In vitro fluoride uptake by enamel and dentin

A comparative study of two varnishes

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The uptake of fluoride in enamel and dentin after application of an experimental fluoride varnish has been studied and compared with the uptake from a commercially available varnish (Duraphat®). Each varnish was applied to 10 extracted human cuspid teeth. Successive etchings with perchloric acid were carried out separately in enamel and root dentin to obtain samples at three different depths. The concentrations of fluoride and calcium in the samples were determined, and the fluoride uptake was calculated. The experimental varnish gave a significantly greater fluoride uptake at all three sample depths in both enamel and dentin.

□ *Dental caries; fluoride varnishes; preventive dentistry; topical fluorides*

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Application of fluoride varnish has been an accepted routine in Sweden for many years. Duraphat®, the fluoride varnish commonly used, contains sodium fluoride (2.26 mg F/l), ethanol, and colophonium and effectively reduces caries under optimal conditions (1–5). The caries inhibition is attributed to the deposition of fluoride apatite in enamel and the formation of surface reservoirs of calcium fluoride (6, 7). Greater fluoride uptake has been shown from varnish than from gel or solution (8, 9).

The above studies have generally concerned fluoride uptake in enamel and effect on enamel caries. However, adults with exposed root surfaces require a cariostatic agent effective on both dentin and enamel.

A new fluoride varnish with improved adhesive properties has recently been developed at the Center for Dental Technology and Biomaterials (CDB), Karolinska Institutet, Stockholm. Clinical pilot studies at the Faculty of Dentistry, Stockholm, and the Preventive Dentistry Service of the Stockholm County Council have confirmed its excellent adhesive properties, and patient acceptance of the appearance of the teeth after application has been high. The aim of the present study was to compare in vitro fluoride uptake by enamel and root dentin from Duraphat and the new fluoride varnish (CDB).

Materials and methods

The material for the investigation comprised 20 extracted human canine teeth, stored in saline. The teeth were randomly allotted to one of two groups for treatment with Duraphat or with the new varnish (CDB). The CDB varnish contains sodium fluoride, ethanol, modified colophonium, zinc chloride, and stabilizing and flavoring agents.

The teeth were longitudinally sectioned buccolingually with a diamond disc, giving a control half and an experimental half. The root dentin was carefully exposed by removal of the cementum with a sharp instrument and polished with pumice and whiting. The specimens were dried with ethyl alcohol. Fluoride varnish was then applied for 10 sec, with a brush of the type routinely used for clinical application, to both the enamel and the root dentin of the experimental halves. After 4 min of drying in air at room temperature the experimental halves were suspended in artificial saliva (10). Four hours later the film of varnish was removed with acetone applied on a gauze swab. The exper-

imental halves were resuspended in artificial saliva for 12 days. The specimens were then immersed in potassium hydroxide for 24 h, to remove CaF_2 from the surfaces. Samples of enamel and dentin were obtained by etching away three successive layers from the surface with perchloric acid (10). The surface area of the biopsy layer was around 7.2 mm^2.

The control specimens were stored in physiologic saline solution throughout the experiment. Samples were obtained by etching as described above.

The sample solution was analyzed for fluoride with a combination fluoride electrode (Orion 910900). Calcium content was determined by atomic absorption spectrophotometry (Pye Unicam SP9). The following values were used for calculation of enamel and dentin mass released into the sample solution: calcium concentration in enamel, 37.03%; in dentin, 26.24% (11); enamel density, 2.95 g/cm^3 (12); and dentin density, 1.9 g/cm^3 (13).

The sample depth obtained with perchloric acid varies from tooth to tooth and from layer to layer. To enable comparison of fluoride content in different etched layers, the fluoride concentration was corrected to the following standard sample depths: 3.5 μm, 7.0 μm, and 10.5 μm for enamel and 6.0 μm, 12.0 μm, and 18.0 μm for dentin. The following regression curve was used (10):

$$\ln(F \text{ mass}) = A + B \ln(\text{enamel or dentin mass}).$$

The factors A and B were determined by the method of least squares. The curve obtained was used as a three-point calibration curve. The adjusted F mass was calculated from the enamel or dentin mass corresponding to the standardized sample depths —that is, 73.0 μg, 146.0 μg, and 219.0 μg for enamel and 80.6 μg, 161.0 μg, and 242.8 μg for dentin.

