SCIENTIFIC ARTICLE

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Comparative uptake of fluoride ion into enamel from various topical fluorides *in vitro*

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Abstract

Background: There are many forms of topical fluoride available today, making the decision as to which is most effective to manage the immediate caries risk problem at hand, very difficult. The objective of this project was to determine the concentration and pattern of fluoride ion uptake into enamel from a variety of categories of topical fluoride recently available in Australia.

Methods: Extracted, intact molar teeth were sectioned to provide six plates of smooth surface enamel. Windows of enamel 2 x 6mm were exposed to a variety of topical fluorides for periods simulating those used *in vivo*. Following drying, the slates of enamel were exposed to 2ml of 0.1M HCl as a chemical biopsy agent for incremental periods of time. The concentrations of fluoride ion in the biopsy solutions for both test and background (control) slates of enamel were determined directly using a fluoride combination selective electrode in conjunction with a high impedance pH meter. Cumulative amounts of fluoride were determined for each topical fluoride agent.

Results: The concentrations of fluoride ion taken up into enamel were generally proportional to those present in each agent. However, those from APF gel greatly exceeded the amounts taken up from NaF gel. Also, the concentrations taken up from some of the highly concentrated metal fluorides were surprisingly low. Prior etching of enamel increased uptake and prolonged application of APF gel provided no extra benefit.

Conclusions: Some topical fluorides, e.g., APF gel, provided a greatly increased uptake and to a greater depth than other self-application products. However, the frequency of its use should be considered with caution where patients have glass-based restorations.

Key words: Fluoride, biopsy, acidulation, etching, uptake.

Abbreviations and acronyms: APF gel = acidulated phosphate fluoride gel; DDW = deionized distilled water.

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INTRODUCTION

A wide variety of topical fluorides is now available to dental professionals for use in clinical practice and for prescribed use by patients at home. Their differing formulas and concentrations make them suitable for therapeutic management of a number of dental problems including prophylactic prevention of dental caries, dentinal sensitivity, control of all caries from incipient to rampant, and reductions in the rate of dental erosion.[1-3]

For the dental professional to be able to choose the formula and concentration of the topical agent which is most effective in managing a specific dental problem for a particular patient, it is necessary to be aware of a number of properties of the agent selected. These include retention rates intra-orally, reactivity of the fluoride with tooth structure, depth and concentration taken up by the tooth, safety to the patient, potential for staining of tooth structure and ease of use. Zero *et al.*,[4] Heath *et al.*[5] and Aasenden *et al.*,[6] have investigated retention rates of a variety of topical fluorides intra-orally, some having described likely patterns of ingestion of fluoride ion following use of some concentrated fluoride gels. Ekstrand[7] thoroughly investigated the safety aspects of ingestion of topical fluorides by children.

It is proposed in this study to investigate the comparative fluoride uptake into enamel from a variety of topical fluorides, using the acid biopsy system refined by Tyler and Comer.[8] The topical fluorides investigated included a selection representing those commonly in use in Australia during the last decade, and some which have been proposed as potential additional sources of fluoride ion.

MATERIALS AND METHODS

Materials

A representative selection of the most commonly used topical fluoride products was initially tested. These represented products most likely to be prescribed for self-application within the last decade. Two experimental forms of fluoride were also tested. All agents tested are listed in Table 1.

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Table 1. Topical fluoride self-application and experimental products tested

Product name	Chemical agent	Concentration % or ppm	Manufacturer at time of use
A. Commercially available products			
Colgate Thixofluor APF Gel	Acidulated phosphate fluoride pH=3.5	1.23% 12 300ppm	Colgate Oral Care, Sydney, Australia
Johnson & Johnson Neupro-Neutral Fluoride Gel	NaF pH neutral	0.9% 9000ppm	Dentsply International Inc, USA
Oral B Fluorinse fluoride mouthrinse	NaF PH neutral	0.2% 1000ppm	Oral B Laboratories Pty Ltd, Sydney, Australia
Colgate Total toothpaste	NaF neutral	0.22% 1000ppm	Colgate Oral Care, Sydney, Australia
Stannous Fluoride concentrated solution	SnF_2 stabilized	48 500ppm	Creighton Pharmaceuticals, Sydney, Australia
Silver Fluoride solution	AgF, stabilized in ammonia	60 000ppm	Creighton Pharmaceuticals, Sydney, Australia
B. Experimental solutions			
Titanium tetrafluoride	TiF_4	30 400ppm	Aldrich Chemical Company Inc., Milwaukee, Wisconsin, USA
Ammonium bifluoride	NH_4F_2	38 000ppm	Obtained from MRC Laboratories, Bristol, England

