Effect of low level fluoride on demineralization kinetics of human dental enamel

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Abstract The purpose of this study was to examine the effect of fluoride levels similar to those reported for saliva from low fluoridated and high fluoridated water areas on the demineralization of human permanent enamel. An adaptation of the method described by Robinson et al. was used. Sections of sound enamel were immersed in a vial containing demineralizing solution [2.0 mM Ca(NO3)2, 1.2 mM KHPO4 in 50 mM acetic acid, pH 4.8] for 1 hour. The demineralizing solution contained 0, 0.02 or 0.05 ppm fluoride, added as NaF was prepared. Twenty microliters aliquots were taken from the demineralizing solution at the time point up to 1 hour, with a sampling frequency bias towards the early time point. The phosphate content of the removed sample was determined by colorimetry. When the mineral loss curves for fluoridated and non-fluoridated demineralizing solutions were compared, there were significant differences between both groups. There was a decrease in the net mineral loss when fluoride was used. This result suggested that salivary fluoride levels of 0.02 ppm and 0.05 ppm had a protective effect against demineralization.

Key words Caries, Demineralization, Enamel, Fluoride, Saliva

Introduction The role of saliva in the mode of action of fluoride is now well recognized such that fluoride in the fluid environment around the tooth is considered to have a significant effect on the process of demineralization even at very low levels1–3. The use of low levels of fluoride at frequent intervals has also been suggested to be more effective than less frequent exposures to high fluoride concentrations1,4. Previous research suggested that fluoride in saliva and plaque fluid, even at very low levels can affect the demineralization and remineralization processes1,5,6. Such fluoride levels in saliva (0.02–0.05 ppm7,8) were similar to those found in areas with either poorly fluoridated or high fluoridated water. The purpose of this study was to examine the effect of two experimental fluoride levels (0.02 ppm F, and 0.05 ppm F) similar to those reported for saliva from low fluoridated and high fluoridated water areas on the early demineralization of human permanent enamel.

Materials and Methods

Extracted 21 premolars, with no visible carious regions were used. It obtained at pediatric dentistry of the hospital affiliated with the Dentistry Department, Aichi-Gakuin University. Patients had not been applied Fluoride before extraction and they lived in non-fluoridated water area. The equipment and method reported by Robinson et al.9 was used. The intact tooth crowns were...
embedded in wax and sectioned buccolingually, and then each section polished to approximately 150 µm in thickness. One set of twelve sections were obtained from one tooth and seven sets of samples were used for each regime i.e. 0, 0.02 and 0.05 ppm fluoridated demineralizing solutions.

The polished sections were observed under the light microscope and the outer edge of the tooth section, the dentine and any carious or other imperfections in the enamel, and were coated with nail varnish. As far as possible, the nail varnish was applied identically to both sides of the section. The area was measured on both sides of the sections and an average of the two values taken. Twelve sections from one teeth were inserted in plastic baskets which were then placed in vials containing 10 ml demineralising solution [2.0 mM Ca(NO₃)₂, 1.2 mM KH₂PO₄ in 50 mM acetic acid, pH 4.8 adjusted by 10 M KOH] for up to 1 hour.

The temperature of solution was maintained at 37°C and the enamel baskets gently agitated within the solution. The model using a historical processor (EMPT, Leika, JAPAN) could set any temperature and agitation level. For the fluoride investigations, 0.02 ppm or 0.05 ppm fluoride was added as NaF.

Twenty microliters aliquots were taken from the supernatant solution following 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 30, 40, 50 and 60 minutes. Mineral loss was evaluated quantitatively on a volume percent basis by the amount of phosphate calculated as hydroxyapatite which was dissolved from the enamel compared with the volume of enamel exposed. It was assumed that all enamel mineral present was in the form of hydroxyapatite. The phosphorus content was determined as described by Chen et al.¹⁰

Mineral loss (%) = 
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\frac{\text{Amount of dissolution P/P content in enamel (18.5)} \times 100}{\text{Specific gravity of enamel (3.1)} \times \text{Total enamel volume}}
\]

 Approval for this study was obtained from the Ethics of the Department of Dentistry, Aichi-Gakuin University (approval No.221).

One way ANOVA and Sheffe test was used to evaluate for statistically significant differences in mineral loss among the 0 ppm and 0.02 ppm solution, 0 ppm and 0.05 ppm solution, 0.02 ppm and 0.05 ppm solution. A P-value less than 0.05 were considered as statistically significant.

