INVESTIGATIONS OF THE ANTI-CARIES POTENTIAL OF FLUORIDE VARNISHES

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DEDICATION

This thesis is dedicated to my beloved family, for all the emotional support throughout my educational quest.
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Laila Adel Al Dehailan

INVESTIGATIONS OF THE ANTI-CARIES POTENTIAL OF FLUORIDE VARNISHES

The majority of currently marketed fluoride varnishes (FV) have not been evaluated for their effectiveness in preventing dental caries. Fundamental research on FVs and how different formulations affect adherence to teeth, fluoride release into saliva and uptake by teeth is virtually non-existent. The objective of this work was to investigate the anti-caries potential, measured as fluoride release into saliva, change in surface microhardness of early enamel caries lesions, and enamel fluoride uptake, of multiple commercially available FVs. We have found that FVs differed in their release characteristics, rehardening capability, and ability to deliver fluoride to demineralized lesions. In addition to our in vitro work, we have conducted a clinical study that aimed to compare saliva and plaque fluid fluoride concentrations following the application of three commercially available FV treatments at predetermined post application time points. We also investigated the change in fluoride concentration in saliva and plaque fluid fluoride from baseline to each post application predetermined time point. We found that FVs varied in their release of fluoride into saliva and plaque fluid but shared common trends in release characteristics. The outcomes of our in vitro and in vivo investigations demonstrate a great variation in anti-caries potential of FVs. This may be attributed to different compositions and physical properties of the tested FVs.

E. A. Martinez-Mier, D.D.S., Ph.D., Chair
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INTRODUCTION
Dental Caries and Fluoride Varnishes

Dental caries remains the most common chronic disease globally, affecting 60-90% of school-aged children and a significant number of adults.¹ Topically applied fluoride has contributed to major reductions in both the incidence and prevalence of dental caries. It has also been shown to be safe and effective.² Fluoride has the ability to inhibit the demineralization process, enhance remineralization, and inhibit bacterial enzymes found in dental plaque.³,⁴ Nowadays, a vast range of professionally applied topical fluoride products exists, including rinses, gels, foams, drops, and varnishes. Fluoride varnishes (FV) are relatively simplistic delivery vehicles for cariostatic amounts of fluoride and typically contain 5% sodium fluoride. FV offers several advantages over other modalities of topical fluoride treatment such as effectiveness, relative safety and ease of application.⁵,⁶ The prolonged contact time with the dentition and extended release of fluoride over a longer period of time compared to other topical vehicles gives FV an advantage over other forms of fluoride delivery.⁷-¹⁰

Current Regulations and Recommendations

In 1994, the US Food and Drug Administration approved fluoride varnishes as cavity liners and dentin hypersensitivity treatments. However, most dental professionals in the US use fluoride varnishes off-label for the prevention of dental caries.⁷,¹⁰

Despite the American Dental Association Council on Scientific Affairs’ evidence-based clinical recommendation for people at risk of developing dental caries that “FV containing 2.26 percent fluoride applied at least twice per year is effective in preventing caries for patients 6 years or older”, the current regulatory situation has created a ‘grey
area’ for manufacturers (a recent search brought more than 30 different manufacturers to daylight). Thus, unlike for fluoride dentifrices and rinses, no efficacy testing is required for FV, or in other words – the majority of currently marketed FV have not been evaluated for their effectiveness in preventing caries or their toxicity. Considering the importance of a professional caries intervention and the fact that FV are typically only applied every 3-6 months, every effort should be taken to ensure a maximum benefit to risk ratio.

**Relevance of Existing Research**

Several recent studies highlighted considerable differences in fluoride release characteristics between FVs from different manufacturers, not only in terms of cumulative amount of fluoride released over time, but also in the kinetics of fluoride release. The clinical relevance of these findings is unknown due to the lack of comparable FV clinical trials on caries progression and reversal. Fundamental research on FV and how different formulations affect adherence to teeth, fluoride release into saliva and uptake by teeth – to name their most important aspects - is virtually non-existent. Thus, further research is required first to establish a baseline before experimental work can commence.

**In Vitro Models and Outcome Variables**

pH cycling models were designed to simulate the dynamic variations in mineral saturation and pH associated with the natural caries process. They mimic specific events of the caries process under controlled conditions and allow the investigation of individual mechanistic variables which would be extremely difficult to do under *in vivo* conditions. At the same time, it is important to recognize the limitations of *in vitro*
experiments in their ability to reproduce the whole complexity of caries dynamics. *In vitro* experiments provide only limited information on the effects of different variables on the caries process. This must be taken into consideration when *in vitro* data are extrapolated to *in vivo* conditions.

Many response variables can be used to investigate the efficacy of fluoride treatments. One of these is hardness measurement, which can quantitatively describe the depth of artificial lesions.\textsuperscript{14} Hardness measurement has been proven to have adequate sensitivity to detect early changes in the outer layer of enamel.\textsuperscript{15, 16} However, this technique has its limitations. The size of the indentation is highly influenced by water and organic content of tissue. This has a bigger impact when analyzing dentin and may affect the analysis of results.\textsuperscript{17} Also, hardness measurement is unable to give a clear explanation on changes that occur deep within a carious lesion.\textsuperscript{18}

Fluoride uptake is a widely used assessment tool to determine the amount of fluoride that has been incorporated in enamel lesions following fluoride treatment.\textsuperscript{18} It is considered as an important research method for testing new formulations for their anti-caries activity. Reduction in dental caries, increased levels of remineralization and elevated resistance to acid challenge, has been linked to increased incorporation of fluoride into enamel, however it is still unclear how enamel fluoride uptake (EFU) correlates with anti-caries activity.\textsuperscript{19-23} One way to assess enamel fluoride uptake is by using the acid etch technique which has demonstrated excellent precision and accuracy.\textsuperscript{24, 25}

The mode of action of FV is not fully understood; however, the bioavailability of fluoride in the oral cavity has been proven to be essential in caries prevention. Low levels
of fluoride over prolonged periods of time have been shown to be effective in preventing
demineralization and enhancing remineralization.\textsuperscript{26, 27} Measuring the levels of fluoride
over time in dental biofilms (plaque, plaque fluid and saliva) is one way to demonstrate
its bioavailability and consequently its effect on caries activity. This method has been
used as a research tool to investigate the anti-caries efficacy of several fluoride
treatments.\textsuperscript{7, 28, 29}

\textbf{Clinical Investigations on Fluoride Release Kinetics}

Current anti-caries models emphasize on the significance of maintaining
cariostatic levels of fluoride in oral fluids, namely saliva and dental plaque.\textsuperscript{30} Therefore,
studying fluoride concentration changes in saliva and dental plaque following the
administration of topical fluoride is one way to determine efficacy as it can be indicative
of the of fluoride levels in the aqueous phase available for interaction with the tooth
structure.\textsuperscript{31}

Very few studies on the kinetics of fluoride in saliva following the topical
application of FV have been reported. A study by (Twetman \textit{et al.}, 1999) showed that
fluoride levels in saliva are significantly elevated following the application of FV. They
also found that fluoride levels in saliva returned to baseline within 6 h for any of the
tested FV. The results of their study suggest a correlation between the concentration of
fluoride in FV and the fluoride levels in saliva following the application of varnish.\textsuperscript{32}

Only one published study investigated fluoride concentration in plaque after
topical application of FVs in adolescents at 3 days, 7 days and 30 days post treatment.
The study investigated FVs with varying concentrations of fluoride. They found an
elevation in fluoride concentration in plaque fluid following the application of FVs that
lasted for up to one week. Some FVs demonstrated an increase in fluoride levels in plaque that lasted for 30 days. Their work suggested different patterns of fluoride retention for different varnishes that is time and dose dependant.33

The use of FV as an effective caries prevention modality is widely accepted. However, there is no evidence as to which in vitro parameters are more relevant in predicting clinical efficacy of FV. Furthermore, there is lack in clinical studies that investigate the efficacy and fluoride release kinetics of different formulations. Therefore, the overall aim of this project was to characterize FVs based on in vitro outcome variables that may predict FVs anti-caries efficacy and to clinically study the kinetics of fluoride release into saliva and dental plaque from different FVs selected based on the results of the initial in vitro investigations.

