Salivary Fluoride Concentration Following the Application of Three Different 5% NaF Varnishes

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Dedication

To my husband Brad and daughter Mia for their patience, encouragement, and inspiration.

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Chapter 1

Background

Fluoride varnish production and use has had a dramatic increase in the last decade following approval by the FDA in 1994 as a cavity liner and root desensitizer.[1] Despite its "off-label" use in caries prevention, research has demonstrated varnishes to be a safe, effective, and efficient way to deliver fluoride to patients at risk for dental caries.[2,3] Accordingly, fluoride varnishes are widely recommended for patients at high risk for dental caries (ADA, CDC, AAPD). Although the FDA did not approve fluoride varnishes until 1994, the first fluoride varnish was developed in the 1960's as a possible mechanism to enhance the treatment duration and uptake of fluoride.[4] Most of the clinical research on fluoride varnishes has been conducted using Duraphat (5% NaF), which was the first commercially available product. Within the last few years, numerous varnishes with similar sodium fluoride concentrations (5%), but with multiple variations in carrier composition, have emerged and have taken a significant portion of the market share. As the number of fluoride varnishes available has increased, each company has created unique changes to the formula in order to improve properties like handling, appearance (i.e., white), flavor, or in

some cases, potentially active ingredients (e.g., tricalcium phosphate, amorphous calcium phosphate, calcium sodium phosphosilicate, xylitol, etc.),[5,6,7,8] leading to a claim of additional preventive benefits. Most of these new varnishes have not been studied in vivo for their caries reduction, efficacy, or safety.

Despite the similar 5% NaF concentration used in most of these varnishes, in vitro data have suggested that some of these secondary ingredients may affect the fluoride ion release of the product.[9,10,11] Fluoride release, and subsequent formation of calcium fluoride is thought to be an essential part of the mechanism of action of fluoride varnishes to prevent and remineralize carious lesions. Differences in fluoride release patterns can potentially enhance or diminish the efficacy and safety of a varnish. Therefore, understanding the differences in fluoride release pattern of varnishes with different formulations in vivo will help us understand which formula modifications have the potential to enhance or diminish the anticariogenicity and safety of the varnish. Several studies have compared fluoride varnishes to other delivery systems such as gels, foams, and pastes; however, a void remains in the literature regarding the comparison of efficacy and safety between the many different new fluoride varnish systems containing 5% NaF widely available today in the US market.

The fluoride varnishes to be used in this study were chosen based on their unique characteristics. Duraphat (Colgate-Palmolive) was chosen because it has the largest body of laboratory and clinical research and it is considered the gold standard. Vanish (3M ESPE) was chosen because it is relatively new, has a large portion of the market share for varnishes, uses a different resin formulation, and contains tri-calcium phosphate. The third varnish, Enamel Pro (Premier) was selected because it is also very popular, has amorphous calcium phosphate added, and in laboratory studies it has shown to release significantly more fluoride than the other two varnishes.[9,10,11]

Comparison of the material safety data sheets for each of these different varnishes reveals three similar ingredients, namely rosin (colophonium), ethanol, and sodium fluoride. However, according to the Hazard Communication Standard 29 CFR 1910.1200 developed by OSHA, only those ingredients that may be hazardous require documentation and therefore many of the unique formulary ingredients of fluoride varnishes remain undisclosed.[12] These ingredients, called "trade secrets" by OSHA, may be withheld from MSDS reports when they are deemed non-hazardous and create some advantage to a company over other companies that do not know of it or use it. Some early investigations have suggested that the

addition of calcium phosphate ingredients used in some dental products may increase both fluoride release from products and subsequent uptake of fluoride in enamel.[13,14] By evaluating the fluoride release from these three varnishes, each containing different calcium phosphate compounds and other ingredients, some insight may be gained as to the complex interaction of these trade secrets and the effect they may have on fluoride varnish efficacy.

Purpose

Determine and compare the fluoride concentration in unstimulated whole saliva at five different time periods following the application of three different 5% NaF varnishes.

Hypotheses

Ho1 - There will be no significant difference in the amount of fluoride release into saliva among the 3 different varnishes.

Ha1 – There will be a significant difference in the amount of fluoride release into saliva release among the 3 different varnishes.

Ho2 – There will be no significant difference in the timing of fluoride release in to saliva among the 3 different varnishes.

Ha1 – There will be a significant difference in the timing of fluoride release into saliva among the 3 different varnishes.

Specific Aims

1. Determine the concentration of fluoride available in whole saliva at different time periods after the application of three different 5% NaF varnishes as measured in unstimulated human saliva.

2. Compare the different patterns of fluoride release from each of the varnishes by comparing the fluoride concentration in saliva at corresponding time periods.

Literature Review

Fluoride Varnish

History

Fluorine is the 13th most prevalent element and is found in water air and soil. Fluoride, which is the ionic form of the element fluorine, is a negatively charged ion that frequently combines with positive ions to form more stable compounds.[15] The discovery of fluoride and its relevancy to dentistry in t he United States, dates back to 1901.[16] According to the National Institute of Dental and Craniofacial Research (NIDCR), it began when Dr. Frederick McKay discovered brown staining on the teeth of Colorado Springs, CO natives. He could not find any mention of this condition in the current literature in 1909, so with the collaborative help of

G.V. Black he was able to make two important discoveries. First, only children with mottled deciduous teeth suffered from a similar mottled appearance of their adult teeth, and second, individuals with mottled teeth were very resistant to dental caries. In 1931, the connection between tooth mottling and naturally occurring high fluoride concentration in water was revealed. In 1945, Grand Rapids Michigan became the first community to have its water fluoridated and after 11 years they reported a 60% decrease in caries rate in children born after the fluoridation implementation began.

According to the 2001 Morbidity and Mortality Weekly Report[1], success following this water fluoridation initiation prompted the Public Health Service (PHS) to develop recommendations in the late 1940's and 1950's regarding community water fluoridation, and later, the development of fluoride-containing products. With the transition from water fluoridation to fluoride containing topical preparations, scientists began to inquire and study the difference between the systemic versus topical effects of fluoride. According to a review by Limeback[17] this question was still not satisfactorily answered by the literature in the late 1990's. The initial thought was that by evaluating the differences in the caries rate of adults that received systemic fluoride before the age of six, an estimation of the protective effects of systemic fluoride could be measured separately from

the topical effects seen in populations that did not receive systemic fluoride but were later exposed to fluoride containing topical applications. Systemic effects such as favorable changes in tooth morphology, and fluoride incorporation into enamel have been hypothesized. As Limeback points out, however, it is difficult to positively say that ingested fluoride is not also acting topically if its introduction to the system is through an oral route as in the case of water. The mechanism of action of topically applied fluorides is not as clear cut either and is still the subject of numerous studies. To accurately answer the question of how effective and by what mechanism do topical and systemic fluorides work, additional controlled studies need to be conducted. Currently, the prevailing evidence points to topical or post-eruptive fluoride administration as the primary means of caries reduction. In 1954, fluoride containing dentifrices became available in the U.S. followed by higher fluoride containing preparations of rinses, gels, and varnishes.[18]

The introduction of fluoride varnish in the dental literature came in 1964 as a report by a German researcher named Schmidt.[4] The journal, *Stoma*, introduced "Fluor-Lack" (fluoride lacquer) as a long-lasting fluoride. Four years later Schmidt and Heuser[19] published the first clinical trial on the efficacy of this varnish and launched it as a commercial product under the name Duraphat (Rhone-Poulenc Rorer, Rorer GmbH Köln, Germany).

Following clinical studies related to the efficacy and safety of Duraphat, other varnishes for topical fluoride application started to become available. Fluor Protector (0.7% F$^-$ at a low pH) was developed in 1975 by Arends and Schuthof (changed to 0.1% F$^-$ in 1987).[20] In 1984 Pharmascience Inc. developed a 5% NaF Varnish called Duraflor/Durafluor (personal communication 2012), and shortly after in 1986 VOCO developed a 6% NaF, 6% CaF varnish called Bifluorid 12.[21] Today Duraphat is marketed in the USA by Colgate-Palmolive, Fluor Protector by Ivoclar/Vivadent and Duraflor by Medicom. In 1994 the FDA approved fluoride varnishes for use as a cavity liner and root desensitizer, however it is primarily been the "off-label" use in caries prevention that has led to the development of the numerous brand names available today.[3]

In 2006 evidence-based clinical recommendations for topical fluoride were published by the American Dental Association (ADA)[22]. Following a Cochrane Systematic Review on clinical studies involving fluoride gels, foams, and varnishes, recommendations for topical fluoride application accounting for caries risk level and age were published. The strongest evidence was shown for topical varnish use in caries prevention in moderate to high risk patients, ages < 6 and 6-18 years, to be applied at either 6 (moderate risk) or 3 (high risk) month intervals. These recommendations for

topically applied fluoride by the ADA are in agreement with the 2008 revised Guideline on Fluoride Therapy by the American Academy of Pediatric Dentistry (AAPD).[22]

Composition

Interestingly, determining the exact composition of each fluoride varnish is not possible because, according to the Hazard Communication Standard 29 CFR 1910.1200 developed by OSHA[12], only those ingredients that may be hazardous require documentation. Therefore, many of the unique proprietary components of fluoride varnishes remain undisclosed. These ingredients, called "trade secrets" by OSHA, may be withheld from Material Safety Data Sheets (MSDS) when they are deemed non-hazardous and create some advantage to a company over other companies that do not know of it, or use it.

Although the original formulations have changed over time, the current MSDS of the first three commercially available fluoride varnishes in the United States list the following active ingredients; both Duraphat (Colgate -Palmolive) and Duraflor (Medicom) contain 5% NaF with a rosin and ethanol carrier.[5,6] Fluor Protector (Ivoclar-Vivadent), differs from the others in the use of difluorosilane as the fluoride source. Fluor Protector, according to the product information guide,[20] and MSDS,[7] contains 0.9%

difluorosilane in a polyurethane varnish base with ethyl acetate and isoamylpropionate solvents.