The adjusted F mass in the first sample layer and the differences between the adjusted F masses of the second and the first and the third and the second sample layers were used to calculate the adjusted fluoride concentration for each sample layer. The difference between the adjusted F concentration in the experimental specimen and the corresponding control half was used to calculate the fluoride uptake in enamel and dentin after treatment with varnish.

The concentration of fluoride in each sample layer was compared with regard to uptake from the two varnishes and differences in uptake for enamel and dentin, using a two-way analysis of variance. The statistical analysis was done with Statgraphics statistical program.

Results

At all three etched levels, specimens treated with CDB fluoride varnish showed significantly greater fluoride uptake than specimens treated with Duraphat ($p < 0.005$ at all three layers).

The uptake of fluoride was greater in dentin than in enamel for both varnishes and in all layers. The difference in fluoride uptake between enamel and dentin was significant for all etched depths, with the same level of significance ($p < 0.001$) (Table 1 and 2).

The partial coefficients of correlation be-

Table 1. Mean values and standard deviation (SD) of in vitro net increase of fluoride (ppm) in consecutive enamel layers

	Layer 1, Depth, 3.5 μm*		Layer 2, Depth, 7.0 μm		Layer 3 Depth, 10.5 μm	
Varnish	Mean	SD	Mean	SD	Mean	SD
Duraphat® ($n = 10$)	1345	811	765	485	657	390
CDB ($n = 10$)	2303	1541	1035	808	779	690

* Referred depth.

Table 2. Mean values and standard deviation (SD) of in vitro net increase of fluoride (ppm) in consecutive dentin layers

	Layer 1, Depth, 6.0 µm*		Layer 2, Depth, 12.0 µm		Layer 3, Depth, 18.0 µm	
Varnish	Mean	SD	Mean	SD	Mean	SD
Duraphat® ($n = 10$)	3575	2172	2634	735	2367	628
CDB ($n = 10$)	6289	3196	5307	3045	5044	3112

* Referred depth.

tween fluoride concentration in the enamel and dentin layers of the control specimens and the fluoride uptake by enamel and dentin layers in the experimental specimens treated with fluoride varnish were calculated. The coefficients for enamel were 0.14 in the first etched depth and −0.17 and −0.11, respectively, in the second and third etched depths. The corresponding coefficients of correlation for dentin were −0.43, −0.06, and −0.18. With the exception of the first layer depth in enamel, fluoride uptake in enamel and dentin in this experiment was thus in inverse relation to the original fluoride concentrations in the teeth.

Discussion

In this study fluoride retention in the outermost layers of enamel and exposed root dentin after treatment with the two varnishes was compared. As a base-line value the initial fluoride concentration in each tooth was determined. It has been shown that the fluoride concentrations in symmetrical buccal and lingual etched samples of a tooth are almost identical (14, 15). This procedure minimizes errors due to variation of initial fluoride concentration in different teeth.

Some variation may, however, still exist because of variation in sample depth. The fluoride-rich enamel may be expected to give a shallower depth of etching. The fluoride concentration decreases with depth, and the fluoride values obtained will therefore be influenced by the thickness of the sample layer.

The CDB varnish gave a markedly greater fluoride uptake than Duraphat in both enamel and root dentin. Although the number of teeth in the experiment was relatively small, statistical analysis showed that the difference was significant. This may be due to the dissimilarities in composition of the varnishes, although the principal ingredients were the same.

Fluoride retention by enamel is in itself no longer considered the only important factor for caries-inhibiting potential. The cariostatic effect is now mainly attributed to the fluoride in the liquid phase within and around the outer enamel layer (16). The fluoride concentration in this phase is considered to be dependent on availability of soluble fluorides at the tooth surface, particularly calcium fluoride. With currently available methods it is, however, difficult to determine the quantity of calcium fluoride deposited during treatment with fluoride varnish.

Earlier studies measuring the fluoride content in the outer layer of enamel have shown good uptake from Duraphat. Its caries-reducing effect is also well documented. Seppä et al. (17) found a positive correlation between caries reduction and fluoride uptake from Duraphat. Therefore in the present study measurements were made in the same manner, and the calcium fluoride that might initially have been present on the teeth was eliminated with potassium hydroxide.

In this initial comparative test of the CDB varnish the fluoride uptake by root dentin was considered of particular interest. Previous investigations have shown a positive correlation between fluoride uptake in dentin and its ability to resist acid attack (18, 19). The new varnish may therefore be expected to have a good cariostatic effect on dentin.

A clinical investigation has been designed to evaluate the clinical effect of the new varnish on root caries and enamel caries.

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