Confirmation of the fluoride concentration in selected products

Diluted samples of four of the products were subjected to a Taves[9] separation of the fluoride ion by acid hydrolysis, to allow subsequent determination of the fluoride ion concentration using an Orion Combination Fluoride electrode (Orion, USA, model EA940). The materials tested were the first four products listed above. In all cases, the fluoride concentrations were as stated in the product information.

Selection and preparation of tooth enamel samples

Approval for the collection of unidentifiable, sound, extracted human molar teeth was gained from the Human Ethics Committee at The University of Adelaide. Teeth were examined for defects and those found with such were discarded. Pairs of enamel slates were cut from the same tooth surfaces to act as test and control for fluoride ion uptake (three pairs from each tooth). Each slate was covered with nail varnish, except for a window 4 x 2mm in size. A dowel stick was then bonded to each slate with sticky wax to facilitate its handling. The samples were stored in deionized distilled water (DDW) prior to testing.

Development and validation of the acid biopsy system

Choice of methods for estimating fluoride content of enamel

Of the variety of lower cost chemical biopsy methods available, the one developed initially by Wetherell and Hargreaves[10] and later refined by Tyler and Comer[8] was chosen for this investigation. This method has the advantage of allowing direct measurement of fluoride concentration, does not require very complex equipment, and uses hydrochloric acid (HCl) as the etchant, compared with the more dangerous perchloric acid. Tyler and Comer[8] found that, if a high impedance pH meter was used, direct measurements of fluoride generated millivolt readings could be made at low pH.

General outline of the biopsy system

Following exposure of test slates to the various topical fluorides, chemical biopsies of fluoride concentration in enamel were taken using 0.1M HCl, which dissolved enamel at six incremental depths over a 20 minute period. The test biopsy solutions and those from the control (background) samples were analysed directly using an Orion Combination Fluoride Specific Electrode (Model EA940), attached to a high impedance pH/volt meter (Orion, USA, model 109), needed for measurement of mv readings at pH 1, as described by Tyler and Comer.[8]

Validation of the biopsy system

A number of aspects of the biopsy system needed detailed analysis prior to the study, to ensure validity of the results. These are described below.

(i) Verification of similar background fluoride content of enamel slates from the same surface of one tooth

This was carried out as the background fluoride levels, even within different locations on a single tooth, had been found to differ slightly.[11] Three pairs of enamel slates were cut from one tooth. Each window was biopsied as described and the background fluoride levels found to show no greater than 35 per cent differences in concentration of fluoride ion for samples from the same surface of one tooth. As biopsy concentrations of fluoride ion were reasonably high in most cases, these differences were considered able to provide a reliable result, provided three sets of samples, each of test and control, were used for each category of fluoride treatment.

(ii) Incremental depths of enamel biopsy using 0.1M HCl

Five molar teeth were prepared as described above. All six enamel slates each from five teeth were subjected to exposure to 0.1M HCl for the following sequential time periods (1, 2, 2, 5, 5, 5 minutes). Following each incremental period of exposure to acid, a section was

Table 2. Cumulative depth of etch of enamel against time

Minutes of etch	Cumulative depth of etch (μ)	Cumulative volume of enamel (mm^3)
1	0-surface etch only	(not known)
3	12.0±3	0.07
5	25.0±4	0.15
10	36.6±7	0.22
15	45.6±8	0.27
20	54.0±7	0.32

made through the end of each window and the cumulative mean depths of etch were recorded using a stereoscopic Wild-Leitz (Germany) microscope with an eyepiece graticule. The cut surface was then recoated with protective nail varnish, and the slate exposed to acid for the next increment of etch. This process was repeated for the six different etch times, and the progressive mean incremental depths of etch determined (Table 2).

