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Salivary fluoride levels following application of fluoride varnish or fluoride rinse

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Abstract – *Objectives:* This study examined the concentration of fluoride in whole saliva over time following the application of a fluoride varnish or a single rinse with a fluoride solution. *Methods:* A two-period, two-treatment randomized cross-over experimental trial with a 2-week washout period was used with 16 adult subjects. In the first period, eight subjects rinsed once with a 0.05% NaF solution and 8 subjects had 5.0% NaF varnish applied to facial and lingual surfaces of 20 teeth. Stimulated whole saliva was collected at baseline, 5 and 15 min, 1, 2, 4, 8, 12, 24, 32, 48, 56, 72, 80, 96, 104 h. After the washout period each subject was switched to the other treatment and saliva was collected at the same intervals. Salivary fluoride content was measured with the micro-diffusion method. *Results:* The NaF levels peaked at 5 min after application for both varnish (mean ± SE 24.5 ± 5.0 ppm) and rinse (3.2 ± 0.8 ppm). Mean NaF levels returned to baseline, on average, within 2 h for the rinse and within 24 h for the varnish. The maximum fluoride levels were significantly greater ($P < 0.01$) with the varnish than with the rinse and remained above baseline levels for a longer duration. *Conclusions:* Salivary fluoride levels with the rinse returned to baseline, on average, in 2 h while they remained elevated for, on average, 24 h with the varnish. Salivary fluoride levels from the varnish were found to be comparable with those in previous studies for 1.1% neutral NaF.

Key words: dental caries; fluoride; prevention; rinse; saliva; varnish

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Introduction

Fluoride products have played an important role in the reduction of caries in the general population. Despite remarkable progress in reducing caries in the USA, 25% of children in the 5–17-year age range had 80% of the caries and 94% of adults had past or present caries (1, 2). In the USA, fluoride has been extensively used in toothpaste and drinking water. Other fluoride products include rinses and topically applied gels. Fluoride varnishes have been widely used in Europe for about three decades, but have been introduced into the USA relatively recently. Numerous *in vitro* studies on uptake and mechanisms of action, as well as clinical studies on efficacy in children can be found in the literature and are reviewed by Helfenstein and Steiner (3, 4), Petersson (5) and Clark (6). Studies of the efficacy of fluoride varnish as a caries preventive agent in adults are lacking in the literature. Topically delivered fluorides are clinically effective in inhibiting the development of dental caries (7, 8). Low concentrations of ionic fluoride (>0.04 ppm) in the oral cavity are considered to play an important role in the effectiveness of these topical fluoride agents (9–12). After application of a topical fluoride agent, the fluoride levels in whole saliva can be considered indicative of fluoride in the aqueous phase available for interaction with the tooth surface at a given time (13). The concentration of fluoride present in oral fluids following fluoride application is influenced by the

initial concentration of fluoride applied, time since the last exposure (14, 15), the method of delivery, factors which influence fluoride clearance and factors which influence fluoride retention (16).

In a previous study (16) it was determined that 0.05% NaF rinse produced an initial rapid spike in the salivary fluoride level that fell back to baseline after 2–4 h. With repeated use of fluoride rinse and/or fluoride dentifrice, salivary fluoride levels increase from baseline levels as a result of fluoride accumulation in the plaque and oral tissues (17, 18). Daily use of a 0.05% fluoride mouthrinse has been shown to eliminate caries around orthodontic brackets (19), whereas fluoride dentifrice used alone did not; this is most likely a result of elevated fluoride levels in plaque and saliva. Furthermore, in xerostomic subjects, daily use of a fluoride mouthrinse eliminated demineralization despite their high caries challenge (20). *In vitro* studies have shown enhanced remineralization with small elevations of fluoride (0.04 ppm and above) during the remineralization phase of pH-cycling studies that simulated caries, further emphasizing the advantage of daily fluoride elevations in saliva, or maintaining this elevation for days or weeks (21, 22). Fluoride levels in saliva above 0.04 ppm have been shown in clinical caries risk assessment studies to be related to lower risk of caries progression (23, 24). Furthermore, salivary fluoride levels of 0.08 ppm and above were related to very low caries progression in a subsequent longitudinal caries risk assessment study (25).