Comparing the MSDS of several commercially available varnishes it is apparent that, with only a few exceptions, the main constituents are quite similar. The common constituents of most varnishes available today are rosin, also referred to as colophony (either natural or synthetic), an alcohol, and sodium fluoride. The amount of each product is estimated on the MSDS, however most of the current top market varnishes advertise 5% neutral sodium fluoride as the active ingredient. This percentage is likely based upon the formulation of the first varnish, Duraphat, which has been extensively studied and boasts a 38% caries reduction in permanent dentition according to meta-analysis.[23]

Rosin, as defined by Merriam-Webster is "a translucent amber-colored to almost black brittle friable resin that is obtained from the oleoresin or deadwood of pine trees or from tall oil and used especially in making varnish".[24] Fluoride salts which are commonly referred to simply as fluorides, according to the U.S. Department of Health and Human Services, are naturally occurring components of rocks and soils.[15] A 5% concentration of sodium fluoride will contain 2.26% of the fluoride ion (22,600 ppm). Therefore, in a typical single dose varnish preparation of 0.4 ml, there are

potentially 9 mg of fluoride available in the oral cavity after application. Ethanol or other alcohols are used as solvents that are intended to evaporate from the solution once exposed to air allowing for the varnish to be fluid enough for application but then become adherent to enamel surfaces for increased length of fluoride exposure.

In addition to these three main constituents, other additives may be present such as an adhesion promoting agents, stabilizing agents, rheology modifying agents, colorants, sweeteners, and flavoring agents.[8] Recent trends, in lieu of data that support the need for calcium and phosphate ions to aid in remineralization and inhibit demineralization, have led to the addition of calcium phosphate compounds to some fluoride varnishes. For example enamel Pro (Premier Dental) markets the addition amorphous calcium phosphate (ACP), while Vanish (3M ESPE) uses tri-calcium phosphate (TCP) in its formulation. While theoretically these additions are included to enhance the product efficacy, the addition of these secondary ingredients may have profound influence on the amount, or pattern of fluoride ion release.

Mechanism of Action

In order to understand how fluoride varnishes are utilized in caries prevention, it is first important to review the mechanism of action of topical

fluoride in general. In 1999 Øgaard discussed possible mechanisms in his review, *The Cariostatic Mechanism of Fluoride*.[25] Originally, it was believed that the most important aspect of caries protection from fluoride was the systemic ingestion that resulted in fluoride being built into the structure of enamel during tooth formation causing reduced solubility of the apatite crystal. However, it is now widely accepted that the main mechanism of action of fluoride is topical. Effects such as the inhibition of bacteria, reduction of demineralization, and increased remineralization have been the focus of current studies. According to Øgaard, available fluoride may exchange F^- for OH^-, promote crystal growth of fluorapatite, or produce calcium fluoride.

Bacterial Inhibition

In 2007, Jeevarathanet al.[26] developed a study to the see if Fluor Protector (Ivoclar, Vivadent) varnish had an effect on the counts of *Streptococcus mutans* in the plaque of caries free children. They used thirty subjects that were separated into a study group of twenty and a control group of ten. Plaque was obtained from similar surfaces of each subject's teeth. Baseline samples were obtained for both the study and control group. The study group then had Fluor Protector applied to clean surfaces of all teeth. After plaque collection 24 hours post varnish application, and incubation for

48 hours, the levels of *S. mutans* were determined using Dentocult SM Strip Mutans (Orion Diagnostica, Espoo, Finland). They demonstrated a significant reduction in plaque of the bacteria *S. mutans* (p=0.000). This study supports the hypothesis that topical fluoride may have bactericidal or bacteriostatic effects. The study did not address the potential that varnish application simply blocked the adherence of *S. mutans* to the enamel surfaces of the sampled areas.

Pandit et al.[27] studied the influence of sodium fluoride on the virulence and composition of *S. mutans* biofilms to determine if perhaps one of the mechanisms of action of fluoride was directly on the bacteria. In this study, they utilized *S. mutans* exclusively to determine what effects NaF at 10, 50, and 125 ppm would have on *S. mutans* biofilm cells. *S. mutans* biofilms were formed on saliva coated hydroxyapatite discs, transferred to a 1% solution of sucrose and given 74 hours to grow and develop. The biofilm discs were treated twice daily with a control (100% H_2O) or NaF (2, 10, 50, and 125 ppm). Following the experimental phase they evaluated glycolytic pH drop, proton-permeability, F-ATPase activity, GTF activity, and biofilm composition. They determined that NaF significantly reduced the initial rate of pH drop at concentrations of 10, 50, and 125 ppm, and that the pH change was NaF dose dependent. Additionally proton permeability was enhanced,

and F-ATPase activity was reduced in the *S. mutans* biofilm cells at 50 and 125 NaF ppm, which both serve to regulate pH homeostasis within the bacterial cells. Despite some of their other findings, at the concentration of NaF used in this study there were no bactericidal activity against *S. mutans*, and the controls displayed similar amounts of CFU's indicating no growth rate inhibition. Overall, this study provided evidence that sodium fluoride may alter the acid production and acid tolerance of *S. mutans* in biofilms. One of the major shortcomings of this experiment was the lack of diversity within the experimental biofilm. It is possible that in vivo interactions between different types of bacteria may significantly alter these results.

A study done by Zickert et al.[28] supported the findings by Pandit, that increased fluoride may not act by eliminating or reducing the actual amounts of *S. mutans*, but rather the increased fluoride may inhibit metabolic activities of the bacteria. This in vivo study used 40 children that were pair-matched for baseline salivary levels of *S. mutans*. Each group participated in the experimental and control arm of the study. The design consisted of two experimental arms. In experiment one, participants had 0.5 ml of 5% NaF (Duraphat) varnish applied to professionally cleaned teeth and were not able to eat for 3 hours or brush for the remainder of the day. In the second arm, participants had the same treatment varnish applied, however it was applied

to teeth without prior prophylaxis. Results showed that there was no significant reduction in the proportion of *S. mutans* in the saliva of either experimental group when compared to the control at 4, 10 or 21 days after the treatment. They hypothesized that it may not have been an adequate concentration to be bactericidal, since some other studies have been able to show a reduction in total bacteria. They did not evaluate salivary bacteria levels during the time frame in which the fluoride varnish concentration would have been the highest in the saliva, however seeing a significant reduction in a microorganism within hours of varnish application was thought to be unlikely.

Fluorapatite Production

In 1984 Øgaard et al.[29] investigated the retention of fluoride in sound and demineralized dentin after treatment with Duraphat 5% NaF. Their aim was to determine if the prophylactic effect of fluoride was in the formation of fluorapatite (alkali insoluble fluoride) or in the formation of alkali soluble CaF_2. Using paired premolars indicated for extraction for orthodontics, they evaluated the effect fluoride had on so und enamel (ten pairs), and demineralized enamel (eight pairs). To create the demineralized enamel group, individuals had orthodontic bands placed on the premolars that allowed for plaque accumulation for 4 w eeks before the Duraphat

application. For the sound enamel samples, the premolar pairs had immediate application of the Duraphat. All teeth were extracted two weeks after the varnish was applied. Using an acid etch technique to r emove enamel at 3 different layers, the samples were then exposed to KOH removing all loosely bound fluoride (CaF_2, or KOH s oluble fluoride), leaving, presumably the KOH insoluble fluoride (fluorapatite). Using a fluoride selective electrode to measure the amount of fluoride, and atomic absorption spectroscopy to measure calcium in the samples, the control teeth were compared to the experimental teeth for amounts of CaF_2 vs. fluorapatite. It was found that in the sound enamel, total fluoride content was greatest in the most superficial layer, and that the majority of the fluoride increase was accounted for by the formation of CaF_2 ($\geq 52\%$) as opposed to fluorapatite ($\leq 16.6\%$). Additionally, the demineralized samples retained more fluoride in all three layers, and in the deeper layers, fluorapatite contributed to as much as 75% increase in the fluoride content. This study demonstrated two possible ways fluoride accumulates in high fluoride environments, and how it is possible that different environments (demineralized vs. sound enamel) may impact this interaction.

In 1992 Cruz et al.[30] evaluated the uptake of KOH-soluble and KOH-insoluble fluoride in enamel after the application of Duraphat or a 2% NaF

solution. The purpose of this study was to quantify the amount of apatitically bound fluoride in contrast to the loosely bound fluoride after the use of two neutral sodium fluoride preparations. Using surgically extracted impacted third molars a 2.5 mm surface area of enamel was exposed to either a 2% NaF solution, pH 7.5, for 5 minutes, a 5% NaF Duraphat varnish for 5 minutes, or left untreated. Both samples were rinsed of the fluoride after the 5-minute testing period. Samples were then subjected to removal of the alkali-soluble fluoride by using 1M KOH which removed both calcium fluoride like material, and adsorbed fluoride. Following the removal of loosely bound fluoride, the KOH-insoluble or apatitically bound F was removed by etching three successive layers and analyzing each layer for fluoride. Results from this study showed that untreated samples showed traces of alkali-soluble fluoride. The samples exposed to 2% NaF solution had the greatest amount (13x) of loosely bound fluoride in comparison to the 5% NaF varnish. Neither groups showed any increase in apatitically bound fluoride during the treatment period. The authors suggested that this type of fluoride incorporation may only occur after pH cycling in plaque. This may also support the hypothesis that it is the loosely bound calcium fluoride that is of greatest importance in the mechanistic action of high fluoride rinses and varnishes. It was interesting to find that impacted third molars had

soluble CaF_2 present on their surfaces. This may indicate that teeth are exposed topically to some amount of fluoride even before eruption.

Grobler and Kotze[31] evaluated the difference in loosely bound alkali-soluble fluoride and insoluble fluoride by observing the relative amounts of each found in erupted and unerupted third molars. This study was novel because the subjects used in this study had minimal levels (<0 .1 ppm) of water fluoridation during ages 1-16, but did have exposure to daily tooth brushing. They evaluated 32 erupted and 22 unerupted third molars removed for various reasons. 19 of the erupted third molars, and 11 of the unerupted third molars were unwashed, while 11 of erupted and 11 unerupted third molars were alkali washed. After their respective treatments, five successive acid-etch biopsies were obtained from specific cusp sites. Using a fluoride ion-selective electrode, the fluoride concentration of a buffered solution containing the etchings was determined. Additionally the Ca concentration was determined using N_2O/C_2H_2 flame atomic absorption spectrometry. The results of this study indicated that for the first two etch depths, significant differences between the mean enamel fluoride concentrations could be found between unerupted unwashed versus washed teeth, and the erupted washed versus unwashed teeth. In both erupted and unerupted teeth, the amount of fluoride in enamel plateaued at about 20 μm into the tooth. In the erupted

tooth, there was an approximate 78% increase in fluoride content compared to the unerupted tooth in the first 3 µm , 53% of which was alkali-soluble or loosely bound, and 47% of which was in a firmly bound form. For the unerupted tooth, no loosely bound fluoride was found. This study indicates that fluoridated toothpaste does increase both the loosely bound and firmly bound fluoride in vivo. Additionally, the fact that they did not find any loosely bound fluoride in the unerupted teeth of this study population that did not have fluoridated water, would support the theory that the unerupted teeth in the study by Cruz, were somehow receiving topical fluoride from systemically ingested fluoride.