It was decided also to test whether the application of high concentrations of fluoride altered the incremental etch depth. Even APF gel made no difference to the rate of etch, as might be expected at pH 1.

(iii) Calibration of fluoride electrode mV readings at pH 1-6

Fluoride standards at concentration 0.005, 0.01, 0.1, 1.0, 10.0, 100.0ppm were prepared each at pH 1-6. The mv readings generated for each concentration of fluoride ion at pH 1-6 are recorded in Fig 1 against the fluoride ion concentration, using a semi-log scale. The classical Nernstian relationships between log (concentrations of fluoride ion) and mv readings (i.e., a reduction of approximately 59mV for each log increase in fluoride concentration), were confirmed to exist. The necessity to ensure that the pH of all samples remained unchanged during testing was clearly demonstrated, and random monitoring of sample pH was maintained to ensure this happened.

(iv) Methodology for collecting biopsy samples

Several hundred vials of 10ml volume had 2ml of 0.1M HCl solution added. These were arranged in groups of 6 x 6, as shown in Fig 2, where they were marked to indicate tooth surface and sample number, and the cumulative etch time for that row. The sticks of doweling holding the enamel slates from one tooth

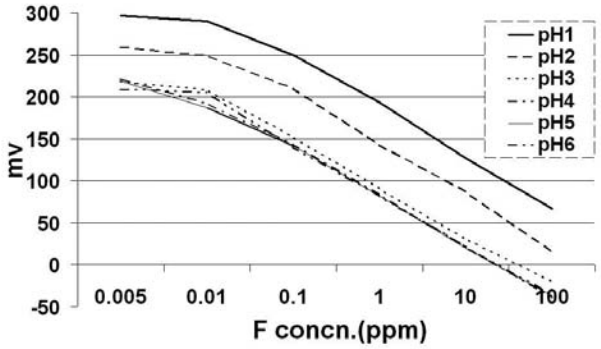

Fig 1. Graph of mv readings against fluoride ion concn, at pH 1-6.

were impaled in polystyrene so as to enable the slates to be immersed in one row of the biopsy solutions at the same time, again as shown in Fig 2.

Application of topical fluoride

The intention was to simulate the method of intra-oral application as closely as possible. Hence each enamel surface, which had been stored in DDW, was kept lightly moist before application of the topical fluoride agent.

The APF and NaF gels were applied to the test enamel windows in excess for four minutes, as recommended by the manufacturers, then removed with copious DDW for 10 seconds and dried with air. The control windows were washed only with DDW and air-dried.

Toothpaste was applied in excess, left for five minutes and then washed for 10 seconds and the surface dried. The five minutes allowed for the maximum time a person might spend toothbrushing.

The test slates were immersed in 'Fluorinse' mouthrinse for one minute, and solutions of SnF_2, AgF_2, TiF_4 and NH_4F_2 for five minutes before the surfaces were washed for 10 seconds and dried. Most of these materials would not be followed by immediate rinsing in practise, the times of application representing those during which the original concentration of fluoride ion might remain in contact with the tooth surface (as shown by Heath *et al.*[5]).

The enamel slates were then immersed in the first row of etch solutions for one minute, removed and

Table 3. Cumulative fluoride recovery profiles for the various topical fluoride agents at incremental biopsy times and depths (ppm or μg/g ± SD, mean of three samples)