Aim

The aim of this two-period, two-treatment cross-over study was to compare the fluoride released into saliva over time by a fluoride varnish (5% NaF, Duraflor; Pharmascience, Montreal, Quebec, Canada) to an over-the-counter fluoride rinse (0.05% NaF, ACT; Johnson & Johnson, Skillman, NJ, USA) in an adult population under a controlled environment where variations in sources of fluoride were reduced. The hypothesis that was tested in the present study was that the fluoride varnish produces elevated levels of fluoride in saliva superior to those from a fluoride rinse and that the fluoride concentration remains elevated for several days.

Materials and methods

Sixteen dental students between the ages of 22 and 30 years in good health were included in the study. This sample size was determined based on a two-sided paired *t*-test with 80% power, a standardized effect size of 3/4 (a difference in mean value of 0.75 standard deviation units), and a 0.05 alpha level (type I error). Variance was estimated using area under the curve (AUC) data from Zero et al. (16) and within-subject correlation was estimated from Ekstrand et al. (26). Inclusion criteria for subjects included:

1 Must have at least 20 teeth (with at least one incisor, premolar and molar in each quadrant) and good oral hygiene (as evidenced by the absence of food debris and heavy plaque accumulations on the teeth).
2 A low past caries experience (no more than one new carious lesion in the past 3 years and no carious lesions in the past year).
3 No current dental caries activity (no demineralized enamel or open carious lesions).
4 No faulty dental restorations.
5 No medications that could affect salivary flow.
6 A stimulated salivary flow rate within the normal range: stimulated whole saliva flow rate between 1.0 and 5.0 ml/min.
7 Live and work in communities with fluoridated water supplies (since most of the communities in the area have fluoridated water).

Participants were third and fourth year dental students at UCSF, allowing the investigators greater access for initial baseline evaluations and follow up visits. Approval from the campus Committee for Human Research and informed consent from participants were obtained.

A small pilot study was conducted on 2 subjects (in addition to the 16 participating in the main study) prior to starting the main study in order to determine the length of time that measurable amounts of fluoride released from the varnish could be detected in the saliva. Salivary samples were collected and tested for fluoride content at baseline. After application of fluoride varnish, measurements were taken at 5, 15 min, 1, 2 and 4 h, then once a day until fluoride levels above baseline levels were no longer detected. Measurements from the pilot study indicated that collection of saliva for 5 days was of sufficient duration.

At the start of the study, a baseline salivary fluoride level was established for each subject so that exogenous sources of fluoride from drinking water and toothpaste could be taken into account. Subjects were asked to refrain during the study from using fluoride products (except the fluoride toothpaste they already might be using) such as

brush-on gels, rinses or fluoride-containing floss and to abstain from eating or drinking foods and beverages that are high in fluoride. A list of products that potentially contain fluoride was given to each participant. Subjects chewed a piece of Parafilm for 2 min and collected the pooled whole saliva in test tubes that were capped. Saliva was collected prior to meals or at least 2 h thereafter.

