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Time dependence of enamel fluoride acquisition from APF gels. I. In vitro study

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Abstract

The objectives of this study were to investigate the effect of application times on fluoride (F) uptake by sound enamel exposed in vitro to APF gels. Two groups of 12 teeth each were treated with one of the following 1.23% APF gels: Minute-Gel™ (gel A) and Nupro® (gel B). The F treatment times were 1, 2, and 4 min and the F concentrations and enamel biopsy depths were determined using an acid-etch biopsy technique.

There was a significant increase ($P < 0.001$) in F concentrations at the depths of 5 and 10 μm following 1, 2, or 4-minute treatments with both gels as compared with their controls. For both tested gels, application time of 4 min significantly increased the F concentrations at all enamel depths as compared with the 1-minute treatment ($P < 0.02$ and $P < 0.001$). The net acquired F in the 15 μm-thick enamel exposed 4 min to gels A and B were 2.8- and 2.4-fold, respectively, more than the 1-minute treatment. There were no significant differences in F concentrations of enamel treated with gels A and B. The results indicate that enamel F uptake is a diffusion-controlled process which is time dependent.

Professionally applied topical fluoride is an important part of the entire program for preventing dental caries (Brown and Konig 1977; Clarkson and Wei 1982). One measure of the effectiveness of topical fluoride (F) therapy is the ability of the agent to deliver and incorporate F into enamel. There are many variables in the topical agents as well as in the therapeutic procedure that may affect the acquisition of F by enamel including the F concentration, pH, temperature, type of agent (gel, solution, varnish, etc.), application time, and frequency of use. Although the effect of most of these parameters on enamel F uptake are well documented (Brudevold et al. 1967; Mellberg and Loertscher 1972; Joyston-Bechal et al. 1973; Retief et al. 1980; Mellberg and Ripa 1983), there is little documentation in the literature on the optimal length of application time to provide a satisfactory F uptake by enamel.

The 40th edition of the *Accepted Dental Therapeutics* (1984) listed 23 acidulated phosphate-fluoride (APF) gels accepted by the American Dental Association. Most of these gels contain 1.23% F^-, from sodium fluoride or hydrogen fluoride, and 1% phosphoric acid in an aqueous medium containing carboxymethyl cellulose as the gelling base. Concerning the mode of application, the Accepted Dental Therapeutics recommends that the APF gel should be applied to the patient's dental arches by means of a tray for 4 min.

A new topical F gel, Minute-Gel™, was introduced by Oral-B Laboratories, Inc. (Redwood City, CA). It was claimed that "Minute-Gel delivers 12 times the minimum fluoride uptake in just 1 minute." The minimum F uptake mentioned in the advertisement (Oral-B Lab 1985) was based on the hypothesis that a concentration of 1000 ppm in the enamel surface is needed to confer protection against dental caries (Muhlemann 1963). The reported F uptake following 1- and 4-minute applications of Minute-Gel were 12,000 and 15,500 ppm, respectively (1985). The implication of the 1-minute application is that dentists can save 3 min of valuable clinical chairside time per F treatment. However, the full data documenting this claim is not published and it is difficult to assess its validity. In view of the great clinical importance to dentists to use the "optimal" treatment time for topically applied F gel, the present study was carried out to clarify this point.

This study aims to test the hypothesis that Minute-Gel produces as much enamel F uptake in 1 min as a conventional APF gel in 4 min. The F uptake by enamel as a function of application time also was evaluated.

Materials and Methods

Preparation of Teeth

Human third molars extracted due to impaction were collected from individuals residing in Hong Kong, which is a fluoridated community with 0.7 ppm F in its water. Teeth with no visible cracks, hypoplastic areas, or

caries on the buccal and lingual surfaces, when examined under a stereomicroscope at 20x magnification, were stored in deionized water containing thymol at 4° C. At the time of use, the teeth were lightly polished with a rotating rubber cup and an aqueous slurry of pumice followed by thorough washing in tap water. Two 4-mm round adhesive discs were placed on the buccal and lingual surfaces of the crown of each molar giving 4 delineated areas for each tooth. The teeth then were entirely covered with nail varnish and dried with air.

