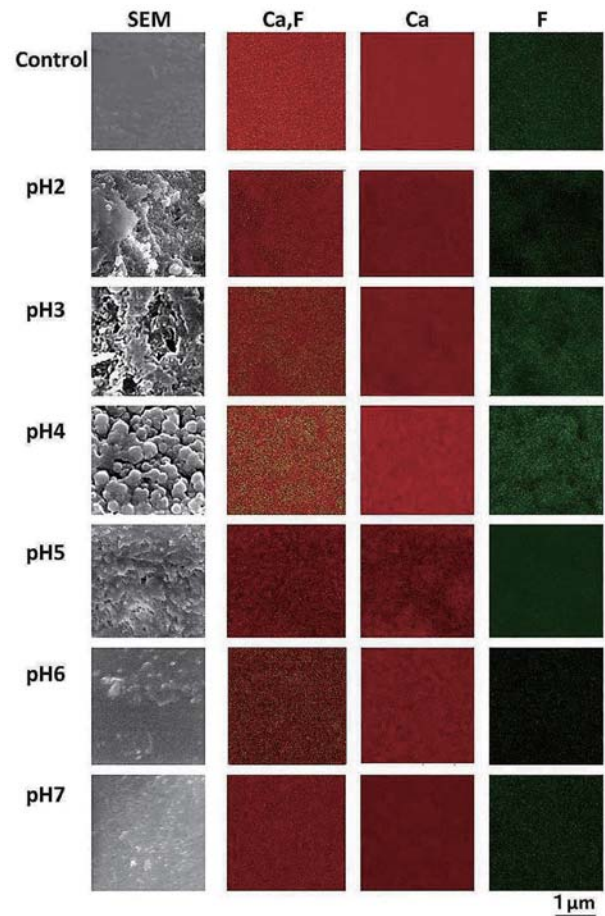


Fig. 2 SEM images and elementary analysis (Ca, F mapping) images using EDX in Sa groups

tration of the pH 7 group was similar to that of the control group. The results of the multiple comparison procedure indicated statistically significant differences among the pH 2–pH 5 groups and control group. Statistically significant differences were observed between the three groups with pH 4 or lower and the three groups with pH 5 or higher. The results showed that the F concentrations were higher in the three groups with pH 4 or lower and were lower in the three groups with pH 5 or higher. In the three groups with pH 4 or lower, no statistically significant difference was indicated between the pH 2 group and pH 3 group and between the pH 2 group and pH 4 group. However, a statistically significant difference was indicated between the pH 3 group and pH 4 group. Statistically significant differences were indicated between the pH 5 group and all other groups. The F concentration of the pH 5 group was higher than the concentrations of the pH 6 group and pH 7 group but lower than the concentrations of the pH 2–pH 4 groups. No statistically significant difference was indicated among the pH 6 group, pH 7 group, and control group (Table 1).

In the Tr group, the Ca concentrations of the pH 2–pH 5 groups tended to be higher than those of the other groups but no statistically significant difference was indicated (Table 1).

