The effect of tooth cleaning procedures on fluoride uptake in enamel

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Abstract

Premolars were cleaned in different ways prior to application of a topical fluoride gel. The teeth were extracted one week later and the fluorine concentrations in the surface enamel were determined by proton activation analysis. The facial and distal surfaces were analyzed. The results showed that a toothbrush and floss cleaning resulted in higher fluorine concentration than did a rubber cup prophylaxis using either a fluoridated or nonfluoridated prophylaxis paste.

The results of numerous clinical trials have shown that the topical application of acidulated phosphate fluoride (APF) solutions or gels on a regular basis is effective in reducing the occurrence of dental caries in test populations, especially in areas that lack optimum fluoride concentrations in the water supply.¹

While evidence for this caries preventive effect is abundant, the exact mechanism by which it occurs remains unclear. When the fluoride is applied at relatively infrequent intervals (three to six months), resistance to dental caries appears to be associated, at least in part, with the amount of fluoride that is deposited in surface enamel as a result of the treatment.²⁴ On the basis of these findings, it seems desirable to use topical application methods that result in the greatest possible fluoride uptake and retention by the surface enamel.

Various treatment regimens have been described for the topical application of APF agents.⁵ A dental prophylaxis using an abrasive paste prior to the topical treatment is a common procedure. In the absence of extrinsic tooth stains, the rationale for use of an abrasive paste has been questioned.⁶ The addition of sodium fluoride, stannous fluoride or APF solutions to these pastes has not been shown to be an effective vehicle for topical fluoride.⁷ effect of various toothcleaning procedures on fluoride uptake by enamel of human permanent teeth.

Methods and Materials

The procedures were completed on 24 informed and consenting patients who were scheduled to have four premolar teeth removed as part of their orthodontic treatment. The premolars were fully erupted and in contact on the distal surface with the adjacent tooth. A history of the subjects' exposure to fluoridated water and topical fluorides was taken.

Three treatment procedures and one control procedure was assigned to the four test teeth. Because of possible differences in chronology of crown development and fluoride exposure between maxillary and mandibular teeth, the upper and lower teeth of a patient were considered as separate blocks. Thus, there were a total of 48 blocks. Because there were only two teeth per block and four treatments, a balanced incomplete block design was used. Treatments were assigned to teeth at random subject to the constraints of this design. Any patients having calculus or stain present on the four test teeth were excluded from the study. The following procedures were assigned and completed on one of the four teeth:

Treatment I: One tooth was thoroughly cleaned with a soft-bristled toothbrush using no dentifrice or prophylaxis paste. Interproximal surfaces were cleaned with unwaxed dental floss.

Treatment II: A prophylaxis was completed on one test tooth with a rubber cup in a slow-speed handpiece using a commercially available prophylaxis paste containing fluoride in the form of APF and a fine-grit pumice and silicon dioxide abrasive.^a Usual clinical pressures were used during the prophylaxis procedure. The tooth was then rinsed and the interproximal surfaces were cleaned upro Lanar Corporation Fast Windsor NL

ne the * Nupro, Janar Corporation, East Windsor, NJ.

The purpose of this project was to examine the

with unwaxed dental floss. The duration of prophylaxis on each tooth was approximately 10 seconds.

Treatment III: A similar dental prophylaxis procedure was completed on another test tooth using a rubber cup in a slow-speed handpiece with the same commercially available prophylaxis paste but without fluoride. The tooth was then rinsed and the interproximal surfaces were cleaned with unwaxed dental floss.

Control: No toothcleaning procedure or topical fluoride treatment was completed on the control tooth. The tooth was covered with orthodontic wax (including the interproximal areas) which was kept in place for 30 minutes following the fluoride treatment to minimize contact with residual APF gel.

The treatment procedures were followed by a fourminute topical fluoride application with an APF gel containing 1.23% fluoride and 0.1M orthophosphoric acid at a pH of 4.5^b in disposable polystyrene trays^c. In the arch in which the wax-covered control tooth was located, the tray was divided in half and that part of the tray that would cover the tooth was discarded. This was to further prevent the fluoride gel from contacting the enamel surface of the control tooth. A sufficient amount of gel was placed in the trays to cover the test teeth, and suction was provided by a saliva ejector to eliminate unnecessary ingestion of excess gel. The patient was instructed to not eat or drink for 30 minutes following the fluoride treatment, at which time the wax on the control tooth was removed.