**Specific Aims**

Specific Aim 1: To investigate the potential anti-caries effect of five commercially available FVs on artificially created early caries lesions through the following outcome variables: a) fluoride 6 h release into artificial saliva; b) microhardness; and, c) enamel fluoride uptake, after pH cycling for 5 days.

Specific Aim 2: To investigate the potential anti-caries effect of fourteen commercially available FVs on artificially created early caries lesions through the following outcome variables: a) fluoride 24 h release into artificial saliva; b) microhardness; and, c) enamel fluoride uptake, after pH cycling for five-day with a second demineralization challenge.

Specific Aim 3: To investigate fluoride levels in saliva and plaque fluid of children aged seven to eleven years after the topical application of three commercially
available FVs selected based on the results of the two previous laboratory investigations.
CHAPTER ONE: THE EFFECT OF FLUORIDE VARNISHES ON CARIES

LESIONS
MATERIALS AND METHODS

Specimen Preparation

Enamel specimens obtained from bovine teeth were used as the hard tissue test substrate. The teeth were cut into $4 \times 4$ mm specimens using a Buehler Isomet low-speed saw. The teeth were stored in deionized water saturated with thymol during the sample preparation process. The $4 \times 4$ mm specimens were ground and polished to create flat surfaces to facilitate surface microhardness testing using Struers Rotopol 31/Rotoforce 4 polishing unit (Struers Inc., Cleveland, PA, USA). The dentin and enamel sides of the specimens were ground flat to a uniform thickness with 500-grit silicon carbide grinding paper. As a final cleaning step, the specimens were sonicated in a detergent solution (Micro-90 concentrated cleaning solution with 2% dilution) for 3 min. The specimens were finally assessed under Nikon SMZ 1500 stereomicroscope at $10\times$ magnification.

Accepted specimens had no obvious cracks, areas of hypomineralization or other flaws in the enamel surface. Specimens were then embedded in acrylic resin (ClaroCit Kit, Struers) using a 1.5 inch mounting mold (Struers FlexiForm). Specimens were arranged to ensure they were not in contact with each other and with the enamel surface facing downwards. The resin was poured carefully over the specimens to a height of approximately 1 to 2 cm. Once the resin had cured, the specimens embedded in the disc (18 specimens per disc) were polished to mirror flatness as described above with a final polishing step using 4000-grit paper followed by 1 µm diamond polishing suspension. Eighteen specimens per FV treatment group were used for this study with a total of 90 specimens.
Early Caries Lesion Creation

The demineralization protocol is based on that by (White, 1987) and has been extensively studied using a variety of techniques over the years. Artificial lesions were formed in the enamel specimens of each disc by a 48 h immersion into a solution of 0.1 M lactic acid and 0.2% Carbopol C907 which was 50% saturated with hydroxyapatite and adjusted to pH 5.0 (using KOH). Demineralization was performed at 37°C at a ratio of 10 ml of solution per specimen. The resulting lesions are early, shallow, subsurface lesions with an average depth of approximately 50 µm.

Demineralization (Baseline) Microhardness

Initial hardness of the demineralized specimens was determined using a Vickers microhardness indenter (M247AT Leco Corporation, St. Joseph, MI, USA) at a load of 200 g for 15 s. The average specimen surface microhardness (VHN$_{lesion}$) was determined from four indentations on the surface of each specimen.

Fluoride Varnish Application

A list of the tested products and their active ingredients can be found in Table 1. Each disc with the polished, embedded specimens was placed back into the mounting mold.

The protective foil from the individual FV dose was removed and the FV mixed using the manufacturer’s application (typically a microbrush) for at least 10 s to homogenize the FV, as sedimentation of NaF and phase separation may have occurred during storage. Subsequently, FVs were evenly applied to the surface of each of the discs using the manufacturer’s applicator. The amount of FV applied was recorded. The
average amount applied to each treatment group consisting of 18 specimens was 0.13 g and ranged between 0.10 to 0.18 g.

**Saliva Incubation**

Immediately after FV application, 7.5 ml of artificial saliva (AS) that had been pre-heated to 37 °C was pipetted carefully over the disc in the mounting mold. The mold was then placed in an incubator set at 37°C. AS formulation was based on that by (Hara et al., 2008) and had the following composition: 2.20 g/l gastric mucin, 1.45 mM CaCl$_2$×2H$_2$O, 5.4 mM KH$_2$PO$_4$, 28.4 mM NaCl, 14.9 mM KCl and was adjusted to pH 7.0 with KOH.$^{36}$

Every 15 min for a total of 6 h, the 7.5 ml AS were renewed by pouring the AS in the mold into a separate pre-weighed container to determine the weight of AS, then carefully pipetting fresh AS into the mold and placing the mold back into the incubator for another 15 min.

These collected AS samples were then processed for fluoride analysis. An aliquot was removed and analyzed for fluoride using an ion-selective electrode (Model 9609BNWP, Orion Research, Boston, MA, USA) and meter by comparison to a similarly prepared standard curve. Fluoride data were calculated as µg F/mg FV.

**Fluoride Varnish Removal**

After the last AS sample collection, 10 ml of chloroform was poured over the disc to dissolve any remaining FV. The mold was placed into a suitable container to prevent evaporation of the chloroform. The mold/container was gently shaken for 5 min to accelerate the dissolution process. This process is repeated at least once and until there are no visible signs of FV left on the specimens.
Enamel Fluoride Uptake (EFU)

The fluoride content of the enamel in each of the discs was determined using a modification of the acid etch technique by Sakkab et al.\textsuperscript{37} Approximately half of the enamel surface of each specimen was covered with nail varnish to protect an area of the specimen for the subsequent pH cycling phase. Each disc was placed back into its mold. Specimens in each disc were acid etched by pouring 5 ml of 1M HClO\textsubscript{4} over each disc for 1 min. The acid etch solution was then collected. Immediately after the etching, the specimens were rinsed thoroughly with deionized water. The acid etch procedure was repeated four more times, with each acid etch solution being collected separately. A sample of each acid etch solution was buffered with TISAB II (0.25 ml sample, 0.5 ml TISAB II and 0.25 ml 1N NaOH) and the fluoride content determined by comparison to a similarly prepared standard curve (1 ml standard+ 1 ml TISAB II). Data from multiple etches for each group were combined to calculate EFU.

pH Cycling Phase

Before pH cycling, the nail varnish that protected half of the specimen during etching for EFU was removed using acetone, and the etched half was painted with nail varnish. The cyclic treatment regimen for each of the five discs containing the demineralized specimens is provided in Table 2. Fluoride treatments were performed using slurries of Crest Cavity Protection (0.243 percent sodium fluoride; Procter and Gamble, Mason, Ohio, USA). The slurry was prepared by adding toothpaste to AS at a ratio of 1:2 w/w (dentifrice:AS) in a beaker with a magnetic stirrer. A fresh treatment for each subgroup was prepared just prior to each treatment. After the treatments, the
specimen discs were rinsed with running deionized water and placed back into AS. The remaining time (~20 h) the discs were in AS. The regimen was repeated for 5 days.

**Post Treatment Microhardness**

The average specimen microhardness was determined, as previously described, from four indentations on the surface of each specimen, next to the baseline indentations (VHN\textsubscript{post}). The difference between the hardness after lesion creation and the pH cycling phase was calculated as follows: ΔVHN = VHN\textsubscript{post} − VHN\textsubscript{lesion}

**Statistical Analysis**

Data analysis was performed using one-way analysis of variance (ANOVA). An overall significance of (α=0.05) was used. Pair wise comparisons between the groups was conducted using Student Newman Keuls test. Pearson correlation coefficients were calculated to investigate associations between the study variables.

**Results**

The results for all study variables can be found in Figures 1 (ΔVHN), 2 (fluoride release profiles), 3 (EFU) and Tables 3 (ΔVHN) and 4 (cumulative fluoride release and peak fluoride concentration).

