Effect of pH on fluoride penetration into natural human plaque

Takeshi Tokura1,*, Colin Robinson2, Philip Watson2, Hani Abudiak3, Takashi Nakano1, Kimihiko Higashi1, Tomokazu Naganawa1, Kazuo Kato4, Osamu Fukuta1 and Haruo Nakagaki4

1 Department of Pediatric Dentistry, School of Dentistry, Aichi-Gakuin University 2-11 Suemori-dori, Chikusa-ku, Nagoya 464-8651, JAPAN
2 Division of Oral Biology, University of Leeds Dental Institute Clarendon Way, Leeds, LS2 9LU, UK
3 Division of Pediatric Dentistry, University of Leeds Dental Institute Clarendon Way, Leeds, LS2 9LU, UK
4 Department of Preventive Dentistry and Dental Public Health, School of Dentistry, Aichi-Gakuin University 1-100 Kusumoto-cho, Chikusa-ku, Nagoya 464-8650, JAPAN

Abstract Since recent studies have demonstrated that penetration profiles of fluoride into plaque falls from the saliva plaque interface towards the enamel, it was hypothesized that charged components may restrict the inward diffusion of fluoride i.e., protonated fluoride and may penetrate more effectively than fluoride ion. Therefore, in this study, we investigated the effects of pH on fluoride uptake and distribution in natural undisturbed human plaque formed in vivo by means of the Leeds in situ device. At pH 3 significantly less fluoride was present throughout the plaque layer compared with pH 7. Similar profiles were seen in the plaque produced over 1 week and 3 weeks. These results may be due to binding to components of the plaque matrix via hydrogen bonding. Protonation of bacterial cell surfaces may also lead to binding of the ionized portion of fluoride. In conclusion, low pH affected the short-term fluoride uptake from a 1,000 ppm fluoride solution.

Introduction Ingress and penetration of therapeutic agents into plaque, such as fluoride, is of tremendous importance to provide effective protection against caries1,2, encouraging remineralisation3, inhibiting both demineralization and bacterial activity4. However, recent work has demonstrated that, the normal distribution pattern for fluoride within dental plaque falls from the saliva plaque interface towards the enamel5. This is presumably generated by fluoride from the diet but more particularly from fluoridated dentifrices6. In terms of penetration of plaque by dentifrice fluoride, during the short periods ≤ 30 seconds associated with tooth brushing7,8, fluoride penetration was seen to be limited to the outer third or so of established plaque layers. Full penetration was achieved only after 30 minutes fluoride exposure although even after this period concentrations never reached those of the applied solution9.

The reasons for such limited penetration of fluoride are not clear. This may be due to the charge on the fluoride ion. Recent autoradiography has demonstrated that amine fluorides, charged molecules, while showing similar overall penetration profiles to fluoride, tended to be concentrated at the periphery of the biomass10. The antibacterial triclosan on the other hand, while again showing similar overall penetration behaviour appeared, unlike amine fluorides, to penetrate into the biomass11.
This may reflect the fact that amine fluorides are charged while triclosan is extremely hydrophobic. Charged components may thus bind to the biomass restricting their inward diffusion. With this in mind, it might be predicted that there would be more penetration of uncharged i.e. protonated fluoride, into the plaque compared with the unprotonated fluoride ion. In addition low pH would result in greater uptake and therefore greater effectiveness against plaque bacteria. This would have important implications with regard to fluoride delivery and the generation of low pH by the plaque itself. However, little is known about this issue in natural undisturbed plaque biofilms. The purpose of this study was therefore to examine the penetration of unionised i.e. protonated fluoride through plaque with the use of fluoride solutions at low pH.

Materials and Methods

Generation of plaque biofilms

Plaque biofilms were generated by means of the Leeds in situ device. Ethical approval was obtained from the Leeds Healthcare/United Leeds Teaching Hospitals Trust Research Ethics Committee and all volunteers participated after giving informed consent to the protocol. Briefly, nylon rings ~5 mm diameters were attached to intact enamel pieces using cyanoacrylate adhesive. Subsequently, the devices were bonded to the intact buccal surfaces of upper first molars on both sides of the mouth with Scotchbond Multipurpose Dental Adhesive (HEMA BIS-GMA resin Scotchbond Multipurpose Dental Adhesive System, 3M, Minneapolis, MN, USA). Surfaces were lightly etched with 10% maleic acid before attaching the nylon rings.

The plaque formation periods in the mouth were 1 week and 3 weeks. With regard to toothbrushing and toothpaste during the formation periods, volunteers were asked to follow their normal daily oral hygiene but if possible to avoid brushing the devices directly in order to obtain undisturbed plaque. Non-fluoride toothpaste was provided.