	1 minute (0 μ)	3 minute (12μ)	5 minute (25μ)	10 minute (37μ)	15 minute (46μ)	20 minute (54μ)
APF gel 12 300ppmF	1.26±0.48	1.77±0.49	2.12±0.61	2.73±0.84	3.15±0.88	3.47±0.96*
NaF Gel 9000ppmF	0.5±0.14	1.33±0.34	1.46±0.23	1.51±0.36	1.52±0.39	1.52±0.24*
F mouthrinse 1000ppmF	0.08±0.03	0.1±0.04	0.13±0.04	0.16±0.04	0.19±0.06	0.21±0.06*
CTotal t/paste (NaF) 1000ppmF	0.01±0.0	0.02±0.0	0.022±0.0	0.025±0.0	0.03±0.01	0.05±0.01*
SnF_2 20% soln 48 000ppm	0.028±0.01	0.039±0.01	0.06±0.01	0.098±0.028	0.127±0.032	0.15±0.03
AgF 40% soln 60 000ppm	0.1±0.02	0.14±0.03	0.17±0.03	0.2±0.05	0.23±0.06	0.28±0.05
TiF_4 soln 30 400ppm	1.2±0.2	1.5±0.4	2.24±0.72	2.85±0.77	2.9±0.9	3.0±0.2
TiF_4 soln 30 ppm	0	0	0	0	0	0
NH_4F_2 soln 38 000ppm	0.4±0.1	1.1±0.3	1.3±0.3	1.4±0.5	1.5±0.3	1.6±0.3
NH_4F_2 soln 38 ppm	0.2±0.0	0.37±0.08	0.48±0.14	0.62±0.16	0.73±0.28	0.82±0.22

(*Indicates a significant difference between adjacent pairs of agents of p=<0.5).

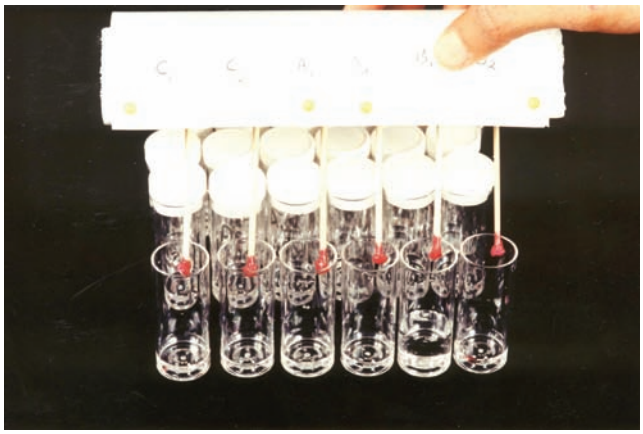

Fig 2. Enamel slates held in sequence to permit bulk collection of biopsy samples.

lightly air dried and immersed in the second row for two minutes. This was repeated for a further two minutes in row three, and for five minutes in each of the next three rows, making cumulative periods of etching at 1, 3, 5, 10, 15 and 20 minutes. All enamel slates and biopsy solutions were stored at 4°C until required for fluoride ion determination.

Fluoride ion estimation

The test biopsy solutions and those from the control (background) samples were analysed directly as described earlier. Standard curves had previously been prepared from the mV readings from the various standard fluoride concentrations at pH 1. Test and background biopsy fluoride ion concentrations were read from the standard curves, and the fluoride concentrations recorded in ppm. As all windows were of equivalent size, and this was a comparative study, it was not considered necessary to determine the concentrations of fluoride ion in mg/unit area. The ppm quantitation was equivalent to an amount of µg/ml of solute from a surface area of $6mm^2$.

To determine the amount of fluoride ion taken up by the test enamel window, background fluoride concentrations were subtracted from those obtained from the test samples at the same etch depths. The results from each depth were added to those from previous depths of etch to provide a cumulative record of fluoride ion taken up from each product. Statistical tests of differences between final cumulative concentrations of net fluoride ion were made using a Student '*t*' test of differences.

Supplementary tests

Two supplementary tests were carried out to provide initial information concerning a variety of questions often asked about the effectiveness of topical fluoride application.

Does increasing exposure time to APF gel result in increased fluoride ion uptake?

In this study, 1.23% APF gel was applied to different slate series for 1, 2, 3, 4, 5, 10, 20 and 40 minutes. Fluoride uptake was estimated following these periods of contact.

Does prior etching of enamel increase fluoride uptake?

Test enamel slates from one tooth were etched with 37% phosphoric acid for 30 seconds and thoroughly washed. Following mild drying, these etched windows and another set which had not been etched were coated with Colgate Total Toothpaste for five minutes and again washed as described previously. Biopsies were then carried out to determine the effect of the acid-etch technique, commonly used in restorative dentistry, on the amount of fluoride uptake into enamel, compared with that occurring in a non-etch situation. The readings were the average from three samples. Statistical significance was estimated using a Student '*t*' test of difference.