Treatment with Enamel Pro resulted in significantly greater lesion surface rehardening compared to all other tested FV. MI Varnish exhibited greater rehardening than Vanish, but was similar to PreviDent and Flor-Opal. There were no differences between PreviDent, Flor-Opal and Vanish.

The fluoride release from FV showed commonalities and dissimilarities. Overall, fluoride release profiles were somewhat similar between FV as all showed a gradual decrease in released fluoride over time. However, differences were apparent in the shape
and slope of the release curves as well as the cumulative amount of released fluoride and the highest released fluoride concentration. For example, while both MI Varnish and Enamel Pro exhibited similar initial fluoride releases, MI Varnish released more fluoride than Enamel Pro during the first 3 h whereas Enamel Pro showed a more gradual decrease and released more fluoride during the latter 3 h of the chosen experimental period. Vanish released less than 1/10\(^{th}\) of fluoride in comparison to MI Varnish and Vanish’ peak fluoride concentration was approximately 1/20\(^{th}\) of that of Enamel Pro. The EFU data was not significantly different for all FVs tested. Enamel Pro had the highest EFU followed by PreviDent with both delivering more than twice as much fluoride compared to the other FV.

There was no linear correlation between the main variables: ΔVHN vs. cumulative fluoride release (r = 0.61; p = 0.28), ΔVHN vs. EFU (r = 0.72; p = 0.17), and cumulative fluoride release vs. EFU (r = 0.01; p = 0.99).
CHAPTER TWO: AN IN-VITRO INVESTIGATION OF ANTI-CARIES

EFFICACY OF FLUORIDE VARNISHES
MATERIALS AND METHODS

Specimen Preparation

Enamel specimens obtained from bovine teeth were used as the hard tissue test substrate. Selection of the tooth for processing was based on the quality of the enamel and whether the particular tooth surface has sufficient size to obtain a large enough specimen to meet the study requirements. Tooth sections with white spots, cracks and other defects were rejected. The tooth sections were cut into 5 × 5 mm specimens using a Buehler Isomet low-speed saw. The teeth were stored in deionized water saturated with thymol during the sample preparation process. The 5 × 5 mm specimens were ground and polished to create flat surfaces to facilitate surface microhardness testing using Struers RotoPol 31 / RotoForce 4 polishing unit (Struers Inc., Cleveland, PA, USA). The bottom side of the specimens was ground flat to a uniform thickness with 500-grit silicon carbide grinding paper. The topside of the specimens was ground using 1200-grit paper until most of the tooth surface is flattened. The specimens were sonicated in deionized water between each grinding/polishing step. As a final cleaning step, the polished specimens were sonicated in 2 % microliquid. The specimens were assessed with a magnification of 10×.

To be acceptable for the study the specimens were required to:

a) have a minimum 5 × 5 mm polished facet across the surface;

b) not have any obvious cracks or other flaws in the enamel surface;

c) have an evenly polished, high gloss enamel surface;

d) have no contamination on the top surface from sticky wax or any other material.
Specimens were then secured, polished enamel side facing upwards, on a one-inch square acrylic block using sticky wax to facilitate surface microhardness measurements. When necessary, an acetone-moistened cotton swab was used to clean the polished enamel surface area to remove any visible debris. Then, all specimen surface areas apart from the polished top surface were covered with a colored nail varnish (Sally Hansen Advanced, Hard As Nails Nail Polish, Red, USA). Prepared specimens were stored at approximately 100% relative humidity and 4°C until further use. A total of 216 specimens were required for the present study (18 groups of 12 specimens each).

**Sound Enamel Microhardness**

Initial surface microhardness of the sound enamel specimens was determined using a Vickers microhardness indenter at a load of 200 g for 11 s (Instron T2100B Vickers Surface Microhardness Tester, Norwood, MA, USA). The average sound enamel microhardness ($V_{HN_{sound}}$) was determined from five indentations on the surface of each specimen. Only specimens with $300 \leq V_{HN_{sound}} \leq 400$ were accepted into the study.

**Artificial Lesion Creation**

Artificial lesions were formed in the enamel specimens by a 48 h immersion at 37°C into a solution of 0.1 M lactic acid and 0.2% Carbopol C907 which was 50% saturated with hydroxyapatite and adjusted to pH 5.0 using KOH. Specimens were placed into air-tight containers (16 specimens fit into one container). Then, the demineralization solution (approximately 30 ml per specimen) was added, lid secured and the container transferred to an incubator. After approximately 8 h, the specimens were checked for bubbles that were moved by shaking the container. After 48 h, the demineralization
solution was decanted and the specimens rinsed with deionized water for approximately 1 min. Specimens were blotted dry with a tissue and stored at approximately 100% relative humidity and 4° C until further use.

**Lesion Baseline Microhardness**

Microhardness of the demineralized enamel specimens was determined as described above. The average specimen lesion baseline microhardness ($VHN_{\text{lesion}}$) was calculated. Only specimens with $25 \leq VHN_{\text{lesion}} \leq 60$ were accepted into the study. Specimens were assigned to treatment groups ($n = 12$) based on a randomization procedure that resulted in treatment groups with statistically significantly indifferent mean $VHN_{\text{lesion}}$.

**Specimen Mounting**

Once assigned to their treatment groups, specimens were removed from their acrylic blocks and mounted onto the inside of a lid of a 12 well microtiter plate as per Figure 4. Acrylic blocks (12×12×9 mm) were mounted onto the inside of the lid using acrylic glue. Then, specimens were mounted enamel side facing upwards onto the acrylic block using sticky wax.

**Fluoride Varnish Application**

The products tested in this study were assigned to groups and labeled as per Table 5. In addition to the test FVs shown above, two additional experimental groups were included, one placebo group (O-; no FV treatment, no toothpaste treatment during pH cycling phase) and one positive control group (O+; no FV treatment, toothpaste treatment during pH cycling phase). The placebo varnish had the following composition (all w/w):
2% shellac; 10% ethyl cellulose; 40% ethyl acetate; 2% polyvinylpyrrolidone; 2% xylitol; 5% NaCl; 39% ethanol and was manufactured in-house especially for this study. The protective foil from the individual FV dose was removed and the FV mixed using the manufacturer’s application (typically a microbrush) for at least 10 s to homogenize the FV, as sedimentation of NaF and phase separation may have occurred during storage. Duraphat (group C) was supplied in a tube. For this FV, approximately 0.5 ml was squeezed into a small weighing cup and processed as described above. The placebo varnish was handled in a similar manner.

Subsequently, FV was applied to the surface of each specimen using a single brush stroke and using the manufacturer’s applicator (typically a microbrush). Any unused FV was discarded.

**Saliva Incubation**

Immediately after FV application, the lid containing 12 specimens was placed onto the microtiter plate containing 4.0 ml of artificial saliva per well. AS had the same composition as previous experiment. Two batches of this solution were prepared, one for the FV incubation phase in all specimens and one for the pH cycling phase for all specimens.

The microtiter plate was then placed in an incubator set at 37 °C. Every hour for 6 h, the lid was removed and rinsed under a stream of running deionized water for exactly 1 min with all specimens on the lid rinsed in an equal manner. After rinsing, the lid was placed onto a new microtiter plate containing 4.0 ml fresh AS per well.

This procedure was repeated until a total AS exposure time of 6 h has been reached. After 6 h, the lid was placed onto a new microtiter plate containing 4.0 ml AS.
per well which was placed in the incubator for 18 h. After 18 h, the lid was removed and rinsed again as described above. All saliva samples were frozen immediately after each cycle and retained for analysis of ionic fluoride.

Then, a soft toothbrush (Oral B P40, Procter & Gamble, USA) was used to brush each specimen. A slurry of Crest Cavity Protection (0.243 percent sodium fluoride; Procter and Gamble, Mason, Ohio, USA) at a ratio of 1:2 w/w (dentifrice:AS) was prepared in a beaker with a magnetic stirrer. The toothbrush was dipped into the slurry briefly (approximately 2 s). Each specimen was then brushed under a stream of deionized water for 20 s with the specimen being rinsed another 10 s after brushing. This procedure was repeated until all specimens on the lid have been brushed. A new toothbrush and slurry was used for each lid. After this procedure, the pH cycling phase commenced on the same day.