All toothcleaning and topical fluoride procedures were completed seven days prior to the extraction of the four teeth, which were always removed at a single appointment. No attempt was made to alter the patients' fluoride exposure, diet, or oral hygiene measures during the time between the fluoride application and the extractions.

The four test teeth were extracted using standard oral surgical techniques with the exception that a gauze pad was placed between the tooth surface and the beaks of the forceps. Contact between the forcep beaks and the tooth surface was made as close to the cementoenamel junction as possible. This was to avoid any mechanical abrasion of the enamel surface which was to be analyzed. After extraction, the teeth were stored in separate bottles containing gauze lightly moistened with 10% formalin until preparation for fluorine analysis.

The method used for the analysis of elemental fluorine in the sample teeth utilized the application of the nuclear reaction 19 F (p, $\alpha\gamma$) ¹⁶⁰. This method,

^b Luride, Hoyt Laboratories, Needham, MA.

which has been referred to as charged particle or proton activation analysis, is a physical method for determining fluorine levels at various depths from the outer surface. The technique has the advantages of being nondestructive to the sample and producing good depth resolution.⁸⁻¹⁰

After removing the root with a carbide bur in a high-speed handpiece, each tooth crown was bonded to a lead/antimony cube with a low-pressure adhesive and placed in a target chamber under vacuum. The cube could be rotated 90 degrees, allowing the proton beam to strike the facial and distal surfaces for analysis. All teeth were coated with a thin layer of gold to prevent artifacts due to charging.

A 2 Mev Van de Graff accelerator was used to produce the proton beam. The beam was collimated to 1 x 1 1/2 mm² at the tooth surface with a current of 80-120 nanoamperes. The beam was centered on a point midway between the cusp tip and the cementoenamel junction on the facial surface, and just below the contact point on the distal surface. A Ge(Li) detector was used to measure the gamma radiation being produced. The duration of gamma-ray detection was a function of the total proton beam charge which was 8 x 10⁻⁶ Coulombs.

The resonance reaction occuring at 872 keV was used since it is the first strong reaction with a high sensitivity.⁸ Resonance energies below 872 keV were considered as background radiation and corrections were made as the gamma-ray yield at these energies was low.

The concentration of fluorine was determined by the yield of gamma radiation occurring at beam energies of 900, 1000, 1100, 1200, and 1300 keV. The increasing energies were necessary to achieve the 872 keV resonance reaction at successively greater depths from the surface. At the energies above 900, secondary resonances occur and corrections were made according to the method described by Kregar et al,⁹ using a hydroxyapatite pellet of known fluorine concentration as a standard.

The specific depth at which the resonance reaction for fluorine occurred was a function of the beam energy and the stopping power (density) of the sample, in this case, hydroxyapatite.¹⁰ The corresponding 872 keV resonance depths for the selected beam energies were as follows:

> 900 keV — .47 microns 1000 keV — 2.34 microns 1100 keV — 4.30 microns 1200 keV — 6.36 microns 1300 keV — 8.48 microns

The estimated error of these depth measurements was $\pm 10\%$.

Statistical analysis of the data was by a general linear model type of analysis.¹¹ This was completed

^c Centrays, Cooper Laboratories, Fairfield, NJ.

on both the distal and facial surfaces at the five depths. Thus, there was a total of 10 dependent variables to be analyzed.

The first analysis was to determine if the two blocks, maxillary and mandibular teeth, could be reduced to one block and so reduce the model to a total of 24 blocks instead of 48 blocks.

Second, the data was tested for any overall differences between the treatments with an $\alpha = .05$. If it was determined that an overall difference was present, the six individual treatment differences were examined. Because there were multiple comparisons on the same set of data, a result was considered significant at a p-value of $\leq .01$.