Experimental Design and Treatment Procedure

Two groups of 12 teeth each were treated with one of two gels: gel A (Minute-Gel, an APF gel containing 1.23% F from sodium fluoride and hydrogen fluoride at pH 3.5, batch no. XHDT); and gel B (Nupro® — Johnson and Johnson Dental Products Co; East Windsor, NJ, an APF gel containing 1.23% F from sodium fluoride and hydrogen fluoride in 0.1 M phosphoric acid at pH 3.0-3.5, batch no. 5M5823). For each tooth, one of the areas was assigned as a control (F–untreated) while the other areas were designated for F gel treatment of 1, 2, or 4 min. In this way each tooth served as its own control. The disc covering the control area was removed leaving behind a varnish-free enamel surface "window" of 12.6 mm^2, for acid-etch enamel biopsy. After the first biopsy on the control window, it was covered with nail varnish. Thereafter the second disc was removed and the F gel was applied to the exposed enamel surface as a layer of gel approximately 2 mm thick, using a cotton bud loaded with the gel. The gel was allowed to stay on the enamel surface for 1 min at room temperature in a relative humidity of 100%. Immediately after F treatment, the enamel surface was washed for 30 sec under running tap water and for 1 min in deionized water. The same treatment procedure was carried out for the third and fourth windows of each tooth except that the treatment time of the F gel on enamel was 2 min for window 3 and 4 min for window 4.

Enamel Biopsy and Chemical Analysis

Acid-etch enamel biopsies were carried out using F-free cotton pellets. The pellet was saturated with 0.1 ml of 0.5 M $HClO_4$ and then held in forceps for 15, 30, and 30 sec consecutively. Immediately after each etching the solution was buffered by directly pipetting onto the enamel surface 0.4 ml of 0.5 M citrate buffer followed by 0.5 ml of deionized water. The residual solution left on the tooth surface was aspirated with a microsampling pipette and small pieces of filter paper and transferred to the original sample solution.

The F concentration in the solution containing the sampled enamel was determined using combination F-ion electrodes (Orion model 960900 — Orion, MA) coupled to a digital ionalyzer (Orion model 901 — Orion, MA). A calibration curve was constructed from sodium fluoride standards containing 0.05, 0.1, 0.5, 1.0, and 5 ppm F, prepared in citrate buffer.

The phosphate concentrations in the samples were determined by a double-beam spectrophotometer (Shimadzu model UV-150-02; Tokyo, Japan) using the one-step malachite green method (Hattab and Linden 1984).

The mass of enamel in each sample was calculated by assuming that human enamel contains 17.5% phosphorus. The thickness of the biopsied enamel layers was estimated from the following formula:

$$\text{Layer thickness in } \mu m = \frac{\text{mass of enamel } (\mu g)}{\text{biopsy area } (mm^2) \times \text{density of enamel}}$$

The density of enamel is assumed to be 2.95.

Because the thicknesses of the biopsied layers are not totally controllable variables and because there is a steep F gradient in the outermost enamel, the mean F concentrations were adjusted to standardized depths of 5, 10, and 15 µm from enamel F profile curves.

An analysis of variance was used to evaluate the differences in enamel F concentrations following the application of each gel at 1-, 2-, and 4-minute application times compared to their respective controls. Comparisons also were made between the two gels on their effect to increase the F uptake in enamel following different application times (1, 2, and 4 min).

