Comparison of Fluoride Uptake into Tooth Enamel from Two Fluoride Varnishes Containing Different Calcium Phosphate Sources

B.R. Schemehorn G.D. Wood

Therametric Technologies, Inc.
Noblesville, IN, USA

W. McHale

Premier Dental Products
Plymouth Meeting, PA, USA

A.E. Winston

R&D for Hire LLC
East Brunswick, NJ, USA

Abstract

- **Objective:** The objective of this *in vitro* study was to compare two 5% sodium fluoride varnishes, each containing different sources of calcium and phosphate, for their ability to deliver fluoride into treated sound tooth enamel and adjacent, but untreated demineralized enamel.
- **Methods:** Six sets of 12 bovine enamel cores were mounted in plexiglass rods and the exposed surfaces were polished. Synthetic lesions were formed in the surface of three sets by soaking in thickened, pH 5.0, 1M lactic acid, 50% saturated with calcium hydroxyapatite. A fluoride varnish containing tri-calcium phosphate (TCP) was applied to one set of sound enamel cores, and a second, delivering amorphous calcium phosphate (ACP), was applied to another. A third set of sound enamel cores was water-treated. Each treated sound core was paired with an untreated lesioned core, and the pairs were soaked in artificial saliva for 24 hours at 37°C. The treated cores, but not their lesioned counterparts, were initially soaked in 1.0 N KOH saturated with calcium phosphate for 18 hours. Each core was separately etched with 1.0 N perchloric acid for exactly 15 seconds, and fluoride measured by an ion-sensitive electrode after neutralizing with NaOH and buffering in TISAB II. The amount of calcium extracted was also determined by atomic absorption spectrophotometry as a measure of etch depth.
- **Results:** Fluoride uptake average was 1677 ± 193 ppm, 455 ± 38 ppm, and 44 ± 5 ppm for the sound enamel cores treated with ACP varnish, TCP varnish, and water treatment, respectively. Fluoride uptake into the demineralized enamel averaged 5567 ± 460 ppm, 2126 ± 126 ppm, and 49 ± 4 ppm for demineralized enamel paired with the sound cores treated with ACP varnish, TCP varnish, and water, respectively. The differences between the ACP varnish, the TCP varnish, and the water treatments were statistically significant ($p < 0.05$).
- **Conclusion:** The ACP varnish formulation delivers statistically significantly more fluoride to both intact and demineralized enamel than the formulation containing TCP.

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Introduction

Over the years, fluoride has proven to be a potent tool in the fight against dental caries. Fluoride is known to increase the resistance of tooth mineral to demineralization by plaque acids.[1] However, fluoride's role in promoting remineralization and the repair of incipient lesions is probably at least as important.[1]

There are many over-the-counter and professionally applied products which deliver fluoride to the mouth. Anticaries fluoride toothpastes and OTC mouthrinses, for regular preventive use by the general population, supply modest quantities of fluoride. The incorporation of fluoride into these products has been largely responsible for the significant drop in the worldwide incidence of caries that has occurred over the last 60 or more years. Fluoride gels, with up to 5000 ppm fluoride ion, are often prescribed for daily use in patients at high risk for dental caries. Fluoride varnishes, with up to 22600 ppm fluoride, are approved by the FDA for the treatment of sensitive teeth, but are also widely applied by dental professionals on a twice-yearly basis for the prevention and treatment of dental caries.[2,3]

Concentrated varnishes contain suspensions of sodium fluoride in a rosin base, which allows them to stick to tooth surfaces and remain in place for up to 24 hours.[2,4] To prevent the adherent film from being prematurely removed, patients are discouraged from eating for two to four hours after application and from brushing their teeth during the evening following treatment. During the post-treatment period, fluoride is gradually released from the varnish and is taken up by the tooth enamel and dentin. Fluoride reacts with tooth mineral, forming either fluoridated apatite or calcium fluoride. Strongly bound fluoride, incorporated onto the surface of the crystals of apatite, can reduce the solubility of tooth mineral and hence inhibit demineralization due to acids generated by plaque bacteria. Loosely bound fluoride provides a relatively slow-release form of ionic fluoride to plaque and saliva. Small amounts of ionic fluoride in solutions around tooth mineral inhibit dissolution but, perhaps more importantly, promote remineralization of incipient lesions, thereby reversing the development of early carious lesions.[1]

There is a significant body of evidence demonstrating the anticaries efficacy of fluoride varnishes.[3] A meta-analysis performed by Helfenstein and Steiner, using the results of eight clinical studies of varying length, showed an overall average 38% reduction for a varnish containing 5% sodium fluoride when applied on a twice-yearly basis.[5] The 95% confidence interval for the magnitude of the reduction was from 19–57%. Further regression analysis projected a 45% reduction over two and a half years with a 95% confidence interval of 35–55%. Another meta-analysis performed by Marhino, *et al.*, which evaluated the data from seven studies, showed an average 46% reduction ($p < 0.0001$) in DMFS.[6] In this study, the 95% confidence interval for the magnitude of the reduction was from 30–63%.