Results

The 24 subjects, 14 females and 10 males, who participated in this study were residents of North Carolina. All of the subjects were in good health and had no history of a serious medical disorder. The dental histories were significant only for minor restorative care and the presence of malocclusions. There was no evidence of hypoplasia, calcification problems, or other defects of enamel formation.

The subjects ranged in age from 10 to 16 years. The mean age was 12.0 years. Fifteen of the subjects lived in a community with an optimum fluoride concentration in their drinking water. Professionally applied topical fluoride treatments, other than those provided during this project, had been received by 23 of the subjects within the past 12 months. The time interval since the last topical treatment ranged from 1 to 24 months. The mean interval was 8.8 months.

The mean values for fluorine concentration at each depth are listed in Table 1. It should be emphasized that the standard deviations shown for these values



Figure 1. Mean fluorine concentration profile in surface enamel (facial surface).

are a reflection of the individual differences among the 24 teeth in each treatment group and not the error in the method of analysis. The mean fluorine concentration profiles for these values are illustrated in Figures 1 and 2.

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	Distal Surface	Fluorine Concentration (Standard Deviation of Mean)			
	Depth in Microns	Control	Treatment I	Treatment II	Treatment III
	.47	2666 (±150)	2947 (±208)	2696 (±148)	2320 (±137)
	2.34	1795 (±100)	1959 (±182)	$1696 (\pm 107)$	1577 (± 95)
	4.30	1410 (± 80)	1559 (±162)	1328 (± 79)	1190 (± 82)
	6.36	1252 (± 73)	1358 (±132)	1173 (± 75)	1038 (± 69)
	8.48	1003 (± 52)	1125 (±102)	943 (± 52)	870 (± 54)
Table 1 Mean enamel fluoring					
concentration at selected depths.	Facial Surface	Fluorine Concentration (Standard Deviation of Mean)			
	Depth in Microns	Control	Treatment I	Treatment II	Treatment III
	.47	2500 (±163)	3106 (±236)	2780 (±230)	$2622 (\pm 228)$
	2.34	1652 (±124)	1922 (±191)	1828 (±177)	1787 (±178)
	4.30	1254 (± 94)	1501 (±148)	1428 (±139)	1389 (±132)
	6.36	1161 (± 89)	1307 (±119)	1222 (±107)	1228 (±120)
	8.48	946 (± 67)	1095 (± 93)	1076 (± 94)	1087 (±128)
	Control — no cleaning, no fluoride treatment.				
	Tr. I-toothbrush and floss cleaning + fluoride treatment.				
	Tr. II-fluoride paste prophylaxis + fluoride treatment.				
	Tr. 111-nonfluoride paste prophylaxis + fluoride treatment.				



Figure 2. Mean fluorine concentration profile in surface enamel (distal surface).

For both the facial and distal surfaces, the highest fluorine concentration was always found at the 0.47 micron depth, regardless of the treatment. The amount of fluorine present in the enamel decreased as a function of increasing depth from the surface. The lowest concentrations were found at the 8.48 micron level.

Concerning only the facial surfaces, ranking of the treatment effects at each of the five measured depths showed a consistent pattern. Treatment I exhibited the highest fluorine levels followed by Treatment II and Treatment III. The control teeth had the lowest mean fluorine concentration at all depths.

On the distal surfaces, while Treatment I again showed the highest mean fluorine concentration at each depth, the ranking of Treatments II, III, and the control teeth differed from the results of the facial surfaces. At the 0.47 micron level, Treatment II ranked slightly higher than the control teeth, but at all successive depths the fluorine concentration of the controls exceeded Treatment II or III. Treatment III consistently showed the lowest fluorine levels at all depths.

In the statistical analysis of the data the mean flourine values were not utilized for comparison. Only the differences found within each subject were analyzed. With regard to the maxillary and mandibular blocks within each patient, it was determined that the model for the distal surface could be reduced to 24 blocks because there was no systematic difference between the maxillary and mandibular arches. There was a difference between the arches on the facial surface, so the 48 block design was retained.

The overall differences were significant at $\alpha = .05$ for the distal surface at all depths but not on the facial surface at any depth.