Ex vivo exposure of biofilms to fluoride

After the plaque formation period in vivo, devices were recovered from the mouths of volunteers using orthodontic de-bonding pliers and immediately immersed in 1 m/l 1,000 ppm solution pH 3 fluoride for 30 sec. pH 3 ensured that ~60% of the fluoride would be in an unionized form. Control devices were immersed in 1,000 ppm fluoride solution pH 7. All ex vivo experiments were conducted at room temperature (approximately 20°C).

The excess solution around the devices was removed by small pieces of tissue under a microscope and the devices were then transferred into Eppendorf tubes to be snap-frozen in liquid nitrogen.

Sample embedding and sectioning

Frozen plaque-containing devices were lyophilised for 24 h removed from the Eppendorf tubes and transferred, nylon ringside up, on to the surface of pre-polymerized methacrylate in flat-bottomed polyethylene capsules. Liquid methacrylate mixture was subsequently added to the devices and vacuum applied for 30 min to ensure removal of all gas bubbles and to achieve complete impregnation of the plaque. To avoid any movement of the device, they were then locked in place using hypodermic needles inserted through the sides of the Eppendorf tubes. Incubation was carried out overnight at 60°C. The needles were then carefully removed and the embedded plaque was completely sectioned from surface to the enamel, parallel to the enamel surface, by means of an ultramicrotome (Reichert, Vienna, Austria). The sectioning sequence was as follows: 5×5 μm sections, followed by 2×2 μm sections and a further 5×5 μm sections.

Each group of ten 5 μm sections was used for fluoride analysis. The 2-μm sections were placed on glass slides and dried for image analysis and determination of biomass fraction.

Determination of fluoride concentrations in plaque sections

After completing the sectioning procedure, plaque sections were removed from cover-slip fragments and transferred into polyethylene capsules, and incubated overnight at room temperature to allow the sections to dry. Each group of sections was subsequently immersed in 50 μl of chloroform for 30 min with lids closed to dissolve and dissipate methacrylate-embedding medium. They were then incubated in an ultrasonic bath for 60 sec and centrifuged (Denlay BR401, Denlay, UK) at 4,000 rpm for 30 min. The groups of sections were then incubated overnight at room temperature (20°C) with lids open, to allow chloroform to evaporate. Ten microliters of 1.0 M perchloric acid was added to each sample for 30 min at room temperature to dissolve fluoride. One point zero molar sodium...
acetate solution (pH 7.0) was then added to each sample, to reach a ratio of 4:1 with the perchloric acid producing a final pH of 5. Capsules were then agitated with a vortex mixer and centrifuged at 4,000 rpm for 10 min. Fluoride concentrations were measured by means of a fluoride-sensitive electrode (Orion EA 940, Orion Research, UK), calibrated using standard solutions of 0.1, 1.0 and 10 ppm F⁻.

**Determination of fluoride concentrations in biomass of plaque sections determination of biomass**

Zero point one percent aqueous toluidine blue was added to each 2 µm section in situ on a glass slide and after gentle heating for about a minute (~60°C) this was incubated at room temperature (15–20°C) for 15 min. Stained plaque sections were finally rinsed with non-sterile tap water and dried at room temperature.

Images of each stained plaque section were captured by means of a video camera (JVC 3-CCD) and analyzed using Zeiss KS300 imaging software (Zeiss, Jena, Germany). For each section pair, the mean area occupied by stained biomass was calculated. Since the section thickness was known, plaque biomass volume within the section could be calculated. By analyzing these data, the determination of biomass distribution throughout plaque could be calculated. Fluoride concentrations in plaque biomass could also be calculated by dividing the total fluoride in each plaque section by the measured biomass fraction.

**Statistical analysis**

Mean fluoride concentrations were compared by one-way ANOVA.

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**Results**

**Plaque biomass distributions formed in vivo**

Figure 1 shows biomass curves for each pH value and plaque age. When comparing the biomass distribution at 1 week, 3 weeks, pH 3 and pH 7 the curves were essentially similar rising gradually towards the enamel. The only differences were seen in the immediate vicinity of the enamel where the proportion of biomass was somewhat variable.

With regard to the base line (non-fluoride exposure), the profiles were a little different, showing no significant difference at pH 3 and pH 7.

**Fluoride concentrations in biomass formed in vivo after 30 seconds exposure to 1,000 ppm F in vitro at pH 3 or pH 7**

Fluoride concentrations in the base line biomass (no F exposure) were almost identically low (0–20 ppm), at both pH 3 and pH 7 although pH 3 fluoride distribution was visually lower and much less variable than pH 7 fluoride.