Demineralization/Remineralization

According to Øgaard et al.,[25] fluoride not only increases the rate of remineralization, but can be reprecipitated with calcium and phosphate ions onto the enamel surface. This benefit is mostly seen when there is a constant low-dose fluoride interaction such as water fluoridation. For concentrated fluoride agents such as varnishes, the mechanism may vary slightly. Øgaard states that these higher fluoride containing agents may "form an intermediate product of calcium fluoride on the tooth surface, in lesions, and in plaque." Sometimes referred to as phosphate-contaminated calcium fluoride, these compounds can be seen under high magnification and may remain present

up to weeks after topical fluoride treatment. These globules which appear to be stabilized by phosphate, may act as fluoride reservoirs that have the ability to release fluoride when the pH is lowered. This fluoride released helps prevent the dissolution of calcium and phosphate from enamel, effectively enhancing the rate of remineralization and slowing demineralization. When the pH returns to normal, phosphates once again protect this calcium fluoride until the next drop in pH.

In 1988, Seppä[32] was evaluating this theory of remineralization as the primary mechanism of fluoride action by testing the ability of pre-softened enamel to remineralize with different concentrations and number of applications of sodium fluoride. In this study 5x5 mm slabs of noncarious human enamel were divided into six experimental groups. The groups were treated as follows; no treatment, 2.26% Duraphat on day 1, 2.26% Duraphat on days 1, 4, and 7, 1.13% Duraphat on day 1, 1.13% Duraphat on days 1, 4, and 7, or a 1 minute treatment with 0.1% NaF solution each of 9 days total. After 24 hours, the varnish was removed and reapplied as dictated by the different treatment groups. Samples were stored in artificial saliva that was renewed daily. Once daily the slabs were immersed in a lactic acid-NaOH buffer (ph 5.0) for 1 hour and then rinsed with water to simulate demineralization. Finally, the acid resistance and fluoride uptake was

measured by immersing the slab in the lactic acid-NaOH solution leaving a 9.6mm^2 area uncovered and determining the amount of dissolved calcium and fluoride. Vickers hardness was evaluated after pre-softening, after the 9-day study period, and after the 1 hour demineralization. Results showed that all treatments were effective at preventing softening of enamel during the 9 day study period. The three treatments with 2.26% Duraphat were slightly more effective than the rest of the varnish treatments, but not at a statistically significant level. The 0.1% NaF solution was the least effective at preventing softening of the enamel. Enamel treated three times with either 2.26% or 1.1% fluoride showed the greatest acid resistance. Because the enamel remineralization was similar between the treatment groups receiving 2.26% Duraphat one or three times, and the group receiving 1.1% Duraphat one or three times, the authors concluded that it is possibly not the concentration of fluoride that is most important, but rather the number of applications. In addition, because a single application of Duraphat 2.26% was able to promote remineralization during the whole 9-day period, it is possible that a formation of soluble CaF$_2$ on the enamel surface was able to act as a fluoride reservoir during acidic challenges. Seppä found that although the higher varnish fluoride concentrations did result in an increased uptake of fluoride in enamel, the number of applications did not increase this

uptake in enamel. Enamel solubility was not directly proportional to the fluoride content of the enamel.

In order to elucidate the effect that CaF_2 may have on the inhibition of enamel demineralization, Tenuta et al.[33] designed a double-blind, crossover, in situ study that evaluated how newly formed CaF_2 on enamel could be released to an *S. mutans* test plaque fluid. Subsequently, this plaque fluid was then used to see the effect it would have on enamel following an acidic challenge. Distinct amounts of CaF_2 were created on enamel slabs by either not treating them (control) or treating them with acidulated 0.5M NaF solution and aging the slabs in 6 hours, or 48 hours in artificial saliva. A representative group of the enamel slabs were tested to determine the CaF_2 from each treatment groups. The remaining enamel slabs were mounted in a palatal appliance of ten subjects with the enamel surface facing a test plaque prepared from *S. mutans*. The enamel slabs remained in contact with the test plaque for 30 minutes, at which time plaque from two of each sample was removed to test the amounts of fluoride, calcium, and inorganic phosphorus. After this, the appliance was reinserted and subjects rinsed for 1 minute with a 20% sucrose rinse. The appliance remained in the mouth for 45 minutes at which time the appliance was removed and another plaque sample was evaluated for fluoride, calcium and phosphorus. The results indicated that

the plaque fluid opposing the fluoride treated slabs had significantly higher levels of fluoride than the control and that this was correlated to the initial CaF_2 concentration in the enamel slab. In addition, the surface micro hardness before and after the sucrose challenge, supported the hypothesis that this increase in plaque fluid fluoride inhibited demineralization. In this experiment the importance of the CaF_2, was demonstrated. An acidic fluoride solution was used in this experiment, which may work in an alternate way to a neutral fluoride compound.

To evaluate the remineralization effect of topical fluoride, Castellano et al.[34] compared how the application of fluoride on a caries-like lesion would compare to the application of the fluoride around a caries-like lesion. In this study, human molars were sectioned and used in pairs for a control vs. experimental sample. Caries-like lesions were created (1x5mm) using an artificial caries solution. A 5% NaF varnish (Duraflor) was used to cover either the entire surface including the caries-like lesion, or the entire surface except the caries-like-lesion. After 30 days, the lesions were photographed under polarized light and compared to the baseline lesions. The mean percentages of remineralization were 9.5% for the group with fluoride surrounding the lesion and 10.8% for the group with the lesion covered with varnish. There was not found to be any significant difference between the

different application techniques in regards to the remineralization potential. These results support remineralization as one of the mechanisms of action of fluoride. Furthermore, this study supports the theory that direct application of varnish to a carious lesion is not necessary for the desired effect.

Fluoride Bioavailability

It was hypothesized early in the history of fluoride use that the prophylactic effect of fluoride was positively correlated with the concentration or amount of fluoride utilized. This protective effect was off-set by the increasing evidence that fluoride, in high concentrations, may pose both an esthetic risk (mottling) and toxic risk to humans. In contrast to the 1900's when the preventative effects of fluoride were discovered, the different sources of fluoride available to people today has greatly expanded. Fluoride is available today in our dentifrice, water, food sources, and professionally applied fluoride rinses, varnishes, and gels. This multi-source availability, in addition to the varying possible mechanisms that these fluorides work, has led to inquiries of how much, and of which types of fluoride containing substances are actually necessary to prevent decay. The initial step in answering this question depends on finding reliable ways to determine the fluoride release and bioavailability from particular products. From there, it may be possible to determine where the available fluoride is

incorporated, or if other fluoride containing compounds are formed that may be crucial in the cariostatic or carioprotective mechanism of fluoride action.

In the case of fluoride varnish, it is not enough to simply rely on the package label stating the ppm or percentage of fluoride that is contained in the product. Interactions between the individual ingredients, the unique environment of the oral cavity, and complex biochemical interactions may have an effect on the bioavailability of fluoride from the product. Several methods for determining the release of fluoride have been utilized. In addition to many in vitro studies, in vivo studies have evaluated release and subsequent storage of fluoride into saliva, oral mucosa, plaque, enamel, blood, urine, etc. It is likely that the fluoride found in whole saliva following fluoride varnish application represents the initial bioavailable fluoride from the product which can be stored in plaque, soft tissue, incorporated into enamel, or bound to other ions or proteins in saliva. Although outside the scope of this review, the storage of fluoride in plaque, oral mucosa, or through formation of fluoride containing compounds adsorbed onto the surfaces of teeth after localized release into saliva is, undoubtedly, an important aspect of fluoride bioavailability.

Fluoride Release into Saliva

Many of the early studies evaluating fluoride release into saliva aimed to determine the exact amount of fluoride released from very different fluoride containing products (gel, foam, dentifrice, varnish). However, using this historical literature to make comparisons between similar products (varnish vs. varnish); it becomes evident that even similar products types and concentrations can release different amounts of fluoride. This unexpected finding prompted studies that compared fluoride release to saliva

A 1983 study by Rytomaa and Meurman[35] evaluated the amount of fluoride in saliva after using different topical fluoride treatments. At the time of this publication, the main cariostatic effect was believed to be the reduction of enamel solubility and the direct action on dental plaque. The aim of this study was to determine the ability of a 2% sodium fluoride solution, an amine fluoride solution (Elmex sol), a sodium fluoride and amine fluoride gel (Elmex gel), and a 5% sodium fluoride varnish (Duraphat) to increase the ionic fluoride concentration in saliva and remain at a significant level. Four subjects were utilized in a cross-over design with a two month wash out period. The difference of amount of fluoride ion applied varied from 9 mg to 63 mg based on the amount applied and the concentration of the product. Stimulated salivary samples were taken at 2, 4,

6, and 8 days post treatment, and the samples were analyzed with a fluoride specific electrode. The results of this study showed that Duraphat gave the highest fluoride peak (10 ppm F^- two hours after treatment), and that although the Elmex gel and solution both produced a mean of 3 ppm of fluoride in saliva 2 hours after treatment, the clearance of the fluoride from the gel solution was slower than the solution. All treatments raised salivary fluoride levels above the baseline levels, however only Duraphat was able to keep this fluoride level above baseline until the next day. Elmex gel contained seven times the amount of fluoride than the other solutions but did not remain in saliva for an extended period of time which may indicate that there was an undesirable amount of fluoride ingested. To investigate this hypothesis further, it would be pertinent to evaluate blood levels of fluoride following the application of these products. It is possible that although the fluoride was not found in saliva the following day from some of these products that it was either bound in plaque, oral soft tissue, or enamel.