In the Tr group, the P concentrations of the pH 2–pH 4

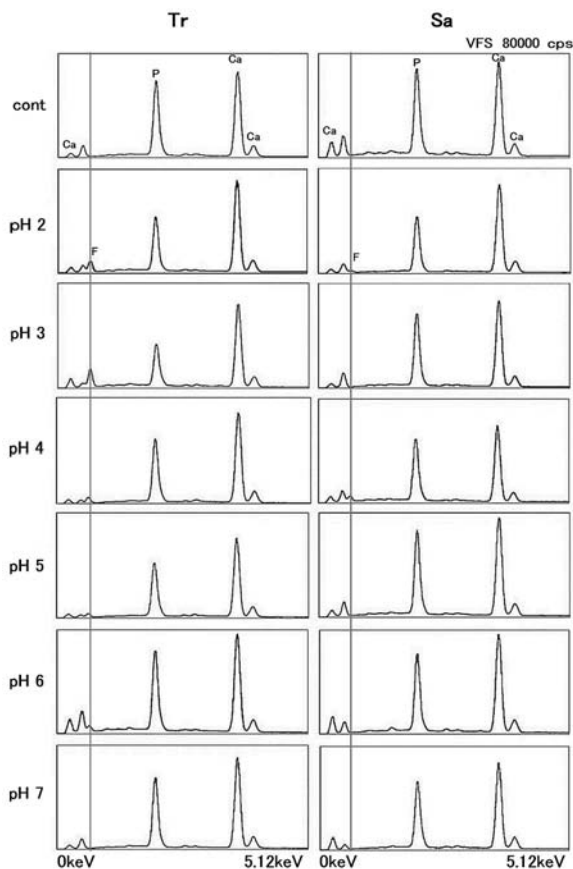


Fig. 3 Qualitative analysis by the point analysis of enamel surface (using EDX)

groups tended to be lower than those of the other groups. The results of the multiple comparison procedure showed that the P concentration of pH 3 group was statistically significantly lower than that of the control group and that P concentrations of the pH 2 group and pH 3 group were statistically significantly lower than those of the pH 5, 6, and 7 group. There was no statistically significant difference among the pH 2, 3, and 4 groups and among the pH 5, 6, and 7 groups. In addition, there was no statistically significant difference between the pH 4 group and all other groups (Table 1).

The above results showed that the pH 2–pH 4 groups of the Tr group had higher F concentrations than the other pH groups of the Tr group, tended to have higher Ca concentrations, and tended to have lower P concentrations. These results are thought to have occurred because a

large amount of  $\text{CaF}_2$  had deposited on the enamel surface due to the treatment with  $\text{NH}_4\text{F}$  solutions, leading to high F and Ca concentrations. In addition, low P concentrations were obtained by point analysis with a beam irradiated from the surface. The above also suggests the presence of  $\text{CaF}_2$  deposition after treatment with fluoride solutions.

The F concentrations were clearly lower in the Sa group than the Tr group for the pH 2–pH 6 groups, indicating statistically significant differences. When comparisons were made among pH groups in the Sa group, the F concentrations decreased in the order of pH 4, 2, 3, 5, 6, and 7 groups. The F concentrations tended to be higher in the pH 2–4 groups than in the control group in the Sa group. The results of the multiple comparison procedure showed statistically significant differences between the pH 4 group and all other all groups (Table 1).

The Ca concentrations tended to be lower in the pH 2–pH 6 groups of the Sa group compared to these pH groups of the Tr group. However, statistically significant differences between the Tr group and Sa group were observed only in the pH 2 and pH 4 groups. The results of the multiple comparison procedure showed no statistically significant difference in the Ca concentration among all groups in the Sa group (Table 1).

The P concentrations in the pH 2–pH 4 group tended to be higher in the Sa group than in the Tr group. In the pH 2 group and the pH 3 group, a statistically significant difference was indicated between the Sa group and the Tr group. The results of the multiple comparison procedure showed no statistically significant difference in the mean P concentration among all groups in the Sa group (Table 1).

## Discussion

Since 1957<sup>8)</sup>, research regarding the preventive effect of  $\text{NH}_4\text{F}$  on caries has been carried out in vitro, in animal experiments, and in vivo. It is well known that the preventive mechanism of  $\text{NH}_4\text{F}$  on caries produces a large amount of  $\text{CaF}_2$  that is difficult to dissolve on the enamel surface<sup>9–12)</sup>.

When the enamel is treated with a fluoride solution, the reaction differs based on the pH of the solution, the fluoride concentration of the solution, and the reaction time. We reported that  $\text{NH}_4\text{F}$  had a greater preventive effect on caries than APF at the same pH and fluoride concentration. Thus, it is possible to shorten the treatment time for  $\text{NH}_4\text{F}$ <sup>1)</sup>. We have also shown that the preventive effect can be expected even with a decrease in the fluoride concentration using the same treatment time<sup>1–6, 13)</sup>.