Results

The mean concentration of F found at standardized depths of untreated enamel (control) and F-treated enamel with gel A and gel B are shown in Table 1 (next page). The data are presented in Figure 1 (next page). There was a highly significant increase ($P < 0.001$) in the F concentrations at the depths of 5 and 10 µm following 1-, 2-, or 4-minute treatment with both gels as compared with their controls. Similar significant increase ($P < 0.001$) also was found at 15 µm depth of enamel treated with gel B. At the depth of 15 µm the differences between 1- and 2-minute treatment with gel B and the control group was significant to a level of $P < 0.01$. Only 1-minute treatment with gel A did not result in significant F uptake. The effect of application times on enamel F concentrations showed that 4 min of treatment with gel A and gel B significantly increased the F concentrations as compared with the 1-minute treatment. The level of significant differences in all depths ranged between $P < 0.02$ and $P < 0.001$. There were no significant differences in enamel F concentrations between gel A and gel B.

The acquired F or fluoride uptake, i.e., treatment

TABLE **Fluoride Concentrations in the Three Enamel Depths of the Control (F-untreated) and Experimental (APF-gel Treatment) Enamel Surfaces After 1, 2, or 4 Min Exposure to Topical F Agent**

Topical Agent and Application Time	*F Concentration (ppm) at Standardized Depths (Mean ± SD)*			*Acquired F (ppm) in the outer 15-μm-Thick Enamel*
	5 μm	*10 μm*	*15 μm*	
Gel A				
Control	1799 ± 254	1345 ± 190	1135 ± 171	–
1 min	3193 817	1867 432	1137 274	1918
2 min	3569 807	2100 488	1550 406	2940
4 min	5162 2415	2686 1056	1842 767	5411
Gel B				
Control	1567 382	1136 273	945 243	–
1 min	3422 696	1848 344	1298 267	2920
2 min	3945 1196	2249 850	1649 676	4195
4 min	5920 ± 1139	2935 ± 890	2025 ± 683	6932

minus the control, from each application time of gel A and gel B also are presented in Table 1. After 1-minute treatment with gel A the cumulative F acquired (ppm) in the 15-μm thick enamel was 44.8%. The corresponding values for 2- and 4-minute treatments were 68.7 and 126.4%, respectively. On the other hand, the cumulative F acquired in the 15-μm-thick enamel treated for 1, 2, and 4 min with gel B were 80.8, 115.0, and 190.0%, respectively. When the percentage values of acquired F at each depth were plotted against the square root of application times, a linear relationship was obtained (Fig 2). For both gels, the coefficient of correlation (r) between the F uptake and the application was strongly correlated, ranging between 0.94 and 0.99 with an average (±SD) of 0.98 ± 0.02. Extrapolation of F concentration to a depth of 30 μm showed that 2- and 4-minute applications of gel A increased F concentrations to 7.7 and 13.1%, respectively, as compared with the control values. The corresponding increases in the F concentration produced by gel B were 37.8 and 54.4%, respectively.

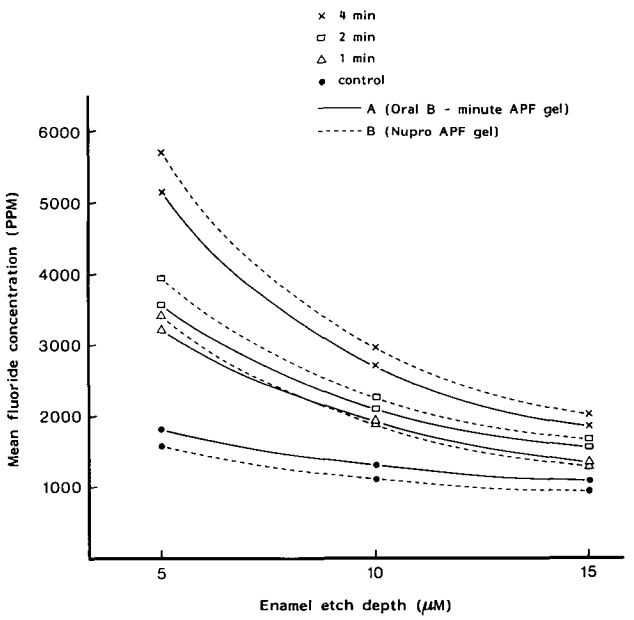

FIG 1. Concentration of F in sound human enamel treated for 1, 2, or 4 min with APF gels and in untreated enamel (control).