Subjects were randomly assigned initially to one of two sequences of treatment: (1) a fluoride rinse and then a fluoride varnish (RV), or (2) a fluoride varnish and then a fluoride rinse (VR). As a result of the nature of the fluoride treatments, blinding/masking of the subjects and practitioner was not possible. However, the fluoride assays were performed blinded to fluoride treatment. All subjects were instructed not to brush with a fluoride toothpaste, use any fluoride products or consume foods having high fluoride content for at least 2 h prior to the baseline salivary sampling. Subjects all had their teeth professionally cleaned with fluoride-free flour of pumice before application of the varnish or rinse. In the varnish group, a single application of fluoride varnish was applied to the facial and lingual surfaces of each of 20 teeth with a minimum of a molar, premolar and anterior tooth in each quadrant. It is known that saliva flows at different rates in different parts of the mouth (27–29). By ensuring a minimum number of teeth in each quadrant, fluoride would be more evenly distributed throughout the various salivary flow zones. Pre-measured amounts of fluoride varnish were used for each subject and applied by a dentist who was familiar with the product. Moisture from the mouth caused the varnish to set. For cleaning their own teeth after varnish application, subjects were supplied with soft-bristled toothbrushes, given oral hygiene instructions and asked to brush very lightly in the sites where the varnish had been applied so as to slow its removal. In the fluoride rinse group, subjects rinsed for 30 s with 10 ml of a 0.05% NaF mouth rinse (ACT, Johnson and Johnson). They expectorated the excess liquid but did not rinse their mouths with tap water. Subjects were instructed not to eat or drink for at least 2 h after treatment. Salivary samples were obtained from each participant immediately after the fluoride treatment (rinse or varnish), after 5, 15 min, 1, 2, 4, 8, 12, 24, 32, 48, 56, 72, 80, 96 and 104 h. In the case of fluoride rinse, we anticipated that the fluoride level would return to baseline by 4 h, but we continued for the same number of days as the varnish in order to make a direct comparison of one day of rinse versus one varnish application that we expected would elevate salivary fluoride levels for several days. Moreover, this helped maintain blinding of the fluoride assay analyses. After the collection of the baseline saliva samples and the application of the fluoride rinse or varnish, the subjects collected their own saliva at the assigned intervals and returned the tubes to a central location. The time each sample was taken was written on a label on the collection tube.

After completion of the salivary sampling, subjects in each group had the surfaces of their teeth thoroughly cleaned with flour of pumice. A 2-week washout period was observed between the last measurement of the first treatment period and the determination of the new baseline salivary fluoride level for each subject prior to the start of the cross-over. Subjects who initially had varnish were now given rinse (VR group) and the subjects originally receiving the rinse were given the varnish (RV group) using the same methods of application as in the first period. Salivary samples were collected at the same time intervals and tested for fluoride content.

The fluoride content of the saliva was analyzed by the micro-diffusion method (30). This method of analysis diffuses all acid-labile, bio-available fluoride from the saliva into an alkaline trap, thereby eliminating interference from other ions and organic molecules usually present in saliva.

The summary statistic approach to repeated measures in cross-over studies was utilized (31). A trapezoidal rule was used to compute the approximate areas under the fluoride-time curves for each subject. Two-sample *t*-tests and Wilcoxon rank sum nonparametric tests compared the within-subject period differences (for treatment effect) and period sums (for carry-over effect) between the VR and RV sequences (28) for maximum concentration (C_{max}), duration above baseline, and AUC concentrations of fluoride. Similar tests were performed using the following two outcomes:

- difference in fluoride concentration between 104 h and baseline;
- last value observed for fluoride (at 104 h).

The log AUC value was used because of its skewed distribution. No other transformations were required to normalize the data. Duration above baseline (equivalent to time value returned to baseline) was determined using baseline plus twice the standard error of the mean of all the baseline values.

The period effect was assessed by comparing the varnish and rinse differences between the two sequences. Although a carry-over effect (residual effect of first period treatment beyond the washout period) of the varnish or rinse was not expected after a 2-week washout period, carry-over and treatment × period interaction were assessed using baseline measures from each period (31).

Results

During fluoride assaying blinded to treatment sequence, notations were made describing potentially problematic values such as potential exogenous fluoride use, assaying problems or protocol incompatibility. For example, 1 person had <2.0 ml of saliva at 104 h in period 1 and 1 person was obviously exposed to an exogenous fluoride source (e.g. fluoride dentifrice) close to saliva sampling four times (12 and 48 h in period 1 and 24 and 48 h in period 2). Such values were removed for analyses. In the 4 cases in which the 104-h sample was not analysable, the previous sample value (96 h) was used. One participant did not provide a baseline sample in period 1 due to his lack of understanding of the instructions, but did provide a new baseline sample after the treatment crossover; his second baseline value was used to calculate his first period AUC and duration above baseline. A total of 37 (7%) of the 512 assays had some potential problem; four (4%) were during the first three time points (≤1 h) of either period (i.e. of 96 assays) so compliance problems increased when subjects were at home. Data analyses were performed with and without these values in the spirit of intention-to-treat and protocol compatible analyses, respectively. As nearly identical results were found, only the intention-to-treat results for AUC, C_{max}, final value and difference from baseline are presented here.