Some reports indicated that  $\text{CaF}_2$  could be produced on an enamel block treated with an  $\text{NH}_4\text{F}$  solution (pH7.0) for

Table 1 Elementary analysis by the point analysis (Ca, P, F concentration) of enamel surface using EDX

solution	F concentration $\pm$ SD				t-test (p<0.05)	Ca concentration $\pm$ SD				t-test (p<0.05)	P concentration $\pm$ SD				t-test (p<0.05)
	pH	Treatment	Bonferroni (p<0.05)	Saliva wash		Bonferroni (p<0.05)	Treatment	Bonferroni (p<0.05)	Saliva wash		Bonferroni (p<0.05)	Treatment	Bonferroni (p<0.05)	Saliva wash	
Control		0.6 $\pm$ 0.1		0.4 $\pm$ 0.4	0.136	36.4 $\pm$ 2.8		34.7 $\pm$ 4.5		0.395	20.6 $\pm$ 1.1		20.1 $\pm$ 1.6	0.546	
pH 2		20.9 $\pm$ 5.9	a,b,c,d	2.0 $\pm$ 2.4	0.000	43.4 $\pm$ 4.7		38.9 $\pm$ 3.6		0.006	15.4 $\pm$ 2.9		18.7 $\pm$ 1.6	0.001	
pH 3		24.3 $\pm$ 11.2	f,g,h,i	0.8 $\pm$ 0.2	c,e,i,m, 0.000	44.8 $\pm$ 8.3		36.7 $\pm$ 5.8		0.072	15.3 $\pm$ 3.5	b,f,g,h	19.6 $\pm$ 2.1	0.026	
pH 4		17.4 $\pm$ 5.5	j,k,l,m	8.8 $\pm$ 5.3	n,o 0.002	43.6 $\pm$ 4.3	n.s.	32.8 $\pm$ 2.7	n.s.	0.000	17.3 $\pm$ 2.9	j,k,l	18.1 $\pm$ 1.2	n.s. 0.488	
pH 5		9.8 $\pm$ 5.3	n,o,p,q	0.7 $\pm$ 0.2	0.000	41.4 $\pm$ 8.6		37.8 $\pm$ 5.6		0.266	19.1 $\pm$ 3.3		18.9 $\pm$ 0.8	0.822	
pH 6		2.1 $\pm$ 2.3		0.6 $\pm$ 0.3	0.027	37.9 $\pm$ 12.4		35.3 $\pm$ 3.0		0.452	19.2 $\pm$ 3.6		19.8 $\pm$ 1.1	0.728	
pH 7		0.5 $\pm$ 0.1		0.5 $\pm$ 0.6	0.798	38.7 $\pm$ 2.3		36.8 $\pm$ 6.8		0.341	19.8 $\pm$ 1.2		19.4 $\pm$ 2.1	0.565	

SD : standard deviation (n=5)  
 Bonferroni : a:Control–pH2, b:Control–pH3, c:Control–pH4, d:Control–pH5, e:pH2–pH4, f:pH2–pH5, g:pH2–pH6, h:pH2–pH7, i:pH3–pH4, (p<0.05) j:pH3–pH5, k:pH3–pH6, l:pH3–pH7, m:pH4–pH5, n:pH4–pH6, o:pH4–pH7, p:pH5–pH6, q:pH5–pH7