Discussion

The present finding showed that the average F concentrations at the outer 5 and 10 μm of untreated enamel (control) were 1683 ± 313 ppm and 1241 ± 332 ppm (mean ± SD), respectively. Extrapolation of the data to the outer 2.0 μm of untreated enamel gave an F concentration of 2514 ppm. These findings are in agreement with those reported in optimally fluoridated communities of 1 ppm F (Keene et al. 1980; Mellberg, et al. 1970; Mellberg 1977; Mellberg and Ripa 1983). Reports from nonfluoridated communities (0.2 ppm F) showed enamel F concentrations at depths of 5 and 15 μm were 893 and 557 ppm, respectively (Hattab and Frostell 1980). In the light of the present findings (Table 1) it appears that an increase of about 790 and 680 ppm F at enamel depths of 5 and 15 μm were obtained in teeth formed in an area with 0.7 ppm F in the drinking water.

The two topical F gels demonstrated, in general, similar abilities to incorporate F into sound enamel (Table 1, Fig 1). Regardless of the gels used, there was a significant increase in the enamel F concentrations at all depths from the 4-minute application compared with the 1-minute treatment. A significant increase in F concentration also was found between 4- and 2-minute applications at the first 5 μm depth. Although the F uptake by enamel treated for 2 min was consistently higher than 1-minute values, the differences did not reach the significant level of $P < 0.05$.

The results showed that the actual thicknesses of the biopsied enamel layers, i.e., prior to standardizing at uniform depths, for the F-treated groups did not significantly differ from the control groups, suggesting that F pretreatment does not affect the dissolution of enamel in 0.5 M $HClO_4$ (Arends and Schuthof 1975; Hattab and Wei 1987).

Several studies were designed to predict the effectiveness of topical F agents through monitoring the ability of the agent to deliver F (Nelson and Farng 1972; Congleton et al. 1978; Hattab and Linden 1985). In a study on the diffusion of F from APF topical gels, using

a continuous flow dialysis, Congleton et al. (1978) found that thixotropic gels released only 15% as much F as did conventional APF gels. These findings were not substantiated by an in vitro F uptake study in sound enamel (Wefel and Wei 1979) or in a clinical trial (Cobb et al. 1980). This might be partly explained on the basis that the diffusion process was carried out under the effect of pressure and stirring forces. Using a diaphragm cell to monitor the diffusion of F from APF gel, Hattab and Linden (1985) found that the diffused F after 4 min of

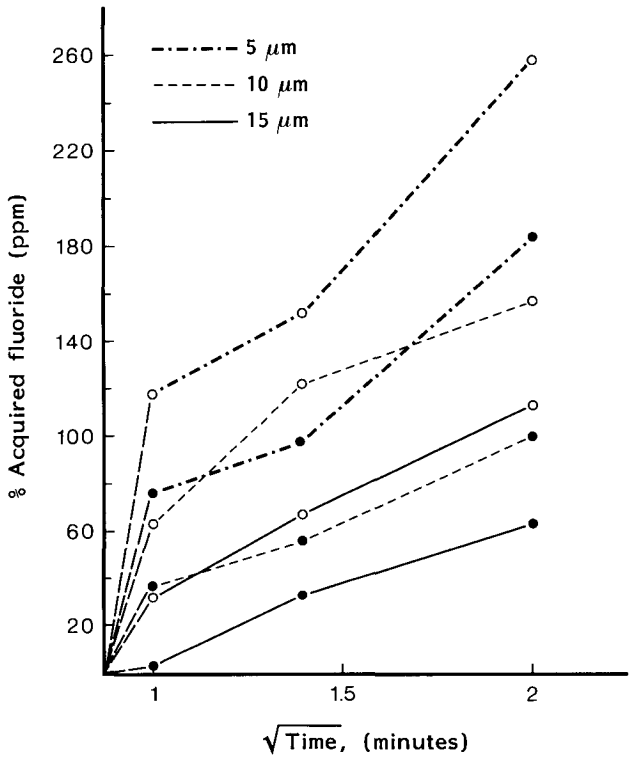

FIG 2. Percentage of the acquired F in enamel as a variable for application times.
(% acquired F in ppm = $\frac{\text{treatment - control x 100}}{\text{control}}$)