Baseline fluoride values had a mean ± SE of 0.022 ± 0.003 ppm at the first period and 0.021 ± 0.003 ppm at the second period; thus, the individual baseline +0.006 ppm was the value utilized to determine duration above (time to return to) baseline for each person. Five minutes after application, salivary fluoride was at mean peak level (±SE) for the varnish (24.5 ± 5.0 ppm) and the rinse (3.2 ± 0.8 ppm); all but 2 of the 16 participants had peak fluoride at five min in each period; the other 2 participants had peak fluoride at 15 min during the varnish period. Average (median and mean) salivary fluoride levels for the rinse returned to baseline +0.006 ppm by 2 h and for the varnish by 24 h. All individuals returned to baseline +0.006 ppm by 12 h (all but 2 by 4 h) following rinse and all except 1 individual returned to baseline by 32 h (all but three by 24 h) following varnish; the other 1 did not return to baseline until 72 h, but had fallen to baseline +0.008 ppm by 32 h. Figure 1 shows the mean fluoride levels for each treatment over the study period. Within-person AUC was significantly greater for fluoride varnish than fluoride rinse ($P < 0.01$), showing that the fluoride values were significantly elevated during the varnish period (Table 1). No carry-over effect was evident ($P = 0.41$), but a period effect was seen ($P < 0.01$) as second period values were slightly higher for both sequences (Fig. 2). Within-person C_{max} was significantly greater ($P < 0.01$) for the varnish than the rinse indicating that the peak fluoride concentration was higher during the varnish period

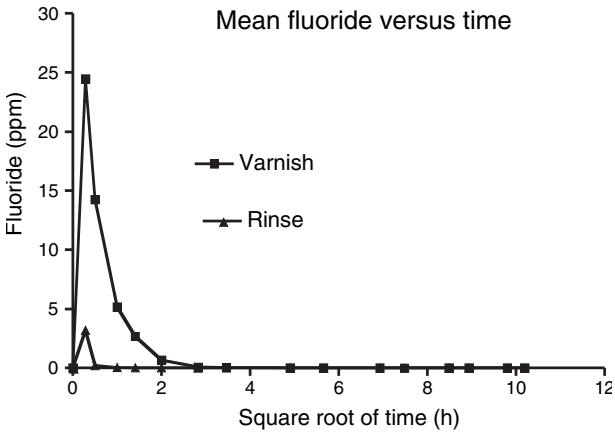

Fig. 1. The mean salivary fluoride levels in parts per million (ppm) are depicted over the course of the study. The square root of time is used to more graphically demonstrate the differences between the varnish and the rinse.

Table 1. Differences in means of log area under the fluoride concentration curve (AUC)

Group	*n*	Period	Mean (ppm)	SD
RV	8	First (R)	2.06	0.89
	8	Second (V)	6.91	1.34
Period difference		(2-1)	4.85	
Treatment difference		(V-R)	4.85	
VR	8	First (V)	5.64	3.22
	8	Second (R)	3.21	1.08
Period difference		(2-1)	−2.43	
Treatment difference		(V-R)	2.43	

Effects: treatment, $P < 0.01$; period, $P < 0.01$; carry-over, $P = 0.41$.