5 minutes, that  $\text{NH}_4\text{F}$  mixed with drinking water had a stronger effect at pH 5.0<sup>(14-16)</sup>. It has been shown that the use of  $\text{NH}_4\text{F}$  resulted in the same degree of fluoride uptake onto the enamel surface as the use of APF, even when the fluoride concentration for the  $\text{NH}_4\text{F}$  solution was approximately half of that of the APF solution<sup>(2)</sup>. Furthermore, at pH 4.2–pH 4.4,  $\text{NH}_4\text{F}$  produced greater amounts of  $\text{CaF}_2$  that adhered tooth enamel more strongly and was more difficult to wash out with KOH and artificial saliva compared with NaF under the same conditions<sup>(9-12)</sup>. There has also been a report on a field study of children who rinsed their mouths with either NaF or  $\text{NH}_4\text{F}$ , pH 4.4, 1,000ppm  $\text{F}^-$ , for 2 years. The results showed that  $\text{NH}_4\text{F}$  had a significantly higher caries preventive effect in newly erupted teeth than NaF<sup>(17)</sup>.  $\text{NH}_4\text{F}$  has been shown to have a caries preventive effect at various pH levels, but comparisons of its influence on the enamel based on pH are difficult because of the differences in fluoride concentration and treatment time. Therefore, we examined the influence of  $\text{NH}_4\text{F}$  solutions at various pH levels (pH 2–pH 7) on human enamel in our present study. In our previous study using chemical analysis and SEM observations, we reported fluoride uptake and acid resistance effects in all groups treated with  $\text{NH}_4\text{F}$  solutions (i.e., in groups with  $\text{NH}_4\text{F}$  treatment and in groups with  $\text{NH}_4\text{F}$  treatment and subsequent artificial saliva wash).

Our present study involved SEM observations and Ca and F mapping analysis and Ca, P and F point analysis of EDX of the enamel surface. In the SEM observations and mapping analysis of EDX, we observed that the pHs of  $\text{NH}_4\text{F}$  solutions were low (pH 2–pH 4) and that the spherical crystals were in greater amounts and adhered more closely. These spherical crystals were considered to be  $\text{CaF}_2$  based on the observations of the aforementioned research studies. In our previous study, we confirmed the deposition of  $\text{CaF}_2$  on an enamel surface that was treated with an  $\text{NH}_4\text{F}$  solution (pH 4.5, 900ppm  $\text{F}^-$ ) for 5 minutes. Furthermore, it was confirmed that there was an increase in fluoride concentration on parts of the enamel surface without deposition of  $\text{CaF}_2$ . The results of state analysis using EPMA suggested the possibility of FAp formation. Therefore the enamel reacted differently depending on the pH of each  $\text{NH}_4\text{F}$  solution<sup>(3)</sup>.

In our present study, the pH 2–pH 6 groups showed  $\text{CaF}_2$  deposition after treatment with fluoride solutions.  $\text{CaF}_2$  was subsequently released slowly into the artificial saliva and reacted with calcium and phosphorus in solution to produce FAp. However, we could not confirm the presence of spherical crystals on the enamel surface of the pH 7 group, as our previous study, the fluoride uptake was shown from the chemical analysis.

In SEM observations, we observed demineralized enamel surfaces in the pH 2 and pH 3 groups after washing with artificial saliva. The remaining spherical crystals were seen in the pH 4 group and to a lesser degree in the pH 2 group. We observed much more spherical crystals in the pH 4 group than in the other groups. When an acidulated fluoride solution is used to treat the enamel surfaces, the surface hydroxyapatite is demineralized. In such a case, it is known that the dissolved Ca and fluoride ion in

the fluoride solution bind to each other to form  $\text{CaF}_2$ . In the pH 2 and pH 3 groups, demineralization occurred into deeper layers due to low pH levels and treatment with the solution. Thus,  $\text{CaF}_2$  formed on the surface was washed away after the artificial saliva wash, enabling the demineralized surface to be exposed and to be observed. It was suggested of the pH 4  $\text{NH}_4\text{F}$  solution could be deposited a lot of  $\text{CaF}_2$  and the based enamel surface has less demineralized damages than pH 2 and pH 3  $\text{NH}_4\text{F}$  solutions during treatment, therefore in the pH 4 group remained lots of  $\text{CaF}_2$ . It has been shown that the restraint effect of an  $\text{NH}_4\text{F}$  solution (pH 4.5, 900ppm  $\text{F}^-$ ) was shown on caries-like lesions at slightly acidic pHs of 4 to 5. The conditions of the  $\text{NH}_4\text{F}$  solution approximated an intraoral condition after using a toothpaste<sup>(3)</sup>.