Due to the presence of an overall difference found for the distal surface, the six individual treatment differences were compared. As previously stated a statistically significant result was considered with a p-value of $\leq .01$ since these were multiple comparisons on the same set of data. The differences between Treatment I and Treatment III at all depths on the distal surface were found to be statistically significant by this criterion.

Discussion

Interpretation of the results from this study includes some assumptions concerning the action of fluoride on tooth enamel. First, the incorporation of the fluoride ion into enamel decreases the caries susceptibility of the tooth surface. Studies involving various methods of fluoride or fluorine determination have been reported using the concentration in enamel as an indicator of the effectiveness of the treatment.^{6,11,12}

Second, increases in fluorine content of enamel appear to be especially important in evaluating topical treatments professionally applied on a semiannual basis. While more frequent application regimens with preparations containing both high or low fluoride concentrations have resulted in caries reductions in clinical trials, the reasons for such cariostatic effects are not necessarily the same as those associated with twice annual applications.¹³ The high frequency application methods are directed towards the effects of labile fluoride on reducing bacterial plaque and increasing the remineralization of decalcified areas.¹³⁻¹⁵While fluorapatite may also be involved in these mechanisms, in the absence of frequent fluoride applications, enhanced caries resistance from semiannual treatments seems to depend on the amount of fluoride more permanently bound in the enamel.¹⁶ The results of animal studies and human clinical trials have shown a residual cariostatic effect from two to five years following various topical treatments.¹⁷⁻²⁰ The interval of seven days between the topical treatment procedures and the extractions in this study enabled us to measure the more stable fluorine component.

Third, it is assumed that the relative concentra-

tions of elemental flourine in the enamel are a reflection of fluoride uptake and retention as a result of the treatments.

It should be mentioned that differences in the fluorine concentrations due to the treatments are the important factors to consider. The absolute fluorine levels are not as useful. Epidemiologic studies have been unable to conclusively determine a specific enamel fluorine concentration threshold, above which, significant caries resistance is attained.²¹⁻²³

The mean fluoride concentration profiles found in this project compare favorably with the results of other studies utilizing charged particle analysis for the fluorine determination.^{12,24} The fluorine gradient showed the highest concentration near the surface (0.47 microns) and this level dropped sharply within the first 8.48 microns. These profiles were also similar to those reported by Petersson and coworkers²⁵ in their ion probe studies of surface enamel. Jones and coworkers,²⁶ using charged particle analysis, found the fluorine concentration to actually increase within the first micron before dropping to lower levels. It should be noted, though, that all of the measurements made by Jones were at depths of less than 1.5 microns in increments of 0.10 microns from the surface.

Absolute fluorine concentrations at all levels were also similar to previous studies utilizing similar treatments.^{12,24} Fluorine concentrations of approximately 2,000 to 3,000 ppm were found at levels near 0.50 microns with values of approximately 1,000 ppm found at a depth of 8.5 μ .

When analyzed at specific depths, the individual treatments and the control did not show statistically significant differences in fluorine concentrations, except for the difference between Treatment I and III on the distal surface. This result was not unexpected because of the experimental design and the limitation on the number of direct comparisons between treatments which could be made within the same patient. Even though 24 patients is a relatively large sample for a clinical study of this nature, the numbers are still small from the standpoint of statistical evaluation.

The inability to reduce the facial surface values to 24 blocks (combine the upper and lower arches) was a severe limitation with respect to those data. However, anticipation of the dissimilarity between the arches in assignment of the treatments and controls avoided a possible design error.

Reduction of the distal surface data to 24 blocks permitted the significance of the overall treatment difference to emerge. The statistically significant difference between Treatment I and Treatment III was then identified.

There was the possibility that the control teeth experienced fluoride uptake from residual fluoride in

the saliva after 30 minutes post-treatment, or from treated teeth via the saliva at longer post-treatment times. This same effect may also have reduced differences between the treatments.