**pH Cycling Phase - Remineralization**

Table 6 highlights the treatment groups per week. As it was anticipated that only a total of six treatment groups can be handled per day, the present study was separated into three phases which each phase containing a placebo group to allow for comparisons between phases.

The cyclic treatment regimen for each lid containing the specimens consisted of a 4 h/d acid challenge in the lesion forming solution and one, one-minute treatment period with a slurry of aforementioned toothpaste (prepared as described above). One slurry per day was prepared and pipetted into each well of the used microtiter plates. The specimens were stored in AS throughout the remainder of the pH cycling phase.
The pH cycling was performed by placing the lid containing the specimens onto different microtiter plates containing 4.0 ml each per well of toothpaste slurry, AS or lesion forming solution. The study was conducted at room temperature. After each treatment, the specimens were rinsed under running deionized water briefly (approximately 2 s per specimen). The regimen was repeated for 5 days. The treatment schedule for this experiment is given in Table 7.

After completion of the pH cycling phase, all specimens were carefully removed from the lids and remounted onto an acrylic block to facilitate microhardness and enamel fluoride uptake measurements.

**Post Treatment Microhardness**

The average specimen microhardness \( (VHN_{\text{post}}) \) was determined again in the same manner it was done while obtaining lesion and baseline microhardness.

**Enamel Fluoride Uptake**

The fluoride content of the enamel specimens was determined using the microdrill technique. The enamel specimens were mounted perpendicular to the long axis of a micro end mill attached to a specially-designed microdrill, and drilled to a depth of 100 µm through the entire lesion. The drilling and sample collection were performed in a static-controlled atmosphere to prevent loss of enamel powder due to charging effects. The enamel powder sample was transferred to a diffusion dish and then analyzed for fluoride. The diameter of the drill hole was determined using a calibrated microscope interfaced with an image analysis system. Indentations for microhardness testing and microdrill holes were placed on enamel specimens according to Figure 5.
**Acid Resistance Test**

To test whether the various FV impart acid resistance to the enamel specimens after pH cycling, a second *in vitro* demineralization treatment was performed and following the same protocol as described above but utilizing a demineralization time of only 8 h. The average specimen microhardness (VHN_{art}) was determined again as described above.

**Artificial Saliva Fluoride Analysis**

The collected, frozen AS samples was thawed. An aliquot is removed and analyzed for fluoride by comparison to a similarly prepared standard curve using an ion-selective electrode (Model 9609BNWP, Orion Research, Boston, MA, USA) and meter. Individual as well as cumulative F release data were calculated to determine [F]_{max} (highest F concentration found in any collected AS sample over the 24 h period) as well as F_{total} (the total amount of F released from FV).

**Statistical Analysis**

One-way analysis of variance (ANOVA) statistical method was used for data analysis. An overall significance of (α=0.05) was used. Pair wise comparisons between the groups were conducted using Student Newman Keuls test. Pearson correlation coefficients were calculated to investigate associations between the study variables.

**Results**

The results for ΔVHN can be found in Table 8, Figures 6 and 7. Nupro was the numerically best performing FV with a ΔVHN (post – lesion)= 24.3. However, Nupro did not result in significant lesion rehardening when compared to other FV under investigation.
Specimens treated with Vella had the least rehardening capability, with a $\Delta VHN_{(post-lesion)} = 11.7$ and was not statistically different than placebo.

For $\Delta VHN_{(art-lesion)}$, specimens treated with Sparkle had the highest rehardening values following the second acid challenge but were only significantly different from those treated with Vella. All other FVs did not statistically affect lesion rehardening differently. All FVs performed better than placebo.

For fluoride release total ($F_{total}$), MI significantly released the highest amount of fluoride over the 24 h incubation period of the experiment ($F_{total} = 14.97 \, \mu g/ml$). Enamel Pro came second with almost 1/3 less fluoride release than MI. Fluoride release total from Enamel Pro was not significantly different than Flor Opal and Nupro. Butler White significantly released the least amount of fluoride ($F_{total} = 0.50 \, \mu g/ml$) and was approximately 1/30th of that of MI. Results are demonstrated in Table 9 and Figure 8.

The highest concentration of fluoride at any given time point was for MI ($[F]_{max} = 9.71$), however, it was not statically different from Enamel Pro ($[F]_{max} = 5.44$). Butler White exhibited the least concentration ($[F]_{max} = 0.17$) and was significantly lower than all other FV under study (Figure 9).

Differences between FVs were less prominent for EFU than for $F_{total}$. PreviDent treated lesions exhibited the numerically highest EFU, which was not significantly different from most of FVs under investigation apart from Cavity Shield, MI, Flor Opal and Butler White (Figure 10).

There was a significant linear correlation between $\Delta VHN_{(post-lesion)}$ vs. EFU ($r = 0.69; p = 0.00135$); however, there was no linear correlation between $\Delta VHN_{(post-lesion)}$ vs. $F_{total}$ ($r = 0.41; p = 0.917$), and between $F_{total}$ vs. EFU ($r = 0.23; p = 0.359$).
CHAPTER THREE: FLUORIDE CONCENTRATION IN SALIVA AND PLAQUE FLUID FOLLOWING THE APPLICATION OF THREE COMMERCIALLY AVAILABLE FLUORIDE VARNISHES: A CLINICAL STUDY
MATERIALS AND METHODS

This was a blinded (from laboratory analysis), randomized cross-over, three-period study in healthy children aged six to eleven years that evaluated the concentration of salivary fluoride and plaque fluid fluoride following the application of three commercially available FVs.

Prior to subject recruitment, approval was obtained from the Indiana University Institutional Review Board (1409221212). Parental informed consent and child assent were collected prior to conduction of this study. The study was conducted at Little Flower Catholic School, Indianapolis, IN.

Based on a previous study on adults (Eackle et al., 2004)\textsuperscript{38}, with a sample size of 16 subjects, the study has a 80\% power to detect a difference of 1.5 for log (AUC) between any two treatments, assuming two-sided tests each conducted at a 5\% significance level, the within-subject correlation is 0.5, and the standard deviation is 2.0. To account for 10\% dropout, the study enrolled 18 subjects.

Subjects had to meet the following inclusion and exclusion criteria in order to be considered to participate in the study:

\textit{Inclusion Criteria}

1. Age and Gender

   Children (boys and girls) must be seven to eleven years old.

2. General Health

   Subjects must have good general and oral health with no clinically significant medical history or oral disease that could interfere with the subjects’ safety or study evaluations during the length of study.
3. Dental condition

   a) Have a sufficient number of teeth to obtain adequate plaque samples (at least 16 teeth).

   b) Have no current dental caries activity, oral soft tissue lesions or periodontal disease including severe gingivitis or cavitated carious lesions that may compromise the health of subjects or study evaluation.

4. Compliance

   a) Subject should understand and is willing, able and likely to comply with study.

   b) Subject must be able to abstain from eating for one hour prior to test visit and for the two hour duration of visit.

**Exclusion Criteria**

1. Allergy/tolerance

   Known or suspected allergy or hypersensitivity to FV or any of their listed ingredients.

2. Fluoride

   Taking fluoride supplements or other fluoride products for medical purposes except for fluoride naturally accruing in diet (Phase 2 only).

3. Antibiotic Use

   Taking any prescription antibiotics for any medical purpose

4. Personnel

   A member of the subject’s immediate family living in the same household as one of the site study staff directly working on the study.
Fluoride Varnish Treatments and Washout Toothpaste

No experimental fluoride varnish was used in this study. Fluoride varnishes contained a standard fluoride level of 5% NaF and were supplied in single dose packages. FV packages were weighed before and after treatment application in order to calculate the amount of varnish applied. The three fluoride varnishes used in this study were (Table 10):

1. CavityShield (CS) 5% Sodium Fluoride Varnish (3M ESPE Dental).
2. Vanish (V) 5% Sodium Fluoride White Varnish with Tri-Calcium Phosphate (3M ESPE Dental).