In the mapping analysis, the demineralized parts in the pH 2 and pH 3 groups had slightly higher F concentrations than those in all the other groups. In addition, the F concentration tended to be higher in the elemental analysis by point analysis. Thus, while the enamel blocks were washed with artificial saliva, fluoride in small amounts was thought to have been taken up into enamel hydroxyapatite that had been demineralized during the treatment with  $\text{NH}_4\text{F}$  solutions. The results of Ca and F mapping analysis and point analysis of EDX analysis suggest that when demineralization occurred, there was more fluoride remaining during washing with artificial saliva in the groups treated with low pH  $\text{NH}_4\text{F}$  solutions (pH 2, pH 3, and pH 4  $\text{NH}_4\text{F}$  groups) than those treated with high pH  $\text{NH}_4\text{F}$  solutions (Fig. 2, 3).

In this present study, the pH 4 group had  $\text{CaF}_2$  remaining even after the artificial saliva wash, albeit in a smaller amount than after treatment alone. This finding suggests that there was a larger amount of  $\text{CaF}_2$  deposition in the pH 4 group than in the other groups, enabling  $\text{CaF}_2$  to be observed even after the 24-hour artificial saliva wash. Speake et al reported that fluoride concentrations of 1,500–2,000ppm on the enamel surface are necessary to acquire suitable resistance to caries. They showed that the  $\text{NH}_4\text{F}$  groups with a pH lower than pH 6 had fluoride concentrations over 1,500ppm after treatment and even after washing with artificial saliva<sup>(18)</sup>. Our results were consistent with their results. In our present study, there was no change in the condition of the enamel surface in the pH 5, pH 6 and pH 7 groups compared with the control group. In the pH 5, pH 6, and pH 7 groups, we speculated that almost soluble  $\text{CaF}_2$  and phosphate compounds, which had loosely combined with enamel, had dissolved into the solution during the 24-hour washing with artificial saliva. Our previous study involved chemical analysis and the same experimental conditions of the fluoride concentration and pHs as our present study. In our previous study, we reported that after treatment, all  $\text{NH}_4\text{F}$  groups (pH 2–pH 7) showed fluoride uptake into the deep enamel layers. In addition, the acid resistance test demonstrated acquisition of higher acid resistance in the deep enamel layers compared with the control group. There was no difference in fluoride uptake and acid resistance based on the pH of the  $\text{NH}_4\text{F}$  solutions<sup>(6)</sup>.

In our present study,  $\text{CaF}_2$  was not observed after treat-

ment in the pH 7 group. EDX analysis did not show any remaining fluoride after treatment with fluoride solutions. Elemental analysis using EDX was performed only for analysis of the enamel surface and not for analysis of the deeper layers. In addition, the accuracy of detection is lower for EDX analysis than for chemical analysis. Therefore, in our present study, we were unable to confirm fluoride uptake resulting from fluorapatite formation, which was suggested in our previous study using chemical analysis. In our present study, the results of mapping analysis showed that fluoride concentrations tended to be slightly higher on the enamel surfaces of the pH 2–pH 4 groups after treatment with  $\text{NH}_4\text{F}$  solutions and after the artificial saliva wash. Thus, we were able to confirm fluoride uptake on the enamel surface using SEM observations and mapping analysis and elemental analysis using EDX just as in morphological observations of the enamel surface.

In our present study, morphological observations of the enamel surface and EDX analysis were performed after enamel blocks were treated with  $\text{NH}_4\text{F}$  solutions in addition to after washing with artificial saliva. The results showed that  $\text{CaF}_2$  formed on the enamel surfaces after treatment with  $\text{NH}_4\text{F}$  solutions at pH 2–pH 6, that there was dense deposition of  $\text{CaF}_2$  in groups treated with  $\text{NH}_4\text{F}$  solutions at pH 2–pH 4, and that there were larger amounts of  $\text{CaF}_2$  deposition. The results of EDX analysis confirmed the presence of remaining fluoride on the enamel surface after washing with artificial saliva. When the  $\text{NH}_4\text{F}$  solution at pH 4 was used, there were large amounts of  $\text{CaF}_2$  deposition and remaining  $\text{CaF}_2$  even after washing with artificial saliva. This solution enabled the highest fluoride uptake onto the enamel surface, and we expect that formation of  $\text{CaF}_2$  and FAp on the enamel surface will have a preventive effect on caries lesions.