RESULTS

Fluoride uptake profiles for the topical fluorides listed, compiled from the mean values at each incremental depth and presented as cumulative data for each incremental period, are presented in Table 2. The concentrations of fluoride ion are in ppm, or µg/ml of biopsy solution, and represent that collected over $6mm^2$ of enamel surface.

Fluoride uptake from APF gel was significantly greater than from NaF gel, even when allowing for the different initial concentrations of fluoride ion. Further more it appeared to have penetrated to a deeper level than that removed by 20 minutes of cumulative etch.

The differences in uptake between gels and rinses/toothpastes appeared to be approximately proportional to initial concentrations of fluoride ion in each product. This was not so for heavy metal salts of stannous and silver fluoride, though uptake from concentrated titanium tetra fluoride matched that from APF gel. Fluoride uptake from ammonium bifluoride was slightly higher than from NaF gel.

Results from supplementary tests are presented in Figs 3 and 4. The uptake profile from APF gel (Fig 3) was most surprising, with a reduction in uptake occurring immediately following four minutes of

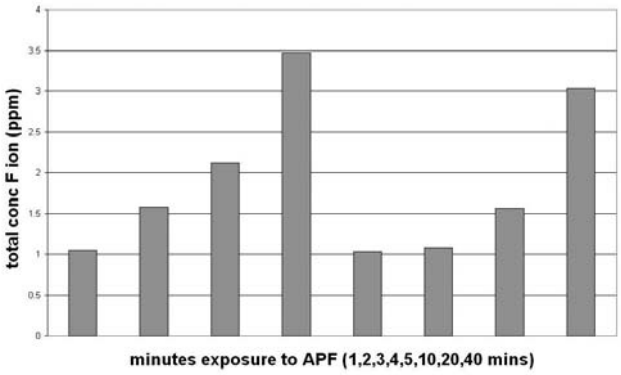

Fig 3. Profile of 20 minute etch readings from 1-40 minutes exposure of enamel to APF Thixoflour gel.

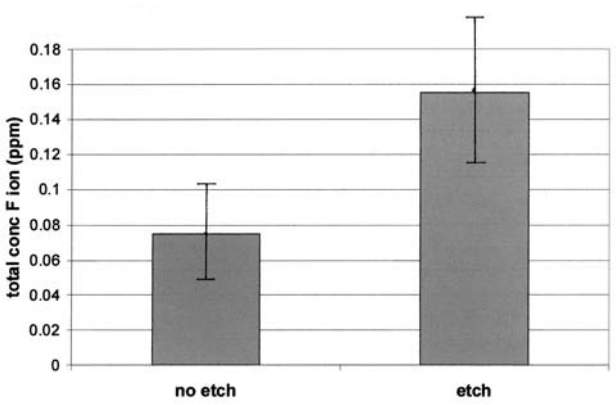

Fig 4. Comparison of 20 minute biopsy concentrations of fluoride ion following 5 min exposure of both non etched and etched enamel (mean of six samples) to Colgate Total toothpaste.

exposure. Following a 37% phosphoric acid etch prior to application of gel, cumulative uptake of fluoride ion increased from 0.08µg/ml to 0.16µg/ml (Fig 4). This difference was statistically significant at the 0.05 level.

DISCUSSION

The results indicate that this method of fluoride extraction and measurement provided relatively consistent and reliable data. The pH of biopsy solutions was closely monitored and found to remain unaltered. The limit of reliability of the fluoride concentration estimations (~0.01ppm) as stated by the electrode manufacturers was not exceeded.

In general, the cumulative values of fluoride ion resulting after 20 minutes of acid biopsy were predictable on the basis of concentration of the topical fluoride agent, except for SnF_2 and AgF. In these cases the cumulative amount taken up by the enamel appeared very low compared with the concentration of fluoride present in the solutions. Alfonso and Gotjamanos[12] also noted that the fluoride ion in AgF did not penetrate deeply into enamel. This was thought to be due to the heavy silver ion forming a dense barrier at the surface and inhibiting deep penetration of the fluoride ion. This may also be so for the tin ion. The metal ions themselves, however, would account for a high level of inhibition of bacterial metabolism, and thus preventive effectiveness.