Subjects were asked to use fluoride-free toothpaste (Tom’s of Maine Fluoride Free Children’s Toothpaste) for a washout period of 2 weeks prior to the administration of the first treatment and for the duration of the study. A two-week washout period is common in the literature for studies involving FV. A study by ³⁸ reported that baseline fluoride values in the second period have returned to values that are very close to baseline fluoride in the first period following a washout period of two weeks.

Randomization Procedures

A unique screening number was used for all subjects screened for study participation. In addition, the study statistician created a randomization schedule to determine the order of treatment application for each subject (Table 11). Due to the uniqueness of each FV (color, flavor, handling properties) the study investigator had the capability to discriminate between varnishes and was therefore blinded to sample analysis rather than varnish application.
Clinical Procedures and Methodologies

The investigator completed an oral soft and hard tissue (OSHT) examination at a screening visit to ensure only subjects eligible are enrolled into the study. At all other visits an oral soft tissue (OST) exam only was performed. Subjects were instructed not to brush their teeth or perform any oral hygiene on treatments days and on 24 and 48 h collection time points.

Subjects provided baseline (BL) five-minute, non-stimulated saliva sample, followed by collection of interproximal/buccal plaque sample from all teeth immediately prior to assigned FV treatment. FV treatment was applied on all teeth surfaces including buccal occlusal third/lingual/occlusal of posterior teeth and facial incisal third/lingual of anterior teeth. The FV treatment was allowed to set then immediately after saliva samples were collected at 30, 60, 120 min, 24 and 48 h following the treatment. Approximately 1 mg of interproximal/buccal plaque was collected immediately after each saliva sample. Subjects remained at school throughout treatment visits. The study investigator brushed the occlusal surfaces of the child’s teeth with water and a new tooth brush (Oral B Indicator Soft, Procter & Gamble, USA) at the end of each visit. A 2 week washout period with fluoride-free toothpaste was observed between treatments to allow fluoride to reach baseline levels.

Saliva Collection Procedure

Unstimulated whole saliva samples was collected at baseline and immediately following treatment at 30, 60, 120 min and at 24 and 48 h. Saliva collection was initiated by having the subjects swallow all the residual saliva in their mouth, and then let saliva pool in their mouths for the five-minute period while their heads are tilted forward. As
the subjects felt the need to swallow, they expectorated into a plastic re-sealable collection vial. At the end of the five-min collection period all remaining saliva was expectorated into the plastic vial.

Saliva samples were stored for in a freezer at -20°C for later fluoride analysis.

**Plaque Collection Procedure**

Immediately before dental plaque collection, subjects were instructed to swallow all remaining saliva and keep their mouth open. Approximately 1 mg of dental plaque was collected from the interproximal and buccal surfaces of teeth of all four quadrants. Plaque samples were collected using a standardized protocol. Pooled plaque samples were collected using a stainless steel periodontal scaler (S. McCall 17/18 or IU 17/18) from each interproximal area from buccal aspect and buccal area starting from the upper right quadrant to the upper left, lower left and ending in lower right quadrant. The pooled plaque sample was transferred into a plastic strip.

**Laboratory Procedures**

*Plaque Sample Preparation*

Prior to plaque sample collection, special centrifuge tubes were constructed by heat sealing 10 microliter (µl) micropipette tips. They were filled with heavy mineral oil (WSM oil). Microcentrifuge tubes containing the plastic strip and plaque sample were centrifuged for 10 min at 10,000 rpm (4,000g) at 4°C.\(^{39}\) Partially oil-filled fine glass micro pipettes were used to recover small aliquots (approximately 5 nanoliters) from the centrifuged tube under a microscope.
Plaque Fluid Fluoride Analysis

The micro analytical method of (Vogel et. al., 1990) was used to analyze plaque fluid samples for fluoride content:40

1. Samples were placed, under mineral oil, on the surface of a specially constructed inverted F electrode. Mineral oil was used to prevent evaporation.

2. Total ionic strength adjusting solution (TISAB III) was added to the samples in a ratio of 9:1.

3. The tip of a micro-reference electrode was placed in contact with the sample to complete the circuit.

Triplicate analyses were performed on each pooled plaque fluid sample. Plaque fluid fluoride was expressed as µg F/g, which was calculated by comparison to a standard fluoride curve, constructed the same day of the analysis.

Saliva Analysis

The concentration of fluoride was measured in all saliva samples. Each saliva sample was analyzed as whole and centrifuged for fluoride level. A 1.4 ml of each saliva sample was centrifuged 10 min at 10,000 rpm (4,000 g) at 7°C. Analysis of saliva was conducted using a modification of the hexamethyl-disiloxane (HMDS,.) microdiffusion method of (Taves, 1968)41 as modified by (Martinez-Mier et al., 2011)42. One ml of centrifuged saliva sample was pipetted into plastic Petri dishes (Falcon 15-cm plastic Petri dishes), adding enough deionized water to bring final volume in each Petri dish to 3.0 ml. A 0.05 sodium hydroxide analytical reagent (NaOH, A.R.) 50 microliters (µl) trap solution was placed in five drops on the Petri dish lid and after the addition of 1 ml of sulfuric acid (H₂SO₄) saturated with HMDS through a small hole in the lid of the Petri
dish, each dish was immediately tightly sealed with petroleum jelly. During overnight diffusion, fluoride was released by acid hydrolysis and was trapped in the NaOH. The trap was recovered and buffered to pH 5.2 with 25 µl acetic acid (CH₃COOH.). The recovered solution was adjusted to a final volume of 100 ml with deionized water. Analyses were performed in sets of approximately 40 samples. Fluoride was measured using a fluoride combination electrode (Model 9609BNWP, Orion Research, Boston, MA, USA) and meter. The fluoride content (µg F) of the samples was calculated from a standard curve constructed from fluoride standards and microdiffused at the same time as the samples.

The amount of total fluoride in the samples was calculated based on the amount of fluoride divided by the volume of the sample and expressed as µg F/ml of sample. The amount of fluoride delivered by the varnish to the saliva over the period of the study, i.e. the area under the salivary F clearance curve, was calculated via the trapezium method.

**Statistical Analysis**

Intra-examiner repeatability and inter-examiner agreement of the fluoride measurements were evaluated using intraclass correlation coefficients. AUC was calculated using the trapezoidal method. Statistical analysis for AUC was performed using a linear mixed-effects model suitable for a crossover design. The model included factors for treatment sequence and baseline fluoride level as covariates, treatment and period as fixed factors, with subject as random factor. Pair-wise comparisons among the three treatments will be made if the treatment main effect is significant, with no multiple comparisons adjustment for the individual pair-wise tests. Analyses of the individual
collection times were made using similar models, with additional factors for time and the treatment-by-time interaction. Correlation coefficients were calculated to evaluate the associations among the fluoride measurements and between the fluoride measurements and amount of varnish applied. Analyses used the log-transformed data to satisfy the model assumptions. All analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC).

**Results**

The results include data from 18 subjects as all subjects completed the study. During fluoride collection and analysis, notations were made to identify potentially problematic values such as any protocol deviation. For example, two subjects were most likely exposed to a fluoride source close to saliva sampling at 24 h. Data analyses were performed with and without the outlier values and the conclusions were nearly identical. Therefore, only the results from the test without the outliers are presented here.

Intra-examiner repeatability (ICC=0.93) and inter-examiner agreement (ICC=0.96) were both acceptable. Table 12 displays the mean and standard deviation of amount of FV applied in g. Mean and standard error of concentration of fluoride in whole and centrifuged saliva, and plaque fluid are shown in Table 13. The baseline fluoride values ranged from 0.02 to 0.03 ppm in centrifuged saliva and from 0.20 to 0.80 ppm in plaque fluid. In general, all values returned to baseline or close to baseline levels after 24 h.

**AUC**

Mean concentration of fluoride over time can be found in Figure 11 (whole saliva), Figure 12 (centrifuged saliva) and Figure 13 (plaque fluid). EP had significantly lower centrifuged saliva fluoride AUC than CS (p=0.0006) and V (p=0.0008) but CS and
V had nearly identical values and were not significantly different from each other (p=0.86). V had significantly higher plaque fluid fluoride AUC than CS (p=0.0116) and EP (p=0.0065) but CS and EP were not significantly different from each other (p=0.27). No significant treatment effect was found for whole saliva fluoride AUC (p=0.79).