### Conclusion

The results of this study showed that when enamel was treated with an  $\text{NH}_4\text{F}$  solution with a fluoride concentration of 900ppm for 5 minutes, the reaction was different depending on the pH of the solution.  $\text{CaF}_2$  was noted on the enamel surface in the low pH  $\text{NH}_4\text{F}$  groups, and more  $\text{CaF}_2$  was remaining in the pH 4  $\text{NH}_4\text{F}$  group even after washing with artificial saliva for 24hours compared with the other groups. It was difficult to observe  $\text{CaF}_2$  in the  $\text{NH}_4\text{F}$  solutions at or near neutral pH (pH 5, pH 6 and pH 7). There could have been more fluoride acquisition in the pH 3, pH 5 and pH 6 groups compared with the control group.

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## pH の異なるフッ化アンモニウム溶液の intact enamel に及ぼす影響 —形態学的観察およびエネルギー分散型 X 線分析装置 (EDX) を用いた検討—

大 橋 たみえ 廣 瀬 晃 子 石 津 恵 津 子 岩 田 幸 子  
小 澤 亨 司 河 野 哲<sup>1)</sup> 磯 崎 篤 則

本研究は pH の異なるフッ化アンモニウム (NH<sub>4</sub>F) 溶液のヒトエナメル質に及ぼす影響について比較検討した。

実験にはヒト新鮮抜去小白歯を用いた。各サンプルを7群に分け、そのうちの6群に対して pH 2~pH 7 の NH<sub>4</sub>F 溶液 (フッ化物濃度 900ppm) を 37°C で 5 分間作用した。また各作用群を任意に 2 つに分類し、一方について 24 時間の人工唾液洗浄を行った。フッ化物溶液作用後ならびに人工唾液洗浄後の各群のエナメル質表面について、SEM 観察、EDX による定性分析および元素分析を行い、各群のエナメル質表面の Ca, P, F の分布および濃度を測定した。

フッ化物作用後のエナメル質表面の SEM 観察では pH 2~pH 6 の NH<sub>4</sub>F 群で顆粒状物質の存在が確認された。次に人工唾液洗浄後の SEM 観察では、人工唾液洗浄後は pH 2 では、エナメル質表層の脱灰された像と、わずかに顆粒状物質が残遺した像が観察された。pH 3 群では、エナメル質表層の脱灰された像が観察された。pH 4 群では顆粒状物質の残留が認められた。

EDX による定性分析と元素分析では、溶液作用後の pH 2~pH 6 群で F 濃度が高く、フッ化カルシウムの形成が確認された。特に pH 2 群、pH 3 群で F 濃度が高いことが観察された。pH 7 群ではコントロールと同様のスペクトルが得られ F は検出されなかった。よって、フルオロアパタイトやハイドロキシフルオロアパタイトの生成については確認できなかった。

人工唾液洗浄後では、pH 2 群と pH 4 群で、他群よりも F 濃度が高かった。しかしその濃度は溶液作用後よりも明らかに低かった。pH 3, pH 5, pH 6 群では、コントロール群よりも若干 F 濃度が高い傾向がみられたがその差は明らかでなかった。

以上より、溶液作用によるフッ化カルシウムの沈着が多く、人工唾液洗浄によっても結晶が残遺した pH 4 群は、本研究の実験条件中では、溶液作用によるエナメル質表面へのフッ化物取り込み効果が最も高く、う蝕抑制効果が期待できることが示唆された。

キーワード：フッ化アンモニウム溶液、pH、人工唾液、顆粒状結晶、形態学的検討、EDX