Nevertheless, the consistent rank order of the mean control and treatment values at each depth on the respective surfaces is rather convincing evidence that the differences are real (see Figures 1 and 2). When the control and treatments on the facial surface were the same as those used by Tinanoff et al.,⁶ they occurred in the same rank order, even though the analytical methods were different. Data from the two studies are in general agreement.

The methods used in this study allowed fluoride assay of the enamel in an interproximal area. This location is important since caries prevention in these susceptible areas is a goal of any topical fluoride treatment. The fact that the mean fluoride concentration on the distal surface of control teeth was usually higher than those treated with either of the prophylaxis pastes was surprising. The result might be explained by fluoride uptake in the control teeth beyond 30 minutes post-treatment, and interference with such uptake in the Treatment II and III teeth by prophylaxis paste retained in the interproximal area. This possibility is consistent with works of Mellberg and Nicholson;¹⁶ Vrbic, Brudevold, and McCann;27 and Vrbic and Brudevold.28 They found that the composition of the prophylaxis paste, without any abrasive action and the addition of humectants and flavoring agents, would decrease the ability of the paste to improve fluoride uptake in enamel. Thorough rinsing and flossing procedures following the use of prophylaxis pastes should be completed to limit a possible action of this type.

The findings of this study and those of Tinanoff and coworkers⁶ and of Stearns²⁹ suggest that, in the clinical setting, the indications for use of an abrasive paste prior to a topical fluoride treatment should be reevaluated. Certainly, leaving the acquired pellicle intact does not appear to interfere with fluoride uptake. When polishing dental restorations or removing extrinsic tooth stains with an abrasive prophylaxis paste, a fluoride-containing preparation should be used. The routine use of an abrasive prophylaxis prior to topical treatments cannot be recommened as the best means of preparing surface enamel to enhance the resulting enamel fluoride concentration. Thorough tooth cleaning with a toothbrush and dental floss appears to be better for that purpose.

Conclusions

The value of charged particle analysis in the study of fluorine concentration profiles in surface enamel was illustrated in this study. Refinements in the method should enhance its value in future studies. On the basis of this study and the one reported by Tinanoff et al.,⁶ it seems clear that cleaning with a toothbrush and dental floss prior to topical APF application results in higher retained fluorine concentrations in surface enamel than does a prophylaxis with either fluoridated or nonfluoridated prophylaxis pastes followed by similar topical treatments. The clinical importance of these differences, if any, must be tested in controlled clinical trials.

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- 1. Horowitz, H. S. and Heifetz, S. B. The current status of topical fluorides in preventive dentistry. JADA 81:166, 1970.
- Brudevold, F., Steadman, L. T., and Smith, F. A. Inorganic and organic components of tooth structure. Ann New York Acad Sci 8:5110, 1960.
- DePaola, P. F., Brudevold, F., Aasenden, R., Moreno, E. C., Englander, H., Baklos, Y., Bookstein, F., and Warram, J. A pilot study of the relationship between caries experience and surface enamel fluoride in man. Arch Oral Biol 20:859, 1975.
- Retief, D. H. Relationships among fluoride concentration in enamel, degree of fluorosis, and caries incidence in a community residing in a high fluoride area. J Oral Path 8:224, 1979.
- Wei, S. H. Y. Topically applied fluorides, in Fluorides: An Update for Dental Practice. New York; Medcom, 1976, pp 20-25.
- Tinanoff, N., Wei, S. H. Y., and Parkins, F. M. Effect of a pumice prophylaxis on fluoride uptake in tooth enamel. JADA 88:384, 1974.
- 7. Horowitz, H. S. Review of topical applications; fluorides and fissure sealants. J Can Dent Assoc 1:38, 1980.
- 8. Dieumegard, D., Maurel, B., and Amsel, G. Microanalysis of fluorine by nuclear reactions. I. $^{19}F(p,x_0)$ $^{16}0$ and $^{19}F(p,\alpha\gamma)$ $^{16}0$ reactions. Nuclear Instruments and Methods 168:93, 1980.
- Kregar, M., Muller, J., Rupnik, P., and Spiler, F. Concentration profile measurements in thin layers using multiplier resonant nuclear reactions. Nuclear Instruments and Methods 142:495, 1977.
- Ahlberg, M., Bawden, J. W., Hammarström, L. E., and Hellborg, R. Proton activation analysis on fluorine and nitrogen content of developing enamel. Odont Revy 26:267, 1975.