Eakle et al.[36] compared the fluoride levels found in saliva after the use of a 5% NaF varnish (Duraphat), to that of a 0.05% NaF rinse (ACT). Their hypothesis was that fluoride levels found in whole saliva represent the fluoride that is available to interact with plaque and enamel and therefore is a logical place to begin measuring the efficacy of fluoride releasing

substances. This study was a two-treatment cross-over design using sixteen subjects. Following pilot study results, they determined that they would test saliva at the following intervals: 5 and15 minutes, and, 1, 2, 4, 8, 12, 24, 32, 48, 56, 72, 80, 96, 104 hours. Although they anticipated that the fluoride rinse group would return to baseline fluoride saliva levels much sooner than 104 hours, they wanted to make a direct comparison to the fluoride varnish group. For the varnish group an unspecified amount of varnish was applied to both the buccal and lingual surfaces of 20 teeth, and for the fluoride rinse group subjects rinsed with 10ml of the 0.05% solution for 30 seconds. Saliva samples were collected in a stimulated fashion by having subjects chew on a piece of Parafilm for 2 m inutes. After the baseline saliva collection, all future collections were done by the subjects themselves and labeled with the time of collection. The fluoride assays were performed blinded to treatment groups and were analyzed with the micro-diffusion method. This study was able to demonstrate that the 5% NaF varnish resulted in higher and more sustained levels of fluoride in saliva than the 0.05% NaF rinse. Salivary fluoride levels remained above baseline for up to 24 hours after the varnish application and for 2 hours after the rinse. For both groups the salivary fluoride levels peaked within 5 to 15 minutes. There was no carry over effect found in this study, and all subjects returned to baseline after a maximum of

32 hours. However, for both groups, a period effect was observed in the Area Under the Curve (AUC) data. Regardless of the order of treatments, the second period AUC's were higher for both the varnish and the rinse. This finding may indicate that some portion of fluoride remained in an un-measurable form, but then was released upon the application of more fluoride.

The amount of fluoride applied to each subject should have been included in the methods for direct comparisons. It is feasible that the collection of stimulated saliva with the Parafilm prematurely removed varnish and increased flow rate, which may have impacted the results. In addition the heavy reliance of subject compliance may have introduced errors.

A study conducted by Seppä et al.,[37] evaluated not only the fluoride concentration in saliva, but in that of the parotid saliva after the application of Fluor Protector and Duraphat fluoride varnishes. This study aimed to not only evaluate the amount of fluoride release into whole saliva, but to evaluate the amount of ingested fluoride that is present when using these slow releasing fluoride agents. Parotid salivary fluoride levels can be used as rough estimate of fluoride plasma values, and are less invasive to obtain. Forty-one participants were randomly divided into the Fluor Protector or

Duraphat group, and each had 0.5 ml of the varnish applied to the teeth according to manufacturer's instructions. Samples of stimulated parotid saliva were obtained at 0.5, 1, 2, 3, 4, 5, 6, 24, 27, and 30 hours after application, while whole resting saliva was obtained at 1, 2, 3, 6, 12, 24, 27, 30 and 48 hours after application. Samples were centrifuged, shaken and then fluoride concentration was determined using a fluoride-specific electrode. For whole saliva, the largest amount of fluoride in the saliva was seen at hour one, where a mean 12.77 µg/ml (SD 4.34) was seen in the Duraphat group, and 3.84 µg/ml (SD 2.12) was seen in the Fluor Protector group. By 24 hours the whole saliva fluoride levels were still elevated significantly above baseline, but there were no differences between the two varnishes. Baseline fluoride values were observed at 24 h, 27 h, and 30 h after application. In parotid gland saliva, the peak fluoride levels were observed 30 minutes after application for both varnishes. Fluoride values were significantly higher in parotid gland saliva after treatment with Duraphat during the first 5 hours. Baseline fluoride values were observed between 24 hours and 30 hours for parotid saliva in both varnish groups. This study effectively showed that differences can exist between two fluoride varnishes, however, it is hard to make a direct comparison because

these varnishes contain very different amounts of fluoride in their formulations (0.1% Fluor Protector, 2.26% Duraphat).

In 1999, Twetman et al.[38] performed a crossover study with eight subjects that evaluated the fluoride concentration in unstimulated whole saliva and paraffin-stimulated whole saliva after the application of Bifluorid 12 (6% F^-, 6% Ca^{2+}), Duraphat (2.26 % F^-), and Fluor Protector (0.1 % F^-). They applied the varnish to the buccal and lingual surfaces of all teeth and the occlusal surfaces of the molars and premolars. For Bifluorid and Fluor Protector they used 0.5 ml, and for Duraphat 0.75 ml. Whole unstimulated saliva was obtained by the passive drooling technique, and stimulated saliva was collected by means of chewing paraffin wax. Samples were centrifuged and the concentration of fluoride was determined using a fluoride sensitive electrode at baseline and 1, 6, 12, and 24 hours after fluoride application. A 6 week washout period was observed between test periods. Within the limitations of their study, they were able to determine that salivary fluoride levels did correlate with the different amounts of fluoride in the varnishes, however the correlation was not linear which indicated that the amount of fluoride availability could not be adequately predicted by simply knowing the amount of fluoride applied. Additionally, their results showed a trend of decreased amount of fluoride found in the unstimulated saliva compared to

the stimulated samples for both Duraphat and Fluor Protector. They hypothesized that chewing paraffin-wax dislodges varnish leading to higher levels of salivary fluoride levels in stimulated saliva. Because this study used varnishes with varying concentrations of fluoride, and they applied a different amount of Duraphat, a direct comparison of fluoride release differences among the three varnishes cannot be made.

Ritwik et al.[9] decided that although several studies were available regarding the release of fluoride over an extended time period, there was a lack of literature describing the immediate release of the fluoride from varnishes which they hypothesized to be the most important aspect from a clinical standpoint. In 2012 they designed an in vitro study to evaluate the fluoride release from Enamel Pro (Premier), PreviDent (Colgate), Vanish (Omni), and Vanish XT (Omni) that happened over a 48 hour time period. The first three products are varnishes while Omni Vanish XT is a light cured resin-modified glass ionomer. The products, described as having the exact same fluoride concentrations (5% NaF), were applied to a 5X5 enamel surface window of extracted teeth. The amount of varnish applied was accounted for by weighing the sample before and after varnish placement. Vanish XT wa s light cured for 20 s econds after placement per manufacturer's instructions. The teeth were immersed in artificial saliva after

treatment, and at intervals of 1, 2, 4, 8, 12, 24, and 48 hours, they were transferred to a new container of fresh artificial saliva. Each vial of artificial saliva was tested using a fluoride specific electrode. From their results, they were able to conclude that despite the similar fluoride concentrations found in each product, there were significant differences regarding their pattern, and amount of fluoride release. Enamel Pro showed the greatest amount of release within the first eight hours compared to the other varnishes, while Vanish XT showed low release in the first 4 hours but maintained the highest fluoride release after the 4 hour time period. They hypothesized that the carrier component of the varnish was responsible for the difference in this fluoride release.

This study was well designed and controlled, however the researchers did not state how they determined the concentration of fluoride in the Vanish XT. The product information guide does not state this is a 5% NaF concentration which has the potential to make direct comparisons with this product invalid.

In a recent study, Jablonowski et al.[10] evaluated in vitro fluoride release of four different fluoride containing products including three varnishes; Enamel Pro, Vanish, and Duraphat, as well as the light-cured resin-modified glass ionomer material, Vanish XT. Using twenty-five third

molars sectioned into four blocks, samples were divided into five different groups, one group for each varnish, plus a control group. Each varnish was applied to only the enamel portion of the tooth sections, a total of 3 mg was applied to each sample, except the control group. Samples were dried for 24 hours and then immersed in 30 ml of artificial saliva. Using a fluoride combination ion-selective electrode, the artificial saliva was tested to determine the concentration of fluoride after 30 minutes, daily, and then weekly until the concentration was below the electrode detection level. By comparing the fluoride concentrations of the different varnish-saliva solutions between specific time periods, a significant difference in fluoride release was found between the varnishes evaluated. It was determined that, for all the varnishes, the greatest rate of fluoride release was from baseline up to three weeks. The differences in rate of fluoride release at each time interval between the products was significant (Enamel Pro > Vanish > Duraphat > Vanish XT). The rate of overall fluoride release was significantly more for Enamel Pro than Duraphat; however Vanish and Vanish XT were not significantly different. Additionally, Enamel Pro displayed a greater cumulative amount of fluoride release than the other products. This study, although in vitro, was well designed demonstrated that there may be complex interactions between fluoride and the carrier

formulations that affect the overall fluoride release. Unfortunately they did not state the fluoride concentration in Vanish XT therefore it is hard to determine if the data from this product can be compared directly to the other products.

Experimental Design

Currently, the literature regarding fluoride release and bioavailability contains large variations in experimental design, limiting the possibility of comparing their results. Some of these experimental design components that will be discussed below include the type of saliva, collection and handling method, and analytical techniques used to determine the fluoride concentration in sa liva. These methods, for various reasons, have the greatest potential to affect variability in the study results.

Saliva Type

In his publication *Clinical Aspects of Salivary Biology for the Dental Clinician*, [39] Walsh describes up to eight major functions of human saliva important to oral health, one of which is to serve as a reservoir for ions involved in remineralization. He states that salivary flow, which can range from 0.03 ml/min up to ≥ 1 ml/min, is affected by stimulation and the time of day it is measured. The components of saliva are a combination of the minor and major salivary glands, and they may be considered a

representative of the blood serum. Whole saliva is 99% water, and is made up of mucinous saliva from the submandibular gland, serous saliva from the parotid gland, with minor contributions from both the sublingual and other minor salivary glands.

Saliva can be classified by either glandular or whole, and by either resting or stimulated. In many cases, analytes of interest to a researcher are produced in different concentrations by the different salivary glands. In this case, there may be a particular reason to sample the saliva coming from only one gland. For example, Seppä et al.[37] evaluated both whole saliva and parotid gland saliva fluoride levels in order to determine what percentage of the fluoride ion calculated came from release directly into saliva from the product, compared to that amount that was coming from the serum or blood represented by in the parotid gland saliva. Studies involving the quantification of systemic or ingested fluoride will commonly evaluate parotid saliva as a less invasive representation of blood fluoride levels. Whole saliva, which represents a mixture of all the saliva available in the oral cavity, is commonly used when calculating the fluoride release directly from a material into the oral cavity; because it represents the fluoride that would be available to act topically, which is fluoride's principal mechanism action. A shortcoming of this method is that in addition to measuring

fluoride released directly into saliva from the product, you may inadvertently be measuring fluoride currently in the blood stream that is released from the salivary glands. If the data collected happens within the first hour, this amount is thought to be negligible because serum fluoride levels peak 20-60 minutes after ingestion and then is excreted from the kidneys within 3-6 hours.[40]

In one study, conducted by Fukushima et al.[40] the differences between parotid duct saliva and whole saliva were examined. The aim of this study was to determine which saliva type, ductal or whole, would be a better indicator of exposure levels to fluoride. Using 300 subjects from five communities with different water fluoridation levels (0.0-1.68 mg/L), unstimulated whole saliva and parotid duct saliva were collected. The amount of fluoride found in each sample was then compared to the known amount of fluoride found in the community's drinking water. Age, gender, and geographical locations of the subjects were also analyzed to determine if any influence of these factors on salivary fluoride levels could be seen. Salivary fluoride levels were determined using an inverted ion-specific electrode. The results showed that the water fluoride concentration was the main factor influencing fluoride levels in parotid duct saliva, but not whole saliva. The authors concluded that parotid duct saliva is a better indicator of

systemic fluoride and can be considered a good biomarker for exposure to fluoride in above-optimum levels from water. This research supports the use of whole saliva rather than parotid saliva to detect fluoride bioavailability from direct release of high fluoride containing products. Ductal saliva analysis would reflect systemic fluoride levels which are not believed to be the main mechanism of action fluoride in caries prevention.