Varnishes with high concentrations of fluoride tend to be more effective than other types of topical fluoride treatment. Thus, Shobha, *et al*. found a 50% reduction in caries for a concentrated varnish compared to a 30% reduction for the APF gel.[7] Tewari, *et al*. obtained a 74% reduction for the 5% sodium fluoride varnish compared with 35% for an APF gel and 26% for a sodium fluoride gel.[8] However, Seppa, *et al*. did not find a significant advantage for a varnish with 5% sodium fluoride compared to an APF gel.[9]

It is important to note that the beneficial effects of fluoride varnishes are not restricted to preventing enamel caries. For example, Tan, *et al*. demonstrated that regular applications of a 5% sodium fluoride varnish every three months over a three-year period reduced the incidence of root caries by 64% ($p < 0.001$) in elderly patients.[10] Also, Schaeken, *et al*. demonstrated that applications of a 5% sodium fluoride varnish to the exposed roots of periodontal patients every three months resulted in a 50% reduction ($p < 0.01$) in the development of root caries in an adult population after one year.[11]

Since calcium and phosphate ions are the primary constituents of tooth mineral, adequate quantities of these ions must be present in the remineralizing medium for remineralization to occur. Saliva provides small amounts of these ions. Recently, more advanced fluoride varnishes, with added calcium and phosphate ions, have been developed to supplement the amounts of these ions in saliva and enhance remineralization by fluoride.

The purpose of this study was to compare two fluoride varnishes, which also contain sources of calcium and phosphate, for their ability to deliver fluoride into tooth enamel. One varnish contained tri-calcium phosphate (TCP), the other, a calcium salt and phosphate salt which combine in aqueous media to form amorphous calcium phosphate (ACP).

Materials and Methods

Six sets of twelve sound bovine incisors were cleaned of all adhering soft tissue. Cores of enamel were prepared from each tooth by drilling perpendicularly through the labial surface using a 3 mm inner diameter hollow core diamond drill bit. During preparation, the tooth enamel was cooled under water. The cores were then mounted into the ends of quarter-inch diameter plexiglass rods of two-inch length using methyl methacrylate. Excess acrylate was removed to expose only the enamel surface. The surface of the enamel was ground flat with 600 grit wet/dry paper and then polished with microfine gamma alumina.

The exposed surface of three sets of twelve tooth cores were demineralized by immersion without stirring in a 0.1 M lactic acid solution at pH 5.0, and which was 50% saturated with calcium hydroxyapatite and contained 0.2% Carbopol 907 to form an artificial incipient lesion in the surface of the enamel. The other three sets of sound cores were not so pretreated. A set of sound cores and a set of demineralized cores were assigned to each test group.

The two varnish test products contained 5% sodium fluoride (22600 ppm fluoride ion). One test product, Omni Vanish™ White Varnish G0151813 (3M ESPE Omni, St. Paul, MN, USA), also contained tri-calcium phosphate, and the other varnish, Enamel Pro® ACP Formula (Premier Dental Co., Plymouth Meeting, PA, USA), contained a calcium salt and a phosphate salt which form amorphous calcium phosphate (ACP) on exposure to an aqueous medium. Deionized water was used as a negative control.

The sound cores for each group were air dried, weighed, and preheated to 37˚C. A thin film of each varnish was then applied to each sound core in its assigned group using the supplied or recommended applicator. Deionized water was similarly applied to the third group of sound cores. The cores were maintained at 37ºC for one minute, air dried, and the amount of varnish which had been applied was determined by reweighing the samples. An attempt was made to equalize the amount of varnish on each core within about 10% by adding varnish to those with a lower weight. However, varnish was not removed from any of the higher weight cores once applied.