**Time Points Comparisons**

Comparisons among FVs at specific time points were also investigated. The time-by-treatment interaction was significant only for centrifuged saliva (p=0.0319) and plaque fluid fluoride (p=0.0312). EP had significantly lower centrifuged saliva fluoride than CS and V at 30 min (p=0.0002 and p=0.0033, respectively), at 60 min (p<0.0001, p<0.0001), and at 120 min (p=0.0050, p=0.0045); EP was not different from CS or V at baseline (p=0.64, p=0.57), 24 h (p=0.85, p=0.49), or 48 h (p=0.88, p=0.91). CS and V did not have significantly different centrifuged saliva fluoride regardless of time (p=0.89).

**Centrifuged Saliva**

For all FVs under investigation, centrifuged saliva fluoride was significantly higher at 30 min than at any other time (p≤0.0001), followed by 60 min (p≤0.0001) and 120 min (p≤0.0001), with no significant differences among baseline, 24 h, and 48 h (p=0.26 for baseline vs. 24 h, p=0.89 for baseline vs. 48 h, p=0.37 for 24 h vs. 48 h).

**Plaque Fluid**

It was found that at 30 min EP had significantly lower plaque fluid fluoride than CS (p=0.0449) and V (p=0.0006) but CS and V were not different from each other (p=0.16). At 60 min, EP (p=0.0045) and CS (p=0.0353) had significantly lower plaque fluid fluoride than V but EP and CS were not different from each other (p=0.48). No treatment effect was found at baseline (p=0.88), 120 min (p=0.11), 24 h (p=0.55), or 48 h.
(p=0.40). For CS, plaque fluid fluoride was significantly higher at 30 min than at any other time (p<0.01), followed by 60 and 120 min (p<0.0001), with no significant differences among baseline, 24, and 48 h (p=0.19 for baseline vs. 24 h, p=0.59 for baseline vs. 48 h, p=0.41 for 24 vs. 48 h) or between 60 and 120 min (p=0.70). For EP, plaque fluid fluoride was significantly higher for 30, 60, and 120 min compared to baseline, 24, and 48 h (p<0.0001), while there were no differences among baseline, 24, and 48 h (p=0.18 for baseline vs. 24 h, p=0.65 for baseline vs. 48 h, p=0.33 for 24 vs. 48 h) or among 30, 60, and 120 min (p=0.26 for 30 vs. 60 min, p=0.78 for 30 vs. 120 min, p=0.29 for 60 vs 120 min). For V, plaque fluid fluoride was significantly higher for 30, 60, and 120 min compared to baseline, 24, and 48 h (p<0.0001), and higher for 30 than 120 min (p=0.0030), while there were no differences among baseline, 24, and 48 h (p=0.44 for baseline vs. 24 h, p=0.82 for baseline vs. 48 h, p=0.24 for 24 vs 48 h) and no differences between 60 and 30 min (p=0.11) or 120 min (p=0.21).

Correlations between Study Variables

Whole and centrifuged saliva fluoride AUC were highly correlated for CS (r=0.84), EP (r=0.82), and V (r=0.88). Plaque fluid fluoride AUC was moderately correlated with whole saliva fluoride AUC (r=0.44, p=0.09) and centrifuged saliva fluoride AUC (r=0.44, p=0.08) for CS but not for the other two treatments. Many of the individual time points had moderate to high correlations between whole and centrifuged saliva fluoride, but plaque fluid fluoride was rarely associated with whole or centrifuged saliva fluoride at the individual time points.
DISCUSSION
The clinical efficacy of fluoride varnishes in preventing dental caries has been well documented in the literature.\textsuperscript{12, 43} Fluoride varnishes last for a limited time in the oral cavity and are quickly removed by the action of mastication and oral hygiene practices. Therefore, varnishes are designed to release their active ingredients in a relatively short time that has been estimated to be up to 24 h.\textsuperscript{28, 44} Since the introduction of the first fluoride varnishes, researchers have been striving to improve FV by testing new formulations that aim to better deliver fluoride in varnishes.\textsuperscript{45, 46} However, fundamental research on how different formulations affect fluoride release into saliva, uptake by teeth, and changes in microhardness of enamel after FV application is needed to establish a baseline for product comparisons.

Our first experiment aimed to evaluate the effect of five commercially available FV products on caries lesions by investigating the amount of fluoride released from each varnish into AS; the amount of fluoride delivered to early enamel carious lesions; and the extent of surface rehardening of these lesions as a result of a FV treatment. To the authors’ knowledge, this is one of the first in vitro studies to employ three response variables (F release, EFU and VHN) and to study correlations among them. The chosen experimental design was based on previous studies\textsuperscript{7, 12, 28, 47} while taking into account findings from preliminary in-house investigations (unpublished data).

Our first study findings indicate that varnishes containing amorphous calcium phosphate (ACP) forming salts and casein-phosphopeptide-amorphous calcium phosphate (CPP-ACP) demonstrated significantly higher ability to reharden early carious lesions than the other tested FV. This may be explained by the higher amounts of available calcium and phosphate ions from varnishes containing ACP forming salts.
Recently, it was shown that ACP forming varnish formulations delivered more fluoride than formulations containing tri-calcium phosphate (TCP) to both sound and demineralized enamel. This was likely due to the non-crystalline structure of ACP that makes it more soluble and reactive compared to TCP that is an insoluble crystalline form of calcium phosphate.\textsuperscript{44}

The results of our first study demonstrate a wide variation in total fluoride release over six hours from the five varnishes under investigation. This wide variation in fluoride release amount and characteristics is difficult to explain since manufacturers are not required to provide exact formulation details. However, this variation may be due to the differences in additives or type of resin carriers (natural vs. synthetic) used. It has been postulated that fluoride ion diffusion is slower in varnishes with a natural resin base; however, this was not observed in this study.\textsuperscript{47, 48} For example, Flor-Opal has a natural resin base (rosin) and released more fluoride than Vanish that has a synthetic resin base.

The highest release from all varnishes was within the first 15 min to 1 h of application and is similar to another study.\textsuperscript{47} In our first study, it was found that the highest total fluoride release over the period of six hours was from a varnish containing CPP-ACP as an additional active ingredient, while the least amount of release was from a varnish with functionalized tri-calcium phosphate. These findings are in agreement with another study and are consistent with the high water solubility and bioavailable nature of CPP-ACP contained within these varnishes.\textsuperscript{49}

The present findings for EFU for our first experiment are in contrast to our expectations for some of the evaluated FVs. For example, MI exhibited the greatest level
of total fluoride release and a high level of rehardening value but a low level of EFU. This FV contains CPP-ACP and was found to release relatively high amounts of inorganic phosphate.\textsuperscript{28} High levels of inorganic phosphate have been found to negatively impact the formation of CaF\textsubscript{2} thereby reducing the amount of bioavailable fluoride ion that is required for remineralization, and this may be an explanation for the lower level of EFU for MI varnish.

In our first experiment, we were unable to observe correlations between the outcome variables. This is in agreement with a prior study in our laboratory which employed a similar range of FVs.\textsuperscript{10} For example, a FV that demonstrated a high fluoride release into saliva did not necessarily result in a high EFU value or enhanced remineralization. It is important to note that while there are similarities in the experimental models between studies, they were inherently different. Most importantly, the present study was concerned with FV effects on lesions after a pH cycling phase to mimic the short term effect of FV on lesions whereas our previous study was solely concerned with the immediate effect of FV on lesions. The observed differences in FV performance but consistencies in lack of correlation between variables highlight some of the shortcomings of laboratory research on FV. In the absence of a clinically validated \textit{in vitro} model to determine the efficacy of FV, results from the present and previous laboratory studies need to be seen with caution.