Fluoride distributions in the biomass formed in vivo after exposures in vitro are shown in Fig. 2. In all cases fluoride concentrations decreased from the plaque surface towards the enamel falling to a plateau about one half of the distance from the surface towards the enamel.

Regarding the distribution of fluoride after a 30 second exposure to 1,000 ppm in plaque produced after 1 week, the concentrations obtained at pH 3 were significantly lower than pH 7 throughout the plaque. Similarly, the distribution of fluoride after a 30 second exposure to 1,000 ppm in plaque produced after 3 weeks showed that the concentrations at pH 3 were significantly lower than pH 7 throughout the
plaque.

Comparison between fluoride uptake at pH 7 for 1-week plaque and 3-week plaque showed that 3-week plaque had significantly lower concentrations in the interior. And at pH 3, likewise, 3-week plaque had significantly lower fluoride concentrations in the interior and at one point near the saliva plaque interface.

Discussion

The effects of pH on fluoride uptake and distribution in plaque biomass treated with fluoride (exposed to 1,000 ppm NaF pH 3 and pH 7 for 30 sec) were examined along with the distribution of biomass. The percentage of biomass i.e. the openness of the structure decreased in all cases towards the enamel similar to data reported earlier9,11. There was no significant differences except perhaps a slightly more open structure i.e. more channels near the enamel at pH 7 compared with pH 3 where the biomass was in any case rather more variable. This may be due to variations in the growth and fusion of initial colonies from the tooth surface.

Fluoride distribution in the biomass in all cases, whether exposed to fluoride or not, were as reported previously5,9 in that they decreased from the outer plaque surface towards the enamel reaching a plateau about half way through the plaque layer. Plaque biofilms exposed to 1,000 ppm fluoride showed a higher concentration in the outer regions compared with controls not exposed to fluoride.

Low pH (pH 3 as opposed to pH 7) affected the short-term uptake from a 1,000 ppm fluoride solution. At low pH i.e. pH 3 significantly less fluoride was present throughout the plaque layer compared with pH 7. This was similar whether the plaques had been produced over 1 week or 3 weeks. Clearly increased amounts of protonated fluoride (60% HF at pH 3) did not result in greater penetration of the plaque layers. In fact less penetration was observed at pH 3 throughout the plaque layer.

This did not support the concept that protonated fluoride might diffuse more effectively than fluoride ion, in fact uptake/penetration seemed to be inhibited.

The reasons may be that protonated fluoride binds more effectively to plaque matrix components perhaps via hydrogen bonding. Carbohydrate and protein of the plaque matrix could be involved here. In addition, protonation of bacterial cell surfaces may also lead to binding of the ionized portion of fluoride present. This has some overall support in the findings of Tanaka and Margolis14 who reported that low pH generated by a sucrose rinse did not increase plaque fluid fluoride concentrations.

Displacement of bound calcium from matrix or bacterial cell surface15 could also result in precipitation of calcium fluoride or calcium phosphate fluoride complexes further reducing penetration15).

While it is possible that low pH had affected biofilm architecture, this seems unlikely since the proportions of biomass present in either 1-week or 3-week plaque biofilms at pH 3 or 7 were not significantly different. This is also in agreement with previous studies, which suggested that low pH had little effect on biomass density16).

There were differences between plaque biofilms formed over 1 week compared with those formed over 3 weeks. When fluoride distributions in 1-week and 3-week plaques were compared, 3-week plaques contained significantly less fluoride than the 1-week plaque. This was apparent at both pH 7 and pH 3.

The reasons may lie in as yet unknown chemical differences between 1-and 3-week plaques. While differences in structure were not detected, however, a previous study did suggest that 4-week plaques were rather more compact, while the basic architecture remained the same17). Whether it is associated with time is unclear. It is thought possible that plaque turnover may be partly the cause. A shift in this direction at 3 weeks therefore could contribute to a reduced diffusion of fluoride.

In conclusion, we found that low pH affected the short-term uptake from a 1,000 ppm fluoride solution, and also found that more plaque formation period provided less fluoride concentration at both pH 7 and pH 3, suggesting that renewal of the outer plaque layers, i.e. plaque turnover reduced fluoride concentrations within plaque. If plaque turnover fairly rapidly happens then fluoride concentrations of plaque or any other therapeutics would decrease to some extent, although few reports have been made about this. Thus, future experiment regarding plaque turnover will be needed because it may play an important role in terms of plaque structure and chemistry.

References

1) Agus, H.M., Schamschula, R.G., Barmes, D.G. and Bunzel, M.: Association between the total fluoride