- Petersson, L. G. Fluorine gradients in outermost surface enamel after various forms of topical application of fluorides in vivo. Odont Revy 27:25, 1976.
- Rajan, K., Raisen, E., Mandler, J. W., Riley, D. L., and Semmlar, R. A. Improved fluoride incorporation and new in vitro fluorine elemental determination. J Dent Res 55:671, 1976.
- Heifetz, S. B., Franchi, G. J., Mosely, G. W., MacDougall, O., and Burnelle, J. Combined anticariogenic effect of fluoride gel trays and fluoride mouthrinsing in an optimally fluoridated community. Clin Prev Dent 6(1):21, 1979.
- 14. Loesche, W. J., Murray, R. J., and Mellberg, J. R. The effect of topical acidulated fluorine on percentage of Streptococcus mutans and Streptococcus sanguis in interproximal plaque samples. Caries Res 7:283, 1973.
- Loesche, W. J., Syed, S. A., Murray, R. J., and Mellberg, J. R. Effect of topical acidulated phosphate fluoride on percentages of Streptococcus mutans and Streptococcus sanguis in plaque. Caries Res 9:139, 1975.
- Mellberg, J. R. and Nicholson, C. R. in vitro evaluation of an acidulated phosphate fluoride prophylaxis paste. Arch Oral Biol 13:1223, 1968.
- Larson, R. H., Mellberg, J. R., Englander, H. R., and Senning, R. Caries inhibition in the rat by water-borne and enamel-bound fluoride. Caries Res 10:321, 1976.
- Englander, H. R., Carlos, J. P., Senning, R. S., and Mellberg, J. R. Residual anti-caries effect of repeated topical sodium fluoride applications by mouthpieces. JADA 78:783, 1969.
- Horowitz, H. S. and Kau, M. C. W. Retained anti-caries protection from topically applied acidulated phosphate-fluoride: 30- and 36-month post-treatment effects. J Prev Dent 1:22, 1974.
- Houwink, B., Backer-Dirks, O., and Kwant, G. W. A nine-year study of topical application with stannous fluoride in identical twins and the caries experience five years after ending the applications. Caries Res 8:27, 1974.
- Richards, A., Joost-Larsen, M., Fejerskov, O., and Thylstrup, A. Fluoride content of buccal surface enamel and its relation to dental caries in children. Arch Oral Biol 22:425, 1977.
- Schamschula, R. G. Associations between fluoride concentrations in successive layers of human enamel and individual caries experience. Arch Oral Biol 24:847, 1979.
- Spector, P. C. Surface enamel fluoride and strontium in relation to caries prevalence in man. Caries Res 13:227, 1979.
- Mandler, J. W., Molev, R. B., Raisen, E., and Rajan, K. S. Determination of the fluoride concentration profile in tooth enamel using a nuclear resonance technique. Solid Films 19:165, 1973.
- Petersson, L. G., Odelius, H., Lodding, A., Larsson, S. J., and Frostell, G. R. Ion probe study of fluorine gradients in outermost layers of human enamel. J Dent Res 55:980, 1976.
- Jones, K. W., Annegard, H. J., and Sellschop, J. P. F. Charged particle analysis of fluorine distributions in the surface of human tooth enamel. Arch Oral Biol 23:31, 1978.
- Vrbic, V., Brudevold, F., and McCann, H. G. Acquisition of fluoride by enamel from fluoride pumice pastes. Helv Odont Acta II:21, 1967.
- 28. Vrbic, V. and Brudevold, F. Fluoride uptake from treatment with different fluoride prophylaxis pastes and from the use of pastes containing a soluble aluminum salt followed by topical application. Caries Res 4:158, 1970.
- Stearns, R. I. Incorporation of fluoride by human enamel: III. In vivo effects of nonfluoride and fluoride prophylactic pastes and APF gels. J Dent Res 52:30, 1973.