In the second \textit{in vitro} study, we aimed to investigate the efficacy of fifteen commercially available FVs using the same outcome variables in our first study. While the two studies investigated the effect of FVs on caries lesions, the study models were fundamentally different. The wide variation in performance of FV in our first experiment
prompted the design of our second \textit{in vitro} study. We aspired to have a better understating of how different formulations affect efficacy of FVs. Therefore, in our second experiment we aimed to investigate a larger variety of FVs in an attempt to have a range of products that represent different formulations. Also, in the second study FVs were left on the specimens for a prolonged period of 24 h vs. the 6 hr period of the first study to better simulate the clinical situation.

The second \textit{in vitro} experiment’s results for $\Delta VHN_{\text{(post – lesion)}}$ and for $\Delta VHN_{\text{(art – lesion)}}$ were unanticipated. As there are numerical differences in lesion rehardening values from different FVs, most of the FVs did not statistically differ from each other. As previously mentioned, it is hard to thoroughly interpret the results due to lack of detailed information on different FV formulations. However, there seems to be a superior effect on lesion rehardening when calcium and phosphate containing ingredients are added to the formulation. This is in agreement with our first \textit{in vitro} study and with data available in the literature, and is explained in detail in the first segment of the discussion. It is important to note that the enhanced rehardening effect from calcium and phosphate containing ingredients did not withstand the second acid challenge. This is in contrast to a recent systematic review that suggested a possible long term effect (> 3 months) of CPP-ACP complexes on early caries lesions, however the results cannot be extrapolated as the review investigated CPP-ACP alone due to insufficient evidence on the complex’s synergistic effect with fluoride.\textsuperscript{50} Another noteworthy finding is that lesions treated with Vanish, a FV containing functionalized TCP (fTCP), had a higher $\Delta VHN_{\text{(post – lesion)}}$ than those treated with FVs containing ACP and CPP-ACP. This may be due to the protective effect of functionalization of the TCP molecule that prevents premature interaction.
between calcium and fluoride and aids in remineralization in a manner similar to that of fluoride.\textsuperscript{51}

The fluoride release data in our second \textit{in vitro} experiment is in agreement with our previously mentioned in house experiment and (Cochrane \textit{et al.}, 2014).\textsuperscript{28} Calcium containing FVs, with the exception of fTCP containing varnishes, were able to release significantly higher levels of fluoride into saliva. This suggests a synergistic effect of adding casein complexes to FVs on fluoride release and may be explained by the bioavailable nature of ACP and CPP-ACP compared to the less soluble fTCP.\textsuperscript{44, 51}

EFU data from our second study extends our results from our aforementioned first \textit{in vitro} investigation. Once again, a CPP-ACP containing varnish delivered less fluoride into caries lesions. Our explanation is noted earlier in the discussion section. Vanish, a FV containing fTCP, delivered more fluoride into lesions compared to CPP-ACP and ACP containing FV in spite of its low fluoride release into saliva. This is in agreement with a study that compared EFU from two varnishes, one containing fTCP and the other containing CPP-ACP.\textsuperscript{44} Also, another study compared EFU with and without fTCP. It was found that lesions exposed to fluoride in conjunction with fTCP had significantly higher fluoride uptake than those exposed to fluoride alone and the effect of fTCP was dose dependent. The mechanism of action of fTCP on enhancing lesion uptake of fluoride is not fully understood but may be attributed to the ability of fTCP to promote fluoride-based nucleation.\textsuperscript{51}

Contrary to our findings in our first \textit{in vitro} study, we were able to demonstrate a significant linear correlation between \(\Delta \text{VHN}_{(\text{post– lesion})}\) and EFU. To our best knowledge, this is the first time a correlation was established between \(\Delta \text{VHN}\) and EFU in FV.
research. More work should be completed before using one of the variables as a predictive factor for the other.

In our clinical study we aimed to compare the differences in AUC of fluoride concentrations in saliva and plaque fluid following the application of three commercially available fluoride varnishes. All FVs had a common fluoride source of 5% NaF and the study did not experience any dropouts.

It is important to note that the literature is very scarce when it comes to clinical studies evaluating the pharmacokinetics of fluoride release into saliva and plaque post FV applications. Also, the available study models vary significantly making comparisons a difficult task. With that being said, a conscious effort was made to critically analyze related studies while designing our model. Also, we believe that we are the first to analyze both saliva and plaque fluid simultaneously in an attempt to find a correlation between fluoride concentrations in both release media.

The amount of CS applied was less than that of the other FVs. This was possibly due to the high viscosity of the varnish that resulted in less varnish being picked up by the application brush. However, when correlations were made, it was found that fluoride AUC measurements for centrifuged saliva and plaque fluid were not associated with the amount of varnish applied.

The fluoride release from FVs tested in our clinical study demonstrated different concentrations but similar release patterns for the collection periods of the study. All FVs resulted in peak fluoride levels in saliva and plaque fluid at 30 min post application followed by a steady decline. Most salivary and plaque fluid fluoride levels returned to
baseline by 24 h. The release pattern is very typical for FV release kinetics and compares well with findings from (Twetman et al., 1999) and (Eakle et al., 2004), where salivary fluoride levels peaked at 1 hr and 15 min after FV application respectively.\textsuperscript{32, 38}

The significantly lower levels of AUC from EP in both centrifuged saliva and plaque fluid maybe related to our subjective finding of its lower viscosity compared to the other FVs under investigation. This extends the findings of (Downey, 2013) were a relatively similar model was used.\textsuperscript{52} We found that EP adhered less to teeth in the process of application and is more likely to seep away from the application zone and therefore maybe swallowed before it reaches its fluoride release potential. This finding is inconsistent with EP’s behavior in our \textit{in vitro} investigations and with the literature as EP consistently released more fluoride \textit{in vitro} conditions.\textsuperscript{7, 10, 28} This is perhaps due to the closed system in an \textit{in vitro} model, where saliva has no pathway to exit the confined area of release. And this may lead us to conclude that EP has the capability of high fluoride release, though it is not sustained in the oral cavity under \textit{in vivo} conditions. Since the levels of fluoride in saliva and plaque fluid are indicative of the amount of fluoride that is bioavailable to interact with tooth structure and is of critical importance in producing an anti-caries effect, we can cautiously state that EP is less effective as a FV than the other tested products, however more research is required before clinical recommendations can be made.\textsuperscript{30, 31, 53}

Another noteworthy finding is the significantly higher plaque fluid fluoride AUC in subjects treated with V. This varnish contains fTCP, a calcium-phosphate ingredient that is well known to enhance fluoride activity by promoting its nucleation.\textsuperscript{51} The calcium added to the formulation possibly acts as a scavenger for fluoride and thereby aids in
increasing the amount of fluoride reservoirs in plaque fluid.\textsuperscript{54, 55} The bioavailability of fluoride in both saliva and plaque is essential in governing the caries process, however salivary fluoride levels quickly fall below levels of fluoride in plaque fluid thus making fluoride ions in plaque the prime factor in the demineralization/remineralization process.\textsuperscript{30, 56} It is important to note that plaque consists of multiple phases, namely plaque solid and plaque fluid. Our analysis focused on the latter and therefore the results and interpretations made here are based on our investigation of the plaque fluid phase only. Based on our recent findings and previous knowledge of factors predicting efficacy of topical fluoride applications and within the limitations of our study, we can conclude that V is likely to have more anti-caries potential compared to CS and EP, however, future evaluation is needed to support this finding.

The concentration of fluoride in saliva was rarely associated with plaque fluid fluoride at the examined time points. This is in disagreement with Vogel et al. that found a strong linear correlation between levels of fluoride in saliva and plaque fluid at 30 and 60 min after administration of NaF rinse.\textsuperscript{57} The high correlation may be attributed to the liquid nature of the rinse in contrast to the viscous formulation of FVs in our study that allows a faster dissociation of fluoride from saliva to plaque from the rinse.

One or all of the investigated variables, in our \textit{in vitro} and \textit{in vivo} studies, may predict the efficacy of FVs. However, it is impossible to foresee at this point the best predictive variable for clinical performance. There is a need to develop and validate clinical and laboratory models that will help us better understand the mode of action of FVs and predict clinical efficacy.
CONCLUSIONS
1. Our *in vitro* investigations revealed that FVs differed in their release characteristics rehardening capability, and ability to deliver fluoride to demineralized lesions.

