Fluoride release from varnishes in two in vitro protocols

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ABSTRACT

Background. The authors conducted a study to evaluate fluoride released from fluoride varnishes that had been applied with two different protocols. Fluoride release information for these two approaches may allow clinicians to vary application intervals to better meet the needs of their patients.

Methods. The authors painted enamel slabs from exfoliated primary molar teeth either in a single application (five samples) or three times within a single week (five samples) with fluoride varnish (Duraphat, Colgate-Palmolive, New York). The samples were immersed in buffered calcium phosphate solution (pH 6) to simulate the oral environment; the amount of fluoride released was measured during a span of six months.

Results. The total release of fluoride was significantly higher in the three-application regimen (34.9 micromoles) than in the single application (23.7 µmol). The rate of release was slower using the three-application regimen. Thus, applying fluoride-release varnish three times in a single week produced greater and longer release of fluoride than did one application.

Clinical Implications. Massed application of fluoride varnish during a single period during the year may be as effective as spaced single applications. This method can be a good alternative to delivering fluoride varnish to high-caries-risk patients who are mobile or difficult to recall.

As a practical matter, it is difficult for some groups of patients to achieve two or more preventive dental visits per year. Thus, it is of interest to know whether the same benefits derived from biannual topical fluoride varnish applications might be achieved using a more intensive "massed" application annually. Such an approach would have particular advantages for dentists and programs serving children from low-income families. While there is ample clinical evidence that biannual application of fluoride varnish decreases dental caries in the primary teeth,[1-5] there are only limited data available suggesting that the massed approach may be efficacious.[6-9]

Massed application of fluoride varnish during a single period during the year may be as effective as spaced single applications.

Previously, we conducted an in vitro study to evaluate the fluoride released from two fluoride varnishes.[10] We conducted the current study, using the same laboratory test system, to determine the amount of fluoride released from fluoride varnishes, but this time we used two different clinical protocols: a single application of fluoride varnish versus three applications of fluoride varnish within a single week.

MATERIALS AND METHODS

Using random assignment, we painted 5 × 5-millimeter primary molar tooth slabs obtained from exfoliated teeth collected in communities with nonfluoridated water in Lima, Peru, with 30 milligrams (35.7 micromoles fluoride) of fluoride varnish (Duraphat, Colgate-Palmolive, New York). Parents gave permission to their children's dentists for collection of the teeth. The research was approved under Federal Title 45, Code of Federal Regulations, Part 46.101, and was exempt from university-level institutional review board review because the investigators had no contact with the children.

In one week, we painted five samples once and five samples three times. Four additional samples remained unpainted so that we could use them as a control. We applied fluoride varnish at baseline and at two and four days after baseline

in the group that was painted three times. All the samples were immersed in a buffered calcium phosphate solution after the baseline application. We painted the samples randomly with varnish obtained from different tubes.

The varnish contained 5 percent by weight sodium fluoride (2.26 percent fluoride) in a neutral colophonium base; it also contained alcohol, shellac, rosin, copal and flavoring. We used Duraphat because it seems to have a good uniformity and low variability in the release mode.[10] We prepared calcium phosphate buffer by mixing 1.5 millimolars of calcium nitrate, 1.0 mmol/L of sodium phosphate monobasic and 0.35 mmol/L of the buffer 2-(N-morpholino) ethanesulfonic acid to achieve a pH 6 solution. We then immersed the samples in this solution at room temperature and placed them on a laboratory shaker table to simulate mouth conditions. Weekly, we prepared a new tube with 20 milliliters of the solution and transferred the sample. We measured the concentration of fluoride from the solution to determine the amount of fluoride released by the sample in each period up to 21 weeks.[11] After 21 weeks, fungal growth made determination of fluoride levels problematic.

Fluoride analysis. We calibrated a fluoride electrode (Orion Fluoride Electrode 9609 BN, Thermo Electron, Beverly, Mass.) with total ionic strength adjusting buffer, or TISAB, solution and fluoride standards. We made two fluoride calibration curves: one for low levels of fluoride using low-level TISAB and another for high levels of fluoride using TISAB III. On semilogarithmic paper, we plotted the concentration (log axis) of the standards against the millivoltage (linear axis). We prepared a new calibration curve with fresh standards each day that we made measurements. We performed a fluoride analysis to determine the concentration of fluoride in the solution, which is the fluoride released by the tooth sample/fluoride varnish sample. We read and converted the millivoltage into micromoles per liter using the calibration curves constructed previously.

Quality control samples (25 µmol/L fluoride) were used to determine the degree of variability of the electrode. At the end of all the experiments, the quality control samples were compared. The average (± standard deviation) fluoride concentration was 23.8 ± 2.0 µmol/L, with a coefficient of variation of 8.15 percent for all measurements, indicating that the results of the study were not influenced by malfunctioning of the electrode.

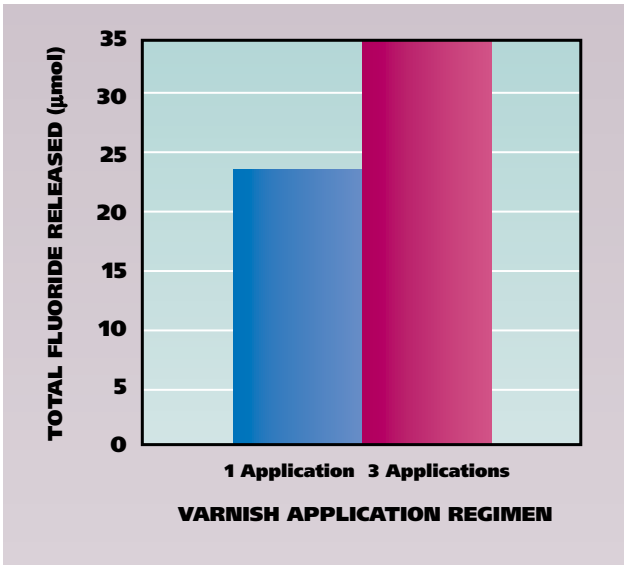

Figure 1. Average cumulative fluoride release from a single application and three applications of fluoride varnish over 21 weeks. The difference is statistically significant (*P* = .003). µmol: Micromoles.