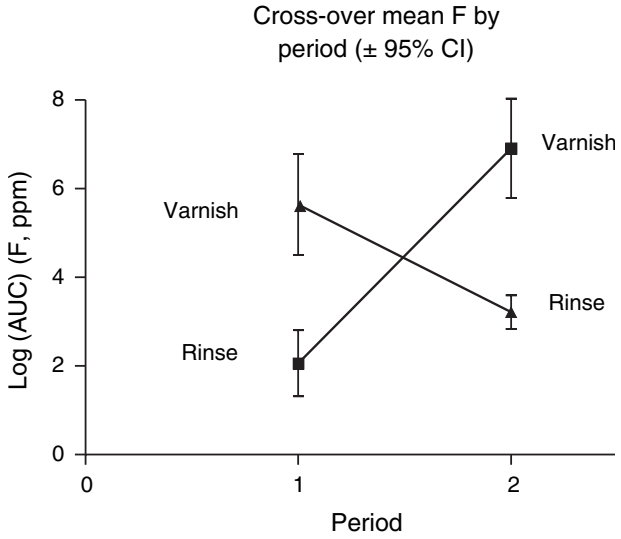

Fig. 2. The effect of switching treatment (cross-over) from varnish to rinse or rinse to varnish can be seen graphically. Mean logarithm of area under the salivary fluoride (F) level concentration curve in parts per million (ppm) dropped when subjects switched from varnish to rinse and increased when they switched from rinse to varnish.

Table 2. Differences in peak fluoride concentrations means for C_{max}

Group	*n*	Period	Mean (ppm)	SD
RV	8	First (R)	0.423	0.315
	8	Second (V)	1.304	0.395
Period difference		(2-1)	0.881	
Treatment difference		(V-R)	0.881	
VR	8	First (V)	1.182	0.512
	8	Second (R)	0.599	0.336
Period difference		(2-1)	−0.583	
Treatment difference		(V-R)	0.583	

Effects: treatment, $P < 0.01$; period, $P = 0.37$; carry-over, $P = 0.76$.

(Table 2). No carryover effects ($P = 0.76$) were observed when subjects switched from rinse to varnish or varnish to rinse.

There was no difference in carryover effects between the two sequences for outcomes (log AUC: $P = 0.41$; C_{max}: $P = 0.76$; difference: $P = 0.75$; and last value: $P = 0.41$). This is not the same test as a zero carry-over effect, but it does examine the robustness of the treatment and period effect results.

There was a significant treatment effect when considering the C_{max} ($P < 0.01$) (Table 2) and the last value ($P = 0.04$). There was no treatment effect when considering the difference in fluoride concentration between baseline and 104 h ($P = 0.17$) (Table 3). Mean values for salivary fluoride levels

Table 3. Differences in salivary fluoride levels between baseline and end of study (104 h); means for difference in fluoride

Group	*n*	Period	Mean (ppm)	SD
RV	7	First (R)	−0.008	0.012
	8	Second (V)	−0.002	0.014
Period difference		(2-1)	0.006	
Treatment difference			0.006	
VR	8	First (V)	−0.005	0.009
	8	Second (R)	−0.004	0.009
Period difference		(2-1)	0.001	
Treatment difference			−0.001	

Effects: treatment, $P = 0.17$; period, $P = 0.08$; carry-over, $P = 0.75$.

Table 4. Salivary fluoride levels at end of study period (104 h); means for last observed fluoride

Group	*n*	Period	Mean (ppm)	SD
RV	7	First (R)	0.012	0.007
	8	Second (V)	0.025	0.020
Period difference		(2-1)	0.013	
Treatment difference		(V-R)	0.013	
VR	8	First (V)	0.017	0.008
	8	Second (R)	0.012	0.004
Period difference		(2-1)	−0.005	
Treatment difference		V-R	0.005	

Effects: treatment, $P = 0.04$; period, $P = 0.36$; carry-over, $P = 0.41$.

at the end of the study are found in Table 4. There was no significant period effect for the outcomes except for AUC.

Discussion

Fluoride has its greatest effect as a topically applied agent. Topical fluoride applications of gels and solutions have been shown in clinical trials to greatly reduce dental caries. However, much of the fluoride was lost in the first 24 h as it leached away. It was found that a longer exposure time to the enamel increased the efficiency of the topical fluoride and produced fluorapatite that is more acid resistant than hydroxyapatite with its naturally occurring carbonate inclusions. Fluoride varnish is a toxicologically safe (26) way of exposing the enamel to fluoride for longer periods of time than gels or solutions, and it results in a deeper penetration of the fluoride into the enamel surface. Clinical trials have shown a significant reduction in dental caries with the use of fluoride varnish. A study by Arends and Schuthof (32) showed that a 24-h exposure of enamel to fluoride after applica-

tion of fluoride varnishes was sufficient to inhibit demineralization completely as determined by microradiography and micro-hardness tests. Our study found that the salivary fluoride levels returned to baseline levels within 24 h, on average, following varnish, presumably offering a similar inhibitory effect on demineralization. A limitation of this study is that we had only one measurement of the baseline salivary fluoride levels, and hence no estimate of normal variation within subjects could be made.