In support of Fukushima's findings, Olivby et al.[42] demonstrated that the concentration of excreted fluoride ion from the submandibular/sublingual, and parotid glands closely reflects the serum fluoride levels and can be a good predictor of systemic fluoride levels. This study also verified that glandular salivary fluoride concentration is independent of the glandular flow rate. This characteristic differs from fluoride analysis done from whole saliva.

The influence of flow rate on the bioavailability of fluoride in whole saliva was examined by Naumova et al.[43] In this study the whole saliva of ten different test subjects was analyzed to determine the concentration of the fluoride ion after delivery of either a 1450 µg/g NaF tablet (DENTTABS) or use of 1400 µg/g amine fluoride (EMLEX) dentifrice. Subjects were identified as either normal or fast salivary secretors based a five minute collection period. Subjects producing 0.3g-0.6 g/min were classified as

normal secretors, and those producing > 0.6 g/min were described as fast secretors. Subject baseline saliva was taken in addition to their saliva immediately after the use of the fluoride containing products. Using a cross-over study design all subjects repeated the two study arms five different times with a three day washout period in between. The result showed that salivary flow rate had a significant effect on the total amount of fluoride ion present in the saliva sample. Individuals with higher salivary flow rates tended to have saliva with a lower amount of fluoride.

Studies conducted by Zero et al.[44] and Brunn et al.[45] demonstrated the same negative correlation between salivary flow rate and fluoride ion concentration in whole saliva following the use of high fluoride products. Clinically, this may have implications in getting the desired amount of fluoride to stay around the oral cavity of individuals that have high salivary secretion rates. Experimentally, this complicates the ability to adequately determine the amount of fluoride release into saliva from different fluoride containing products due to possible flow rate differences among subjects. For this reason, cross-over study designs using this methodology of fluoride concentration analyses are indicated.

Finally, determining the preference for stimulated versus unstimulated saliva for sampling, there are several considerations. According to Walsh,[39]

the majority (60%) of resting saliva is derived from the submandibular gland which produces mostly mucinous saliva high in mucin proteins and calcium. Parotid saliva, in contrast, comprises only 20% of resting saliva, is high in bicarbonate and amylase and termed serous saliva. Submandibular salivary secretion rate increases by stimulation of chemoreceptors, while parotid gland secretion is mainly influenced by mechanoreceptor stimulation. Walsh contends that the protein content found in submandibular saliva has the potential to interfere or bind to free ions in saliva. For fluoride varnish, which is intended to adhere to tooth surfaces for prolonged fluoride release, the use of stimulation by chewing has the potential to prematurely dislodge the varnish, and increase salivary flow rates thereby leading to altered results. Stimulated saliva collection is advantageous in some cases because of the ability to rapidly collect larger amounts of saliva.

Collection and Handling

The method of saliva collection can play a large part in the acquisition and subsequent analysis of an analyte of interest. Salimetrics,[46] a corporation based in Pennsylvania, offers one of the most extensive, research supported literature for determining methods to obtain clean salivary samples for analysis of several different analytes. In their handbook *Saliva Collection and Handling Advice*, they outline proper ways to collect saliva, avoid

contaminants, and store samples for future use. For some analytes, variations can be seen based on diurnal cycle, stress levels, or salivary flow rates. Additionally, contaminants such as alcohol, food, caffeine, nicotine, medications or blood, must be considered. They recommend avoidance of food 60 minutes prior to sample collection and documentation of the subject's use of alcohol, caffeine, nicotine, or medications. They indicate that the passive drool technique is a cost effective way to obtain whole saliva that can be used for almost all analytes. In this method, the subject tilts his head forward and allows for unstimulated saliva to pool before letting the saliva flow into a collection vial. Saliva samples are then stored at -20ºC to -80ºC until analyzed.

Recently the handling techniques used in salivary fluoride analysis studies have been an area of interest. Historically, many studies centrifuged the salivary samples and analyzed the supernatant alone as a way to eliminate interference from fluorides found in salivary sediment which Gron et al.[47] hypothesized to reflect levels more similar to that found in plaque. Additionally, analysis of the supernatant has the advantage of creating clean samples with minimal interference from mucin protein globules which tend to become suspended in saliva creating a non-homogeneous solution.

Naumova et al.[48] conducted a study to determine if differences existed between the analyses of fluoride in the different phases of saliva. They hypothesized that the sediment, which contains cellular, proteins, and food debris, may be an important source of fluoride bioavailability, despite the fact that several studies examine the supernatant alone. Their specific aim was to assess the fluoride content found in the sediment and supernatant phases of saliva after centrifugation. In this cross-over study design, seven subjects either brushed with an amine fluoride dentifrice (EMLEX) containing 1400 ppm F⁻, or chewed a sodium fluoride tablet (DENTABS) containing 1450 ppm F⁻ at least 10 times and then brush with the particulate. All subjects completed both experimental arms twice and had a minimum seven day washout period between. Saliva samples were obtained at baseline, 3, 30, 120, and 360 minutes after brushing. 1.5 ml of saliva was centrifuged for 10 min at 3024 x g, and 1 ml of supernatant was removed from the sample, leaving 0.5ml of sediment. For analysis, equal parts of TISAB II were added to the samples. The samples of sediment and supernatant were analyzed with a fluoride-sensitive electrode. The results indicated three main things. First of all, there were significant differences between the amine and fluoride group in the total amount of fluoride found in both phases of saliva. Secondly, the dispersion of fluoride found in the

different phases was significantly different, with the amine fluoride group having more fluoride recovery from the sediment than the supernatant. Lastly, the overall amount of fluoride found in the different phases was significant for both groups, and the ratio of the mean sediment to supernatant fluoride ranged from 0.07-31.6 across the time periods. In general, the amount of fluoride found in the sediment was greater than that found in the supernatant for all time periods, for both fluoride groups. This study indicates that centrifugation prior to fluoride analysis may be missing an important portion of the total fluoride available from products.

Analytical Methods

Several methods exist to analyze trace levels of fluorides in materials. Well established methods include the potentiometric, gas chromatography, and the rapid diffusion techniques.

Evaluation of the literature suggests that for biological samples, the most common method is the potentiometric technique using an ion selective electrode (ISE). In this method, a lanthanum fluoride plate doped with europium^{++} is used at the base of a probe to quantify fluoride activity in a solution. This method is commonly used because of its rapid analytical capabilities, relative accuracy and ease of use. Frant and Ross[49] first described the use of this probe for evaluation of fluoride ion activity in 1966.

They described that the lanthium fluoride plate is impermeable to other ions and therefore the resulting relationship of fluoride ion activity to millivoltage readings follows Nernstian behavior from 1- 10^5 M. Aside from the hydroxide ion, which can be adjusted for by changing the pH of the solution, other ions do not appreciably interfere with the electrode. The method of pH adjustment used today is by use of a Total Ionic Strength Adjustment Buffer (TISAB) as described by Frant and Ross[50] two years later in 1968.

Frant and Ross were experimentally looking for a solution that would have three main purposes for its use in fluoride ion analysis. First they wanted a solution to buffer the solution to a pH less than 8.5 to a void hydroxide ion interference. Second they needed to increase the ionic strength of the water by adding more ions than were normally present in the solution. Finally they needed citrate present in order to complex Fe^{3+} and Al^{3+} to displace any bound fluoride to these ions. Their TISAB reagent consisted of 57 ml Acetic Acid, 58 g of NaCl, 0.30 g sodium citrate, and 500 ml H_2O. Experimentally, Frant and Ross were able to determine that the addition of this TISAB in a 1:1 ratio with their samples allowed for the lower direct determination of concentration in water to $\pm$ .005 ppm. Without the use of TISAB, 25% of their lower limit samples fell below the best fit

standard curve line. At the time they hypothesized that this solution would allow direct readings for low fluoride concentrations in a variety of aqueous solutions. The TISAB reagent is currently still used for both the direct and indirect analysis of low fluoride ion detection in solution, including saliva.

According to t he Department of Health and Human Services[1], the most accurate technique in fluoride analysis is the microdiffusion technique. In 1968 Taves[50] described this method using hexamethydisiloxane saturated acid as a way to speed up the process of diffusion. Although very accurate, this method is technique sensitive and requires more time than the direct method. According to Taves, greater than 97% recovery in one hour at room temperature is possible. Following diffusion of the fluoride ion into a sodium hydroxide trap, further analysis is required to determine the amount of fluoride trapped. This can be done with one of the other techniques of fluoride analysis such as the direct technique using a fluoride ion specific probe.

In an attempt to standardize the most common methods of fluoride ion analysis, Martinez-Mier et al.[52] conducted an experiment which evaluated a total of nine different labs analytical techniques and results from a set of standardized fluoride samples. These labs were all using either the direct technique, or the microdiffusion technique. Broken down into three phases,

labs were first instructed to describe their current technique and provide results for a set of biological and non-biological samples. After this, inconsistencies between the different techniques were identified and labs reanalyzed the samples using the various techniques documenting the results. Finally these results from the various techniques were distributed to the nine labs and a plan was made to standardize the techniques providing the most accurate results. After the technique was agreed upon, each lab re-analyzed the biological and non-biological samples and the intraclass correlation coefficient (ICC) was found to be 0.93 for the direct analysis and 0.90 for the microdiffusion technique. The specific recommendations for each technique were further outlined in this paper, which also concluded that samples below 0.0105 μmol F/g could be analyzed either with a blank correction or two-term polynomial regression equation. The authors concluded that ion-selective potentiometric methods were the technique of choice due to the ubiquitous use, ease of accessibility, and acceptable lower detection limit.

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Chapter 2

Abstract

Objective: To compare the release of fluoride into unstimulated whole saliva in vivo after the application of three different 5% NaF varnishes: Enamel Pro, Vanish, and Duraphat.