To simulate the effect of what might happen to both a varnish-treated sound tooth and to an untreated adjacent tooth with a synthetic incipient lesion, each treated whole core was paired with an untreated demineralized core by attaching them side by side. The paired specimens were then immersed in 10 ml of artificial saliva at 37˚C for 24 hours, with constant agitation to simulate what might happen in the mouth.

Following the 24-hour treatment period, loosely bound fluoride was extracted from each treated sound core by immersing it for 18 hours in 10 ml of 1.0 N potassium hydroxide saturated with tribasic calcium phosphate. The cores were then rinsed well in deionized water. All cores (sound and lesioned) were then individually immersed in 0.5 ml of 1.0 N perchloric acid for exactly 15 seconds to remove a layer of enamel. A 0.25 ml aliquot of this solution was neutralized with 0.25 ml of 1.0 N sodium hydroxide, and 0.5 ml of TISAB II was added to buffer the sample to pH 5.2. The fluoride content in the dissolved layer of enamel was determined using a fluoride electrode which had been standardized using standard concentrations of fluoride buffered with TISAB II. The depth of enamel removed by the perchloric acid treatment was determined by measuring the calcium content of a 50 µl aliquot of the treatment solution using a PerkinElmer AAnalyst 200 atomic absorption spectrophotometer (PerkinElmer, Waltham, MA, USA).

The complete sets of fluoride uptake data and etch depth data, respectively, for the sound and lesioned enamel specimens were analyzed for significance at $p < 0.05$ by means of a one-way analysis of variance (ANOVA). Since the comparisons of interest were those comparing fluoride uptake and etch depth for the three sets of intact enamel specimens (three comparisons) and for the three sets of lesioned enamel specimens (three comparisons), we ran an additional ANOVA on the data from the intact specimens and a separate ANOVA on the data from the lesioned specimens. Where these ANOVAs indicated significant differences, the individual means were compared using a Student-Newman-Keuls (SNK) test.

Results

The fluoride uptake results into sound enamel are shown in Table I. Both of the varnishes increased the fluoride content of the sound enamel over the fluoride-free water treatment ($p < 0.05$). The ACP varnish delivered almost four times the quantity of fluoride to the enamel than the TCP varnish, and the difference was statistically significant ($p < 0.05$).

Table I
Fluoride Uptake into Sound Enamel

	ACP Varnish (n = 12)		TCP Varnish (n = 12)		Water Control (n = 12)
EFU (ppm) ± SEM	1677 ± 193	>	455 ± 38	>	44 ± 5
Etch Depth (μ) ± SEM	16.81 ± 0.22	<	18.41 ± 0.36	<	19.39 ± 022

All differences between groups were statistically significant at $p < 0.05$.

It may also be noted that the etch depths were less in the fluoride varnish-treated enamel than in the water-treated enamel ($p < 0.05$). The etch depth was statistically significantly lower for the enamel treated with the ACP varnish than the TCP varnish-treated enamel ($p < 0.05$).

The fluoride uptake into lesioned enamel is shown in Table II. Both of the varnishes statistically significantly increased the fluoride content of the lesioned enamel compared to the fluoride-free water treatment ($p < 0.05$). The ACP varnish delivered about 2.5 times the quantity of fluoride to the lesioned enamel than the varnish containing TCP, and this difference was statistically significant ($p < 0.05$).

Table II
Fluoride Uptake into Lesioned Enamel

	ACP Varnish (n = 12)		TCP Varnish (n = 12)		Water Control (n = 12)
EFU (ppm) ± SEM	5567 ± 460	>	2124 ± 126	>	49 ± 4
Etch Depth (μ) ± SEM	17.80 ± 0.49	<	19.46 ± 0.36	<	21.14 ± 0.47

All differences between groups were statistically significant at $p < 0.05$.

Likewise, both varnishes decreased the average etch depth for the incipient lesion enamel compared with the water control ($p < 0.05$). The etch depth for the enamel treated with the ACP varnish was statistically significantly less than for the enamel treated with the TCP varnish ($p < 0.05$).

Discussion

There is a growing body of literature showing how calcium and phosphate supplementation of fluoride treatments can enhance fluoride uptake. For example, pretreatment of tooth enamel with acidic solutions containing calcium and phosphate ions prior to application of various sodium fluoride solutions has been shown in several studies to enhance fluoride uptake.[12-15]

Schemehorn, *et al*. demonstrated that a fluoride toothpaste, simultaneously delivering calcium and phosphate salts, released about two and a half times as much fluoride into lesioned enamel as a reference toothpaste containing a similar content of fluoride ($p < 0.001$).[16] They also showed that the tooth enamel treated with the toothpaste containing fluoride, calcium, and phosphate was significantly less soluble than that treated with the reference fluoride toothpaste.