In addition to the direct effect on surfaces to which it has been applied, there is likely an overall effect on the teeth as the fluoride diffuses out of the varnish and circulates around the mouth in the saliva. *In vitro* studies suggest that low levels of fluoride in the saliva can inhibit development of dental caries and promote remineralization (9, 11, 33). After application of a topical fluoride agent, the fluoride level present in saliva is indicative of the fluoride available for interaction with the surfaces of the teeth (13). Our study showed a significant increase in the salivary fluoride levels following application of a fluoride varnish and use of a fluoride rinse. The varnish, however, produced greater levels of fluoride in the saliva for a longer period of time. The fluoride rinse produced elevated levels of salivary fluoride that returned to baseline levels on average in 2 h while it took 24 h, on average, for the salivary fluoride levels with the varnish to return to baseline levels. During this 24-h period, there was adequate time for a significant uptake of fluoride in dental plaque and in demineralized tooth structure. The concentration of fluoride released into saliva from the varnish in the present study was very similar to the levels and duration released by a 5000 ppm F neutral NaF (1.1% NaF) gel applied in trays in the study by Zero et al. (18). Furthermore, the concentration and duration from the 0.05% NaF mouthrinse used in the present study were very close to those reported by Zero and others for the same treatment. It is, therefore, reasonable to speculate that the varnish treatment releases fluoride in a comparable fashion to the clinically effective high concentration topical fluoride tray treatment.

Our study found that the salivary fluoride reached peak levels within 15 min for all subjects with the varnish treatment. This compares favorably with the findings of Twetman et al. (34) that showed significantly elevated fluoride in saliva within an hour after application of fluoride varnish. They found the elevated salivary fluoride to last for 6 h. Our study showed most subjects returned to baseline fluoride levels within 24 h. As a result of the nature of this study, subjects could not be blinded as to the treatments they received. However, randomization of treatment order or sequence tends to balance measured and unmeasured factors that would influence salivary fluoride levels. Subjects received each treatment at approximately the same time of day to offset factors related to variations in salivary flow rates during the course of a day.

A recent *in vitro* study by Castillo et al. (35) showed that Duraflor released fluoride for 19 weeks after application to primary molar enamel slabs stored in buffered calcium phosphate solution. Our study showed that the level of salivary fluoride returned to baseline levels, on average, within 24 h. This large disparity between the *in vitro* and the *in vivo* studies is likely because of the factors present in the oral cavity that were missing in the laboratory. Factors that would tend to leach fluoride from the varnish include the flow of saliva and dietary acid challenges. Other factors present in our *in vivo* study that would tend to diminish the volume of fluoride varnish present on the teeth include tooth brushing and flossing and abrasion by foods and by movements of tongue, lip and buccal mucosa over the varnish. Subjects were advised to brush gently and were given soft-bristle toothbrushes (Oral B 35: Oral B Laboratories, Boston MA, USA) and were asked to avoid hard or sticky foods to help reduce these effects. Still the discrepancy between *in vitro* and *in vivo* studies even in this controlled study setting indicates such factors can be substantial.

Conclusions

In our study, the fluoride released into saliva by the varnish applied to coronal surfaces of teeth was compared with fluoride found in saliva from a fluoride rinse over comparable time periods using a cross-over design. The maximum (peak) concentration levels of fluoride (C_{max}), the duration of salivary fluoride elevated above baseline, and the AUC were measured for both sources of fluoride. The varnish produced higher levels of fluoride in the saliva and for a longer period of time than the rinse. Salivary fluoride levels with the rinse returned to baseline, on average, in 2 h while they remained elevated for, on average, 24 h with the varnish. Salivary fluoride levels for

the varnish in our study have been compared with levels reported in the literature that have been found to be effective against caries development and for remineralization. Salivary fluoride levels from the varnish were found to be comparable with those in previous studies for 1.1% neutral NaF.

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