Experimental Methods: Following IRB approval, 15 subjects were recruited and consented based upon the inclusion/exclusion criteria of the study. A four-treatment randomized cross over study design with a 2-week washout period between treatments was used. Treatment consisted of the application of 0.4 ml of either a 5% NaF varnish, or a placebo (no fluoride) varnish applied to the buccal surfaces of all the teeth. After a minimum of 2 weeks washout period, the next randomly assigned treatment was given. All subjects received the 4 different treatments and during each treatment unstimulated whole saliva was obtained at baseline and 1, 4, 6, 26, and 50 hours. Following storage, saliva samples were centrifuged and the supernatant salivary fluoride was measured using a fluoride ion specific electrode to compare unknown values with a standard curve. Mixed linear effects models were used to evaluate the effects of varnish and time on salivary fluoride concentration. Significance was determined at 5% level for all tests.

Results: For time periods 1, 4, 6, and 26 hours, treatment with Duraphat and Vanish resulted in significantly higher mean concentrations of salivary fluoride than Enamel Pro, but were not different from each other. 50 hours after treatment, mean salivary fluoride levels for Duraphat were greater than all other treatments. For all the fluoride containing varnishes, the maximum amount of fluoride was measured at the 1 hour time point [D (18.94±9.95), V(19.78±14.57), EP (6.19±4)] and the fluoride concentration decreased at each time point thereafter. When treated with Enamel Pro and Vanish, mean baseline salivary fluoride concentrations were reached by 26 hours. Mean salivary fluoride concentrations with Duraphat treatment was still above baseline at the 26 hour collection point.

Conclusions: Salivary fluoride concentrations after treatment with Duraphat and Vanish are similar over 26 hours. Treatment with Enamel Pro resulted in significantly less fluoride in saliva over 26 hours. Despite the similar fluoride concentrations, the fluoride release into saliva from these varnishes over time differs. The reasons for this difference warrant further investigation.

Introduction

Fluoride varnish production and use has had a dramatic increase in the last decade following approval by the FDA in 1994 as a cavity liner and root desensitizer.[1] Despite its "off-label" use in caries prevention, research has demonstrated varnishes to be a safe, effective, and efficient way to deliver fluoride to patients at risk for dental caries.[2,3] Accordingly, fluoride varnishes are widely recommended for patients at high risk for dental caries (ADA, CDC, AAPD). Although the FDA did not approve fluoride varnishes until 1994, the first fluoride varnish was developed in the 1960's as a possible mechanism to enhance the treatment duration and uptake of fluoride.[4] Most of the clinical research on fluoride varnishes has been conducted using Duraphat (5% NaF), which was the first commercially available product.

Within the last few years, numerous varnishes with similar sodium fluoride concentrations (5%), but with multiple variations in carrier composition, have emerged and have taken a significant portion of the market share. As the number of fluoride varnishes available has increased, each company has created unique changes to the formula in order to improve properties like handling, appearance (i.e., white), flavor, or in some cases,

potentially active ingredients (e.g., tricalcium phosphate, amorphous calcium phosphate, calcium sodium phosphosilicate, xylitol, etc.),[5-8] leading to a claim of additional preventive benefits. Most of these new varnishes have not been studied in vivo for their caries reduction, efficacy, or safety.

Despite the similar 5% NaF concentration used in most of these varnishes, in vitro data have suggested that some of these secondary ingredients may affect the fluoride ion release of the product.[9,10,11] Fluoride release, and subsequent formation of calcium fluoride is thought to be an essential part of the mechanism of action of fluoride varnishes to prevent and remineralize carious lesions. Differences in fluoride release patterns can potentially enhance or diminish the efficacy and safety of a varnish. Therefore, understanding the differences in fluoride release pattern of varnishes with different formulations in vivo will help us understand which formula modifications have the potential to enhance or diminish the anticariogenicity and safety of the varnish. Several studies have compared fluoride varnishes to other delivery systems such as gels, foams, and pastes,[12,13] however, a void remains in the literature regarding the comparison of efficacy and safety between the many different new fluoride varnish systems containing 5% NaF widely available today.

The aim of this study was to evaluate the fluoride release from three different 5% NaF varnishes. By comparing the fluoride release as measured by concentration of fluoride in whole unstimulated saliva of participants, we wanted to gain some insight to the complex interaction of these trade secrets in vivo and the potential effect they may have on fluoride varnish efficacy.

Hypotheses

Ho1 - There will be no significant difference in the amount of fluoride release into saliva among the 3 different varnishes.

Ha1 – There will be a significant difference in the amount of fluoride release into saliva release among the 3 different varnishes.

Ho2 – There will be no significant difference in the timing of fluoride release in to saliva among the 3 different varnishes.

Ha1 – There will be a significant difference in the timing of fluoride release into saliva among the 3 different varnishes.

Methods and Materials

Subject Recruitment

Prior to subject recruitment, approval was obtained from the Institutional Review Board, University of Michigan Medical School (HUM00062943). The clinical trial was registered at ClinicalTrials.gov.

(NCT01629290). Subjects were subsequently recruited at the University of Michigan School of Dentistry primarily by word of mouth. Individuals who had signed the informed consent qualified for participation in the study unless they met any of the following criteria for exclusion: having less than 20 teeth, having significant untreated medical conditions, being pregnant or lactating, requiring pre-medication prior to dental treatment, having known allergies to fluoride varnishes, and having no history or current carious lesions. Additionally, subjects that stated they would not be available for each cycle of the study did not qualify for participation. Subjects meeting the inclusion criteria were further screened for adequate salivary production. Those subjects able to produce 2 ml of unstimulated saliva in a two minute time period were included in the study and given a subject number based on their order of consent.

A total of seventeen subjects were included in the study. Fifteen were given a number 1-15 and the remaining two served as back-ups. There were no drop outs during the study period, and the original fifteen subjects completed the 4 experimental arms.

Varnish Selection

The different fluoride varnishes were chosen for this study based on their identical concentration of sodium fluoride (5%), commercial

availability, presumed popularity, and clear use of different carrier materials and clinical appearance **(Table 1)**. Although all subjects lived in fluoridated communities and reported using fluoridated toothpaste, a placebo varnish, made by the Premier Dental Company, with no sodium fluoride was utilized as a means for comparison of background salivary fluoride concentrations. The placebo varnish was tested and the absence of fluoride in the formulation was verified. Like the other varnishes, the placebo varnish had unknown amounts of other ingredients but was presumed to be similar to the components found in the experimental varnish Enamel Pro.

Table 1. Experimental Varnishes

Varnish	Company	Active Ingredient	Marketed Additives
Vanish	3M ESPE (St.Paul, MN)	5% NaF	Tri-Calcium Phosphate (TCP)
Enamel Pro	Premier (Plymouth Meeting, PA)	5% NaF	Amorphous Calcium Phosphate (ACP)
Duraphat	Colgate-Palmolive (New York, NY)	5% NaF	None
Placebo	Premier (Plymouth Meeting, PA)	N/A	N/A

Varnish Application

Using the unique subject number and the different varnishes to be applied, a randomized table was created using a random number generator service (www.random.org) to determine the order of application for each

subject **(Table 2)**. The subjects were blinded to the order of varnish application and to the name of the varnish, however because of the uniqueness of each varnish (flavor, color, adherence etc.) the subjects had the potential to discriminate between the varnishes. Varnish application, saliva collection, and saliva analysis was carried out by the same person who was not blinded to varnish application, but was blinded to sample analysis.

Table 2. Randomized Treatment Sequence

Subject	Treatment 1	Treatment 2	Treatment 3	Treatment 4
1	Placebo	Enamel Pro	Duraphat	Vanish
2	Enamel Pro	Duraphat	Placebo	Vanish
3	Duraphat	Enamel Pro	Vanish	Placebo
4	Duraphat	Enamel Pro	Placebo	Vanish
5	Enamel Pro	Vanish	Placebo	Duraphat
6	Vanish	Enamel Pro	Duraphat	Placebo
7	Enamel Pro	Duraphat	Vanish	Placebo
8	Duraphat	Enamel Pro	Vanish	Placebo
9	Duraphat	Vanish	Placebo	Enamel Pro
10	Vanish	Enamel Pro	Placebo	Duraphat
11	Duraphat	Placebo	Enamel Pro	Vanish
12	Enamel Pro	Placebo	Vanish	Duraphat
13	Vanish	Duraphat	Placebo	Enamel Pro
14	Enamel Pro	Placebo	Duraphat	Vanish
15	Enamel Pro	Placebo	Vanish	Duraphat

Each active varnish (3) and the placebo varnish (1) was applied one time to each subject and saliva was collected over a total of 50 hours after application. Between different varnish applications a minimum of two weeks was allowed for wash-out from prior varnish applications, this protocol

applied to the placebo as well. In total, all subjects completed four rounds of varnish application and saliva collection. Varnish containers and brushes were weighed prior to the application and afterward for determination of the actual amount of varnish applied.

On the morning of varnish application, subjects were instructed to refrain from brushing. Subjects were given a new toothbrush when they presented to the clinic between 8 am and 10 am and were instructed to brush with a non-fluoridated toothpaste (Fruit Splash Training Toothpaste, Orajel Toddler, Chrurch & Dwight Inc. Princeton, NJ) for a total of 1 minute. Health history was reviewed and updated as necessary and each subject was given a soft tissue exam noting any pre-existing lesions or deviations from normal. Following the exam, subjects provided 2 ml of unstimulated, whole saliva using the drooling technique to serve as a baseline comparison (Heintze et al., 1983). In this technique subjects were seated in a quiet operatory and told to allow saliva to pool at the base of their mouth without sucking or stimulating flow. Saliva was then allowed to passively flow from their mouth into a medicine cup. This technique was used for all saliva collection times.

Using a soft tissue retractor (Optragate®, Ivoclar Vivadent, Amherst, NY) for isolation, teeth were lightly air dried with an air water syringe tip,

and 0.4 ml (actual amount determined later) of varnish was applied to the buccal surface of each tooth. In cases where varnish was leftover in the container after initial layer, another layer was applied over the first layer until no more was available for application. Following application, the Optragate® was removed, and the remaining varnish container, plus the application brush was saved for determining the actual amount applied.

Subjects were given instructions to avoid hard foods, alcohol, or warm liquids for 24 hours. These instructions were a combination of post application instructions provided by the different varnish companies. Additionally, aside from water, subjects were instructed to refrain from eating or drinking 1 hour prior to any of the saliva collection times, and subjects were given a list of foods potentially high in fluoride to avoid throughout the three day study period (i.e., sardines, green tea). All oral hygiene procedures were forbidden during the first 26 hours. After the 26 hour collection time, subjects were allowed to brush and floss with the non-fluoridated products provided. Each subject was provided with a new fluoride free toothbrush at the 26 hour collection period to avoid the use of their personal fluoride contaminated toothbrushes.