dialysis, without stirring or pressure forces, was 2.2-fold more than after 1 min of dialysis. Interestingly, the acquired F after 4-minute treatment with gel A and gel B (Table 1) were 2.8- and 2.4-fold more than the 1-minute treatment. In a recent study on F uptake by polished (abraded) enamel surfaces from different topical F agents, Hattab and Wei (1987) found a strong correlation (r = 0.98) between F released by dialysis and F uptake in enamel, after relating the data to the F content in the applied agent. The present findings suggest that the uptake of F by sound enamel from agents containing the same F concentration and having very similar physicochemical properties is time dependent.

The relationship between the percentage acquired F [(treatment - control) x 100/control] and application times is illustrated in Figure 2. The strong correlation (r = 0.98) between the two variables indicated that the rate of F penetration in sound enamel from APF gels is diffusion controlled, i.e., the depth of penetration of F is time dependent. Figure 2 also shows that the pattern of F uptake in the outer 5 μm enamel departs from linearity as demonstrated for inner enamel depths. This probably could be due to the vigorous reaction of F at the outer enamel surface and the deposition of CaF_2 as the main reaction product. The dissolution of the CaF_2 layer during the first enamel biopsy may account for the discrepancy in the pattern of F uptake between the outer and inner enamel layers. In a study on the uptake of ^{18}F by human enamel from APF solutions, Joyston-Bechal et al. (1973) showed that the uptake of F was very rapid during the first 4 min, and that the uptake after 4 min was about 1.2-fold more than after 1 min. Wefel and Wei (1979) reported that 4-minute treatment with APF gels increased the F uptake at enamel depths of 1.9-3.6 μm to an average of 1.4-fold more than a 1-minute treatment.

The present findings indicate a total net increase of F uptake, from both gels, after 4 min to be 2.6-fold more than a 1-minute treatment (Table 1 and Fig 2). The higher F uptake (as a function of time) in this study as opposed to previous studies is due to the differences in the experimental design, type of topical F agents (gels vs. solution) and techniques in measuring the F uptake (Joyston-Bechal et al. 1973) or due to the effect of different washing procedures following the topical F application (Wefel and Wei 1979). The outcome of this study confirms the earlier report (Duckworth and Braden 1967) indicating that the uptake of F by enamel is a diffusion process accompanied by simultaneous chemical reaction.

Conclusion

The claim that Minute-Gel produced as much F uptake after 1 min as some brands after 4 min is misleading. The amount of F uptake by dental enamel from APF gel preparations appears to be a diffusion process and is strongly time dependent. Significantly more enamel F uptake was produced after 4-minute treatment with APF gels than after 1 min.

A linear relationship between the percentage acquired F and the square root of application times was established. Dentists should continue to use 4 min rather than 1 min as suggested by the manufacturer of Minute-Gel.

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Dr. Wei is a professor and head, and at the time of the study Dr. Hattab was senior research assistant, children's dentistry and orthodontics, University of Hong Kong. Presently, Dr. Hattab is affiliated with the Dept. of Pedodontics, Jordan University of Science and Technology, Irbid, Jordan. Reprint requests should be sent to: Dr. Stephen H.Y. Wei, Dept. of Children's Dentistry and Orthodontics, Faculty of Dentistry, University of Hong Kong, The Prince Philip Dental Hospital, 34 Hospital Rd., Hong Kong.

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