**Results**

Figures 1 and 2 shows the effect of 0.02 ppm and 0.05 ppm fluoridated solution on the kinetics of
permanent enamel demineralization over a period of 1 hour, compared with a non-fluoridated demineralizing solution. There were a significant differences between 0 ppm and 0.02 ppm ($P < 0.001$), 0 ppm and 0.05 ppm ($P < 0.001$), 0.02 ppm and 0.05 ppm ($P < 0.001$) shown in Fig. 1. There were a significant differences between 0 ppm and 0.02 ppm at 30, 40, 50, 60 minutes and 0.05 ppm at 10, 12, 14, 16, 18, 20, 30, 40, 50, 60 minutes shown in Table 1.

When sound enamel was demineralized at pH 4.8, in a non-fluoridated solution which was under saturated with respect to hydroxyapatite, demineralization proceeded steadily for 1 hour. The final mineral loss was (mean ± S.E.) 2.45 ± 0.10%. When the final mineral losses for fluoridated and non-fluoridated dematerializing solutions were compared however, there were obvious differences. There was a significant decrease in the final mineral loss (2.45 ± 0.10% for 0 ppm, 1.06 ± 0.17% for 0.02 ppm, 0.84 ± 0.14% for 0.05 ppm).

**Discussion**

There was a significant decrease in the final mineral loss when the non-fluoridated solution was compared with both fluoridated experiments. There was, however, little difference between 0.02 ppm fluoridated and 0.05 ppm fluoridated experiments.

The demineralization profiles, while not statistically different showed distinct trends during early
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For both non-fluoride solution and 0.02 ppm fluoridated solution began almost immediately on exposure to demineralization solution. The initial rapid loss in the 0.02 ppm fluoridated solution may correspond with the loss of carbonate and magnesium rich material on which fluoride may have little effect\(^1\), the subsequent slowing down due to an effect on apatite.

On the other hand, the rate of demineralization in the 0.05 ppm fluoride increased very little up to about 10 minutes and then followed the 0.02 ppm curve. This data suggests that the effect of 0.05 ppm fluoride differs from that of the 0 ppm control and the 0.02 ppm F during the first few minutes of demineralization and is consistent with previously reported work\(^{12}\).

This intriguing result may be an indication of the mechanism of action of 0.05 ppm fluoride which has been reported to be much more effective than 0.02 ppm in reducing caries.

The reason for this possible lag period is intriguing. It may be related to the effect of higher supernatant fluoride concentrations first on the more soluble magnesium and carbonate materials as described above, but also on the initial protonation of the enamel mineral\(^1\). This process is thought to precede ultimate release of calcium and phosphate. This is consistent with the findings of Arends and Davidson\(^1\), who demonstrated that over 30 h calcium was preferentially removed presumably in the first instance in exchange for protons. More recent data using AFM (atomic force microscopy) pK titrations technology has also demonstrated direct protonation of enamel apatite as pH is reduced in the presence of fluoride\(^4\). After a brief fluoride treatment protonation was rendered more difficult i.e. occurred only at much lower pH values. In addition, erratic data at very low pH due to removal of phosphate by adherence to the AFM tip was dramatically reduced after fluoride treatment.

This would be consistent with stabilizing the lattice by fluoride which rendered the protonation of phosphate and removal of both calcium and phosphate more difficult. The mechanism of initial protonation is unclear while direct replacement of calcium is possible replacement of both sodium and/or magnesium may also be important.

From the point of view of the oral cavity such a delay in mineral loss could be an extremely protective measure and it has an important role in caries prevention. The time period for acid in the oral cavity following ingestion of fermentable substrate is around 20 minutes. It would thus only be necessary to delay or reduce demineralization for this short period following the appearance of acid. From the data presented it seems that fluoride at levels present in saliva may well be capable of effecting a delay of 8–10 minutes which coupled with the subsequent slower demineralization could have a profound effect on net demineralization.

The possible lag period in early release of mineral ions from the tooth surface is a hitherto unexplored area, which focuses on an effect relating fluoride to episodic acid production. It would seem reasonable that if the fluoride induced delay in release of mineral from the enamel surface, it is similar to the time taken to eliminate acid from the mouth. Additional work needs to be done concerning the dynamics of acid induced mineral release into plaque biofilms coupled with studies of local fluoride concentration.

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References

6) Feagin, F.F. and Jeansonne, B.G.: Effective fluoride concentrations to promote apatite mineralization at the enamel surface. *Alabama Journal of Medical*