RESULTS

Total release of fluoride. Figure 1 illustrates the release of fluoride from both modes of application during the 21 weeks. The average total amount of fluoride released from the samples was 23.7 ± 1.6 µmol (64.9 percent) for a single application and 34.9 ± 0.3 µmol (31.9 percent) for the three-application regimen. The *t* tests for samples with unequal variances were used to examine differences between the two experimental groups ($P = .003$). Less than 1 µmol of fluoride was detected in the unpainted samples.

The fluoride release in the three-application samples decreased more slowly than that in the single-application samples.

Fluoride release by week. Figure 2 shows the release of fluoride by week. Fluoride release was the highest in both protocols at week one: 5.73 ± 1.47 µmol for the single application and 9.56 ± 1.70 µmol for the three-application regimen ($P < .003$). The release of fluoride started decreasing from week two to the end of the experiments for both protocols. From week two to week seven, we did not find any significant difference between the protocols. From week eight to week 21, the samples that received the three-

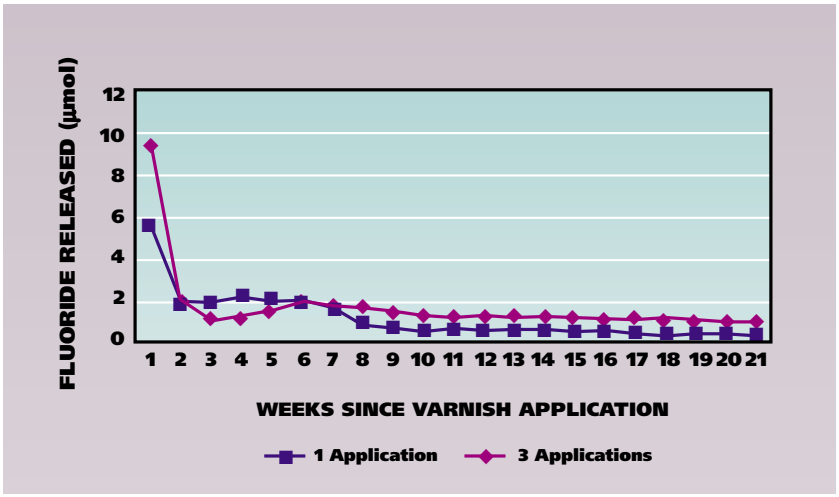

Figure 2. Fluoride release by varnish applied according to the two protocols and by the control during the 21-week study period. µmol: Micromoles.

application regimen released more fluoride than did the samples painted only once, and the difference was statistically significant ($P < .05$).

Rate of release of fluoride. We determined the rate of release (slope) of fluoride after the application for each one of the samples during the 21 weeks. We found that single-application samples had an average slope of –0.058 ± 0.014 and the three-application samples had an average slope of –0.026 ± 0.005 (t test, $P < .005$). Analyzing the second period of the experiment (weeks 11 through 21), we found that the release for both modes of application was at a steadier rate; the fluoride release in the three-application samples decreased more slowly than that in the single-application samples ($P = .027$).

DISCUSSION

Maintaining low levels of fluoride release over long periods is important in the inhibition of demineralization and promotion of remineralization.[12,13] The three-applications-in-one-week protocol resulted in a higher, and probably longer, release of fluoride. The average absolute level of fluoride released in that regimen was consistently higher and the amount remaining to be released after 21 weeks also was markedly higher.

This in vitro system mimics the conditions of the mouth using human primary teeth and solutions with pH similar to that of saliva. Consistent with clinical findings, we found that the release of fluoride from varnish applied in both regimens was extended for a long period, about six months. There are, of course, differences between the experimental model and clinical practice. In working with small children, it is difficult to achieve the degree of moisture control as that achieved in these experiments, and the amount of varnish adhering to the teeth may vary. In addition, oral functions such as swallowing, chewing and salivation, and mechanical functions such as brushing and flossing are not present in an in vitro model. Thus, in vivo, there might be greater variation between people and the release of fluoride might be faster. The shaker table mimics some of the continuous mechanical forces on the tooth.

Our analysis of the behavior of both protocols over time yielded some interesting findings. Apart from the higher overall fluoride release from the three-application regimen, the behavior and the rate of release of both protocols also were different. The rate of release (how fast the fluoride was released), measured by means of the slope, was slower in the three-application regimen than in the single-application approach.

Thus, about one-third of the fluoride applied was released within the first 21 weeks in the three-application regimen, while two-thirds of the fluoride applied was released in that same period in the single-application approach. That means a greater percentage of fluoride still may be available to be released for the remainder of the year if the three-application approach is used. These findings may be clinically useful. The three-application regimen provides higher amounts of fluoride, slower rates of release and availability of fluoride for a longer period. Because caries prevention requires certain concentrations of fluoride for longer periods, this may explain why the three-application protocol has demonstrated better caries-reduction rates than has the one-application protocol.

CONCLUSION

The results of this experiment and our previous in vivo study suggest that fluoride varnish applied three times in one week every year may be a good alternative to spaced single treatments. This protocol can be very useful in areas in which access to dental care is limited or populations are mobile. ■

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