Sample Collection

At time periods of 1h, 4h, 6h, 26h, and 50h after varnish application,

subjects returned for saliva collection. At each collection period, a minimum of 2 m l of unstimulated, whole saliva was obtained by the drooling technique over a 5 minute time period. In total, subjects provided six 2 ml samples at each of the following time periods; baseline, 1 h, 4h, 6h, 26h, and 50h. Following a two week or greater wash out period, subjects returned for the application of a different varnish. All treatments were randomized and each subject participated in four study arms (three experimental, and one placebo) in which each subject ultimately received all treatments. Saliva was collected in a medicine cup and transferred to an eppendorph tube where it was stored within an hour of collection to a -18°C freezer. At the end of the day all samples were transferred to a -80°C freezer for future analysis. All known deviations from the time periods or protocol were recorded.

Sample Preparation

Saliva samples were removed from freezer and thawed at room temperature (24 ºC) for one hour. After thawing, samples were loaded into a centrifuge machine (Spectrafuge 16M, Labnet, Edison NJ) and centrifuged at 10,000 x g for 10 minutes. After removal, 0.7 ml of the supernatant was removed and added to a scintillation vial containing 0.7 ml of TSAB II (Orion TISAB II with CDTA, Thermo Scientific, Waltham, MA) for a total volume of 1.4 ml.

Creating a Fluoride Standard Curve

Prior to sample analysis, serial dilutions of a 0.1M fluoride standard (Orion Ionplus® Fluoride Standard, Thermo Scientific, Waltham, PA) were made to produce nine samples of known fluoride concentrations (0.02-10.0 ppm) The mV readings were obtained using the fluoride specific ion probe (Orion 4 Star pH ISE Benchtop, Thermo Scientific, Waltham, MA). The mV was then used to create a fluoride standard curve where determination of the fluoride concentration of the unknown samples could be compared. For the unknown samples that fell below 0.02 ppm based on the linear standard curve, a second two term polynomial regression curve was constructed to closer approximate the fluoride concentrations. This curve was remade each day prior to sample analysis, and was re-checked after every 2 hours of sample analysis to check for electrode sensitivity changes (drift). According to manufacture directions, drift of less than 3% is acceptable. There was no drift greater than 2% during the analysis of the samples.

Sample Analysis

To the 1.4 ml samples, a magnetic stir bar was added and they were placed on a magnetic stirrer (2 Mag Mix 15 Eco, Scragenhofstr, Muchen Germany) for a minimum of fifteen minutes before analyzing. The fluoride specific electrode (Orion 4 S tar pH ISE Benchtop, Thermo Scientific,

Waltham, MA) was submerged into the mix while the mix was being continuously stirred and the mV reading was recorded. This mV reading was compared to the fluoride standard curve made that day for the determination of ppm F⁻ in the sample. All samples were analyzed with this technique. All analyses were carried out at room temperature of 24 ℃.

Statistical Analysis

To determine if there was a significant difference in the amount of varnish applied to the subjects , a Kruskal-Wallis One Way Analysis of Variance on Ranks followed by a Pairwise Multiple Caparison Procedure (Tukey Test) was performed using a 5% significance level ($p < 0.05$).

To evaluate the effects of varnish and time on salivary fluoride concentration, linear mixed effects models were used. The models included the order of varnish application as a covariate, random effects for subject, and an unstructured variance/covariance matrix for the repeated measurements within each study period. The analyses were repeated using amount of varnish applied as a covariate. A natural logarithm transformation (not displayed) of the saliva fluoride measurements was used in the analyses. No adjustments were made for multiple comparisons, and a 5% significance level was used for each test.

Results

The results include data from 15 subjects, each having a total of three 5% NaF varnishes and a placebo applied during the course of the study. Table 3 displays the mean and standard deviation in g of product applied for each treatment. The amount of Vanish applied was found to be significantly less than that of the other varnishes. There was no significant difference in the amount of Duraphat, Enamel Pro, or Placebo applied.

Means and standard deviations of the concentration of fluoride in saliva (ppm) over time are shown in **Table 4.** Comparisons among the varnishes at specific time periods are displayed in rows. For 1 hour through 26 hours after treatment, subjects treated with Duraphat and Vanish had significantly higher salivary fluoride levels than those treated with Enamel Pro and Placebo. Treatment with Enamel Pro gave significantly higher levels of fluoride in saliva than the Placebo, but subject fluoride saliva levels after treatment with Duraphat and Vanish were not different from each other. After 50 hours, subjects treated with Duraphat had significantly higher amounts of fluoride in saliva than those treated with Vanish and Placebo, while none of the other treatments were different from each other. Although there was found to be a statistically significant lesser amount of Vanish applied compared to the other varnishes, this did not change any of the

results when taken into account in the analyses.

Table 3. Actual amount of product applied (± SD; in g)

Varnish	Mean (SD)
Duraphat	0.30 (0.07)[a]
Vanish	0.25 (0.04)[b]
EnamelPro	0.29 (0.04)[a]
Placebo	0.26 (0.05)[a]
Groups with same letters superscripts were not significantly different (p<0.05)	

Table 4. Mean concentration (± SD; in ppm) of fluoride in saliva over time

Time	Duraphat	Vanish	Enamel Pro	Placebo
B	0.07(0.04)[a1]	0.09(0.07)[a1]	0.09(0.07)[a1]	0.09(0.08)[a1]
1 HR	18.94(9.95)[a2]	19.78(14.57)[a2]	6.19(4.09)[b2]	0.02(0.02)[c2]
4 HR	3.39(4.83)[a3]	4.12(3.80)[a3]	0.67(0.36)[b3]	0.01(0.01)[c2]
6 HR	1.82(1.98)[a4]	1.15(0.79)[a4]	0.37(0.20)[b4]	0.03(0.04)[c2]
26 HR	0.19(0.15)[a5]	0.20(0.33)[a1]	0.04(0.02)[b5]	0.02(0.04)[b2]
50 HR	0.04(0.03)[a6]	0.02(0.03)[b5]	0.02(0.03)[ab6]	0.01(0.01)[b2]
Data in rows with the same letter superscript are not significantly different (p< 0.05) Data in columns with the same numeric superscripts are significantly different (p<0.05)				

Time point comparisons for each varnish are also shown in **Table 4**

displayed by column. For Duraphat, the fluoride concentration in saliva was

significantly higher than baseline for 1 hour through 26 hours after treatment

but was lower than baseline 50 hours after treatment, and decreased significantly between each time point after treatment. For Vanish, the fluoride concentration in saliva was significantly higher than baseline for 1 hour through 6 hours after treatment, was not different from baseline 26 hours after treatment, was lower than baseline 50 hours after treatment, and decreased significantly between each time point after treatment. For Enamel Pro, the fluoride concentration in saliva was significantly higher than baseline for 1 hour through 6 hours after treatment, was lower than baseline 26 and 50 hours after treatment, and decreased significantly between each time point after treatment except for no significant change between 26 and 50 hours. For Placebo, the concentration of fluoride in saliva was significantly higher at baseline than at any time point after treatment, and there were no significant differences among any time points after treatment.

The differences in the concentration of fluoride in saliva are displayed graphically in figures 1 and 2. **Figure 1** displays the mean and standard deviation of the entire sample for each varnish (n=15). **Figure 2** displays the individual variation in salivary fluoride concentration of the subjects for each of the different varnishes applied.
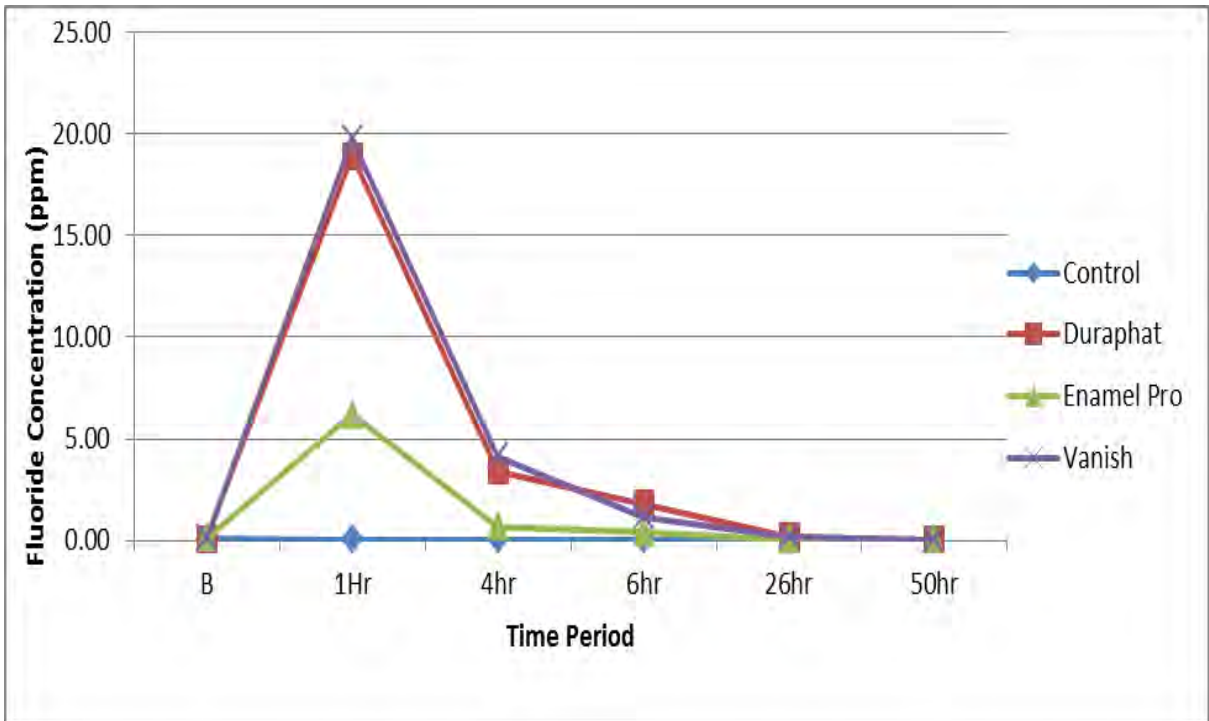
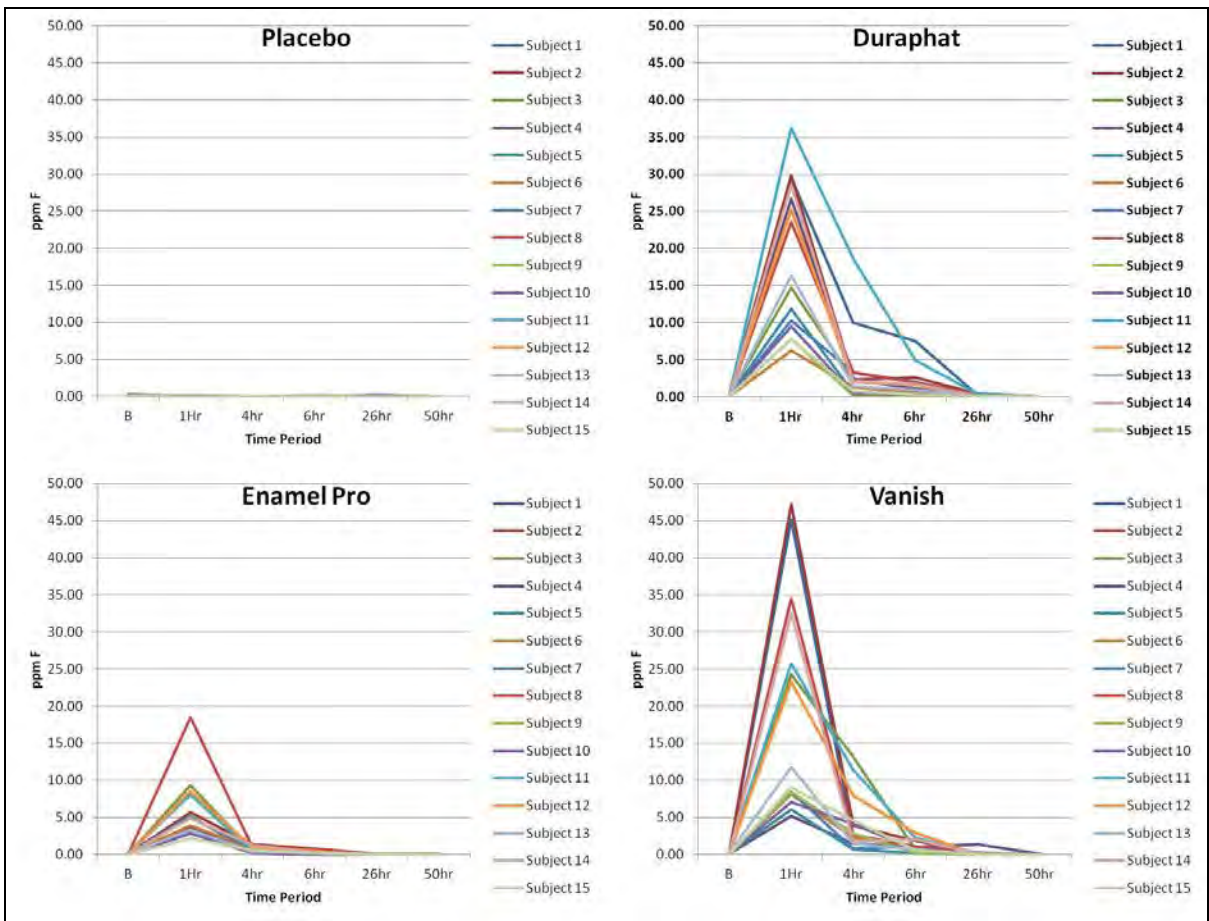**Figure 1. Mean concentration of fluoride in saliva varnish over time**