The high level of uptake of the fluoride ion from acidulated fluoride gel compared with neutral gel at relatively similar concentrations was obtained in a number of repeats of this test. Furthermore, it appeared that the ion had penetrated more deeply than the 54µm depth achieved in 20 minutes of acid biopsy, as the concentrations appeared to be increasing to this point. The cumulating concentrations of fluoride from NaF appeared to have plateaued by 10 minutes of biopsy at 36.6µm. However, even with some low concentration materials, like toothpaste and mouthrinse, there appeared to be a slow initial uptake followed by increasing amounts even at the 45–54µm depth levels.

The high level of fluoride taken up by enamel exposed to APF gel may explain its superior ability in reducing both erosion and caries when compared to NaF gel (Jones *et al.*,[2] Mok *et al.*,[3] McIntyre and Blackmore[13]). However, this difference has not been supported in population studies in which there is no difference in effectiveness between APF and NaF gels in preventing dental caries. It appeared that the difference was only evident when there was a greater acidic challenge, which might occur in only a small proportion of the population. Furthermore, the potential damage caused by APF gel to glass-based restorative materials makes it a less attractive choice in many clinical situations.

The fluoride uptake by enamel exposed to TiF_4 and NH_4F_2 confirms findings by both Clarkson and Wefel,[14] and Tyler and Poole,[15] that these high fluoride content salts may be also useful clinically. The toxicity of TiF_4 appears to be greater than for existing fluoride salts although it could be useful at slightly lower concentrations. Their potential for staining should be lower than the silver and tin salts. This aspect needs further investigation.

Whilst the supplementary tests were of a pilot nature, and need more widespread investigation, the interim results are of clinical significance. The results obtained when Colgate Thixofluor APF gel was in contact with enamel for a wide range of times were puzzling. This test was repeated a number of times to confirm the data, with consistent results. The result supports the recommendation for the four minute application period as achieving the maximum uptake of fluoride ion from the APF gel. However, the reduction in stored fluoride with longer uptake is difficult to understand. It may be that the maximum uptake level is achieved at this point, and establishes a negative equilibrium gradient of fluoride concentration between the tooth structure and its surface within the acidic environment, resulting in a reverse of fluoride ion transport to the surface until a point is reached when the ionic fluoride equilibrium is reversed, resulting in further uptake. The result indicates that there is no advantage in prolonged contact between APF gel and enamel.

No evidence of acid etching of enamel from the prolonged exposure to APF gel prior to biopsy was noted. In previous pilot studies in this laboratory, even three months continual storage of enamel in APF gels resulted in no discernable etch microscopically.[5]

The increased uptake of fluoride from toothpaste when the enamel is first acid-etched prior to resin bonding, confirms that found by De Paula *et al.*[16] in that it resulted in a doubling of the amount of fluoride ion taken up. This does not indicate the need for such etching of teeth prior to fluoride contact, but rather confirms that it may be useful to coat a tooth with a neutral topical fluoride agent following extensive acid-etching for resin placement.

CONCLUSION

The results from this study provide the dental professional with a better understanding of the uptake patterns of fluoride ion into enamel following both professional and home use of a representative group of topical fluoride agents which have been available in Australia over the last decade. They point to the very high uptake particularly from acidulated fluoride gel, though the difficulty of this gel being able to etch glass-based restorative materials limits its potential for routine use. Research is currently underway to explore other non-damaging acidic systems for acidulating fluoride products as acidulation appears to provide such a greater level of protection against strong acidic challenges than with neutral products. Even so, the present form of this acidulated fluoride gel is clearly very useful where strong acidic challenges are causing rapid and extreme demineralization, as in rampant caries and erosion. It is recognized that the level of uptake of fluoride ion in itself is only one factor contributing to the demineralizing inhibiting effect of topical fluoride therapy. However, it is considered a significant one.

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