However, there are many salts of calcium phosphate with different calcium and phosphate ion availabilities. Tri-calcium phosphate is a fairly insoluble crystalline form of calcium phosphate, and is similar to apatitic calcium phosphate in tooth enamel. On the other hand, amorphous calcium phosphate (ACP) has a similar, though less defined ratio of calcium to phosphate ions; but, importantly, it is non-crystalline and has no systematic structure. As a result, ACP is more soluble, and hence is more reactive than other crystalline calcium phosphates.[17]

In this study, the ACP varnish delivered statistically significantly more fluoride into both sound and lesioned enamel than the fluoride varnish which contains TCP, probably due to the greater availability of calcium and phosphate ions in the product delivering ACP.

In agreement with Attin, *et al*., who found *in vivo* that untreated teeth close to fluoride varnish-coated teeth can receive some of the beneficial effects of the varnish treatment,[18] the untreated demineralized tooth specimens in this study took up a considerable amount of fluoride. In fact, in this study there was greater fluoride uptake by the adjacent demineralized specimens than the treated sound enamel itself. One reason for this might be that fluoride is unlikely to be directly taken up by tooth mineral from the varnish. Rather, fluoride ions can only be taken up by tooth mineral once the ions are released from the varnish and dissolved in the aqueous salivary medium. The demineralized enamel specimens would actually be the first to be exposed to dissolved fluoride. Secondly, the accessible surface area for reaction with fluoride is much greater in lesioned enamel than sound.

In determining fluoride uptake in this study, we elected to treat the sound enamel differently than the demineralized enamel. Thus, prior to the acid etch, any loosely bound fluoride was extracted from the sound enamel specimens by soaking the cores in potassium hydroxide solution saturated with calcium hydroxyapatite. The measure of fluoride uptake into sound enamel in this study, therefore, only includes strongly bound fluoride. The reason for performing the study this way is that we believe loosely bound fluoride is much less important in protecting sound enamel. Firstly, as shown *in vivo* by Dijkman, *et al*., most, if not all of the loosely bound fluoride deposited onto sound enamel by a varnish is lost within a week.[19] Secondly, the remineralizing activity of loosely bound fluoride is not very relevant to its activity in sound enamel since no incipient lesions are present. In sound enamel, it seems likely that high levels of fluoride incorporated into the surface of the apatitic mineral crystals would be more protective against the development of an incipient lesion due to a challenge with plaque acids.

In lesioned enamel, after treatment, both loosely bound and strongly bound fluoride were analyzed as a combined total since both are extractable into perchloric acid. In contrast to the type of fluoride in sound enamel, the total fluoride uptake seems more relevant to an incipient lesion where the dual roles of fluoride are probably operative. Immediately after deposition into a lesion, loosely bound fluoride can actively promote remineralization using calcium and phosphate from saliva or, in the case of a calcium and phosphate-containing varnish, using these supplemental ions to restore tooth mineral. Also, as with sound enamel, high concentrations of localized fluorapatitic mineral on crystal surfaces can inhibit dissolution when the tooth is exposed to plaque acids.

We did not include a calcium and phosphate-free varnish formulation in this study. Therefore, we cannot assess whether TCP is effective in promoting fluoride uptake from a varnish formulation from the results of this study.

As noted in the Results section, both varnish treatments slightly reduced the etch depth when the cores were submerged in perchloric acid. The ACP varnish decreased the etch depth to a greater extent than the TCP varnish. Since the etch times used were identical throughout the study, it would seem that the fluoride uptake from the varnish treatments increased the resistance of the enamel to the acidic challenge. However, presumably because of the strength of the perchloric acid used for etching, although statistically significant ($p < 0.05$), the inhibitory effect of increasing fluoride levels was relatively small, *e.g.*, about 13% for the substrates treated with the ACP varnish.

The results of this study indicate that the ACP varnish formulation delivers statistically significantly more fluoride to both intact and demineralized enamel than the formulation containing TCP. It is likely that this outcome results from the greater availability of the calcium and phosphate ions in the ACP formulation.

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For correspondence with the authors of this paper, contact B.R. Schemehorn—BSchemehorn@therametric.com.

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