Figure 2. Subject variation in treatment response

Discussion

Saliva is the initial medium into which fluoride that is released from dental products is able to achieve the desired effect intra-orally. This study evaluated saliva in order to make comparisons between different brands of topical fluoride varnishes with similar fluoride concentrations in order to observe potential differences in their in vivo fluoride release. The primary null hypothesis was rejected based on the statistical analysis indicating a significant difference between the fluoride concentrations found in saliva after treatment with the different 5% NaF varnishes. The primary alternate hypothesis failed to be rejected because subjects had significantly less fluoride in saliva when treated with Enamel Pro compared to the other products. These findings, although different than the findings in several in vitro studies [9,10,11] in which Enamel Pro released more fluoride, still support the notion that factors other than the concentration of fluoride may play a role in the overall fluoride release of a varnish.

Unlike many of the previous studies evaluating fluoride release into saliva,[12,14,15] a conscious effort was made to apply equal amounts of the fluoride product to each subject in order to determine if properties other than the total amount of fluoride applied affected the fluoride concentration found in saliva. Despite this effort, statistical analysis showed that the amount of

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Vanish applied was significantly less than that of the other products. This finding was not a surprise to the operator as it was noted in some early trial runs that in addition to color and odor differences, the high viscosity of Vanish made it more difficult to remove from the product container and application brush in comparison to the other varnishes. When the difference in amount of varnish applied was used as a covariate in the analysis of varnish and time effects on salivary fluoride levels, it was not found to have a significant influence on the results.

Although differences in previous study designs make direct comparisons to the current study difficult, the studies conducted by Seppä[14] and Twetman[15] allow for some comparisons. In both these studies, Duraphat 5% NaF was used, whole unstimulated saliva was collected at various time intervals, and the samples were centrifuged prior to analysis. While the one hour time period mean salivary fluoride in the current study was found to be 18.94 (±9.95) ppm, Twetman and Seppä's were 13.37(±4.70), and 12.37 (±4.34) respectively at the same time period. While this difference may not appear large, Twetman applied nearly twice as much (0.75 ml) Duraphat than in the current study (0.4 ml). It would seem reasonable that Twetman should have recorded significantly higher one hour time period salivary fluoride levels than in the current study, however study design differences,

such as the application technique, post application instructions, and population, may explain these differences. Twetman applied fluoride to all surfaces of the teeth, including the occlusal. He did not dictate any pre or post application avoidance of food or drink, and his population consisted of school aged children. In contrast, the current study dictated that subjects refrain from eating or drinking one hour prior to collection times, which meant that after initial application subjects did not have any food or drink before the 1 hour collection time period. Additionally, in the current study, the varnish was only applied to the buccal surfaces of teeth in adults. By applying varnish to the occlusal surfaces of school aged children and allowing for post application eating and drinking, a large portion of the varnish may have abraded or dissolved away by the one hour time period accounting for the unexpectedly lower fluoride levels (considering the relatively high amount of product application) at the one hour time period of Twetman's study. Seppä reported applying nearly similar amounts of Duraphat as the current study (0.5 ml vs. 0.4 ml), however she did not report using any form of isolation during application. As a result, the lower amount of fluoride in saliva at the 1 hour time period may have been from early ingestion or inadvertent application of varnish on the soft tissues.

The mean ppm of fluoride in saliva for the individual varnishes over time demonstrates that the overall pattern of fluoride release among the products was very similar. The highest fluoride concentration in saliva was seen for all the products in the first collection period after application (1 hour) and the fluoride concentration in saliva began to decline after this at each subsequent collection period. This finding, which supports the secondary null hypothesis, is consistent with the findings by other authors that have found a peak in salivary fluoride levels within minutes of application followed by a steady decrease.[12,14,15] Although the logistics of this study prevented saliva collection minutes after application, it is likely, based on the study by Eakle et al.,[12] that the 1 hour salivary fluoride concentration in this study represents a point on the declination of the ppm curve. Eakle et al. measured peak levels of fluoride in saliva five to fifteen minutes after fluoride application, which was not evaluated in this study.

Although it has been demonstrated that fluoride can be stored in soft and hard oral tissues as well as dental plaque,[16,17] it is presumed that saliva represents the initial medium into which fluoride is released. The relative ease of collection and measurement make saliva a great candidate for in vivo fluoride release studies; however fluoride activity likely continues long after the 50 hours it was measurable in saliva. One assumption that has been made

when conducting salivary fluoride concentration studies is that the distribution of fluoride released in saliva to other oral tissues and plaque is similar. As future studies begin to demonstrate the complexity of these products and their reactions in biological systems, we may find that the distribution of fluoride to enamel, soft tissue, plaque, etc. differs among varnishes.

In addition to the portion of fluoride that potentially becomes bound to other oral structures or plaque, in vivo studies involving saliva also are complicated by the fact that the medium is constantly being ingested. In vitro, it is possible to evaluate total fluoride release from the products because the fluoride is released into a closed system (artificial saliva). Because the total fluoride release in vivo cannot be accounted for, we cannot conclude that Enamel Pro releases less fluoride, we can only conclude within the limitations of this study that the amount of fluoride released into saliva is less for subjects treated with Enamel Pro. If the distribution of fluoride to other oral tissues and plaque are directly related to salivary fluoride levels, this lesser amount of release from Enamel Pro may diminish its potential efficacy as a topical fluoride agent, however this requires further evaluation.

Differences in viscosity, which was a subjective finding in this study, may be one of many differences in physical properties of interest when evaluating fluoride release from varnishes in vivo. Abrasion resistance, solubility, adhesion, etc. may also account for differences between the in vivo and in vitro results. Teeth are undoubtedly subjected to many mechanical forces unaccounted for in laboratory studies that may affect the retention and subsequent release of fluoride into saliva. It is possible that although Enamel Pro has the potential to release more fluoride as demonstrated in vitro, it was mechanically unable to withstand the oral environment as well as the other varnishes and therefore was dissociated from the teeth and swallowed before reaching this potential.

It is important to appreciate that before any conclusions regarding the efficacy between different fluoride varnishes can be made, several future investigations must be considered. Only by accounting for the total amount of fluoride released in vivo, by both systemic fluoride measurements after varnish application and measurements of levels in soft tissue, hard tissue, plaque etc., can true comparisons of fluoride release between products be made. Chemical, physical, and mechanical properties studies may also help to determine if there is a link between the total fluoride release and particular properties of the varnish. These types of studies, paired with studies further

evaluating the mechanism of action of topical fluoride, may advance our

understanding of varnish efficacy.

Conclusions

From this study which evaluated the fluoride concentration of saliva after the application of three different 5% NaF varnishes, the following conclusions can be made:

1. Despite similar concentrations of fluoride, the amount of fluoride released into saliva from Enamel Pro is significantly less than that of Vanish or Duraphat up to 26 hours after application.

2. Vanish and Duraphat release similar amounts of fluoride up to 26 hours, but Duraphat sustains a higher release than Vanish up to 50 hours after application.

3. All three varnishes maintain above baseline salivary fluoride levels up to 6 hours.

4. Duraphat and Vanish maintain above baseline salivary fluoride levels up to 26 hours, although Vanish was not significant due to a higher standard deviation.

5. Enamel Pro, Vanish, and Duraphat release the maximum amount of fluoride into saliva after application and the levels decrease at each time period thereafter.

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