2. Our *in vitro* studies showed significantly higher fluoride release in AS from FVs with an additional calcium phosphate source.

3. Our laboratory and clinical investigations demonstrated that fluoride release profiles were somewhat similar between FVs as all showed a gradual decrease in released fluoride over time.

4. In vitro models are not suitable for predicting fluoride release behavior and therefore may not be a good choice for studying the kinetics of fluoride release from FVs.

5. The observed differences *in vitro* and *in vivo* may be attributed to different compositions and the presence of other active ingredients besides fluoride in the FVs tested.
<table>
<thead>
<tr>
<th>Fluoride Varnish</th>
<th>Manufacturer</th>
<th>Fluoride Source and Concentration</th>
<th>Carrier</th>
<th>Other Active Ingredient</th>
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</thead>
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<tr>
<td>Enamel Pro</td>
<td>Premier Dental</td>
<td>5% NaF</td>
<td>Rosin</td>
<td>Amorphous calcium phosphate (ACP), Xylitol</td>
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<tr>
<td>Flor-Opal</td>
<td>Ultradent</td>
<td>5% NaF</td>
<td>Hydrogenated Rosin</td>
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<td>MI Varnish</td>
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<td>Casein phosphopeptide-ACP (CPP-ACP, Recaldent)</td>
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<tr>
<td>PreviDent</td>
<td>Colgate-Palmolive</td>
<td>5% NaF</td>
<td>Synthetic resin</td>
<td>Xylitol</td>
</tr>
<tr>
<td>Vanish</td>
<td>3M ESPE</td>
<td>5% NaF</td>
<td>Pentaerythritol glycerol ester of colophony resin</td>
<td>Functionalized tri-calcium phosphate (fTCP), Xylitol</td>
</tr>
</tbody>
</table>
**Table 2.** Daily pH cycling treatment schedule.

<table>
<thead>
<tr>
<th>Time</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00-8:01 a.m.</td>
<td>Toothpaste treatment</td>
</tr>
<tr>
<td>8:01-10:00 a.m.</td>
<td>Artificial saliva</td>
</tr>
<tr>
<td>10:00 a.m.-2:00 p.m.</td>
<td>Acid challenge</td>
</tr>
<tr>
<td>2:00-4:00 p.m.</td>
<td>Artificial saliva</td>
</tr>
<tr>
<td>4:00-4:01 p.m.</td>
<td>Toothpaste treatment*</td>
</tr>
<tr>
<td>4:01 p.m.-8:00 a.m.</td>
<td>Artificial saliva</td>
</tr>
</tbody>
</table>

*On the last day, this treatment was not given; the test ended with the AS treatment at 4 pm.*
### Table 3. Mean ΔVHN and SD.

<table>
<thead>
<tr>
<th>Fluoride Varnish</th>
<th>n</th>
<th>Mean ΔVHN (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enamel Pro</td>
<td>15</td>
<td>32.3 (5.8)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flor-Opal</td>
<td>18</td>
<td>20.4 (7.4)&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>MI Varnish</td>
<td>18</td>
<td>25.9 (12.5)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PreviDent</td>
<td>16</td>
<td>24.7 (6.2)&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vanish</td>
<td>16</td>
<td>18.9 (11.3)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Superscript letters represent significant differences of ΔVHN means.*
Table 4. Cumulative fluoride release and peak fluoride concentration (n=1).

<table>
<thead>
<tr>
<th>Fluoride Varnish</th>
<th>Cumulative Fluoride Release [µg/ml]</th>
<th>Peak Fluoride Concentration [µg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enamel Pro</td>
<td>216.7</td>
<td>76.9</td>
</tr>
<tr>
<td>Flor-Opal</td>
<td>153.0</td>
<td>56.7</td>
</tr>
<tr>
<td>MI Varnish</td>
<td>303.0</td>
<td>72.9</td>
</tr>
<tr>
<td>PreviDent</td>
<td>84.3</td>
<td>14.6</td>
</tr>
<tr>
<td>Vanish</td>
<td>27.6</td>
<td>4.2</td>
</tr>
<tr>
<td>Group</td>
<td>Product</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>-------</td>
<td>--------------------------------------------------</td>
<td>---------------------------------------</td>
</tr>
<tr>
<td>A</td>
<td>CavityShield 5% Varnish</td>
<td>3M ESPE</td>
</tr>
<tr>
<td>B</td>
<td>Vanish 5% NaF Varnish w/ TCP</td>
<td>3M ESPE</td>
</tr>
<tr>
<td>C</td>
<td>Colgate Duraphat Varnish</td>
<td>Colgate Oral Pharmaceuticals</td>
</tr>
<tr>
<td>D</td>
<td>Colgate Prevident Varnish</td>
<td>Colgate Oral Pharmaceuticals</td>
</tr>
<tr>
<td>E</td>
<td>Sparkle V Varnish</td>
<td>Crosstex International</td>
</tr>
<tr>
<td>F</td>
<td>Nupro 5% Fluoride Varnish</td>
<td>Dentsply Professional Division</td>
</tr>
<tr>
<td>G</td>
<td>Kolorz Clearshield Varnish</td>
<td>DMG America</td>
</tr>
<tr>
<td>H</td>
<td>MI Varnish</td>
<td>GC America</td>
</tr>
<tr>
<td>I</td>
<td>Duraflor Halo 5% Sodium Fluoride Varnish</td>
<td>Medicom</td>
</tr>
<tr>
<td>J</td>
<td>Enamel Pro Varnish Clear</td>
<td>Premier Dental</td>
</tr>
<tr>
<td>K</td>
<td>Vella Fluoride Varnish</td>
<td>Preventive Technologies</td>
</tr>
<tr>
<td>L</td>
<td>Butler White Fluoride Varnish</td>
<td>Sunstar Americas, Inc.</td>
</tr>
<tr>
<td>M</td>
<td>Flor-Opal Varnish White Fluoride Varnish</td>
<td>Ultradent</td>
</tr>
<tr>
<td>N</td>
<td>Waterpik UltraThin Varnish</td>
<td>Waterpik Technologies Inc.</td>
</tr>
<tr>
<td>O</td>
<td>Placebo Varnish</td>
<td>(Manufactured in house)</td>
</tr>
</tbody>
</table>
Table 6. Treatment groups for pH cycling phase

<table>
<thead>
<tr>
<th>Week</th>
<th>FV treatment groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A, B, C, D, E, O-</td>
</tr>
<tr>
<td>2</td>
<td>F, G, H, I, O-, O+</td>
</tr>
<tr>
<td>3</td>
<td>J, K, L, M, N, O-</td>
</tr>
</tbody>
</table>
Table 7. Daily pH cycling treatment schedule.

<table>
<thead>
<tr>
<th>Time</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:00-10:01 a.m.</td>
<td>Toothpaste treatment*</td>
</tr>
<tr>
<td>10:01-12:00 a.m.</td>
<td>Artificial saliva</td>
</tr>
<tr>
<td>12:01 a.m.-4:00 p.m.</td>
<td>Acid challenge</td>
</tr>
<tr>
<td>4:01-10:00 a.m.</td>
<td>Artificial saliva</td>
</tr>
</tbody>
</table>

*Specimens in placebo group did not receive this treatment and were stored in deionized water instead.
Table 8. ΔVHN \(_{\text{post} – \text{lesion}}\) Mean and SD (n=12).

<table>
<thead>
<tr>
<th>Fluoride Varnish</th>
<th>ΔVHN (_{\text{post} – \text{lesion}}) Mean (SD)</th>
<th>Statistical Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butler White</td>
<td>42.1 (9.2)</td>
<td>A,B,C</td>
</tr>
<tr>
<td>CavityShield</td>
<td>48.9 (10.8)</td>
<td>A,B</td>
</tr>
<tr>
<td>Duraflor Halo</td>
<td>39.4 (8.9)</td>
<td>A,B</td>
</tr>
<tr>
<td>Duraphat</td>
<td>46.0 (9.4)</td>
<td>A,B</td>
</tr>
<tr>
<td>Enamel Pro</td>
<td>46.0 (10.0)</td>
<td>A,D</td>
</tr>
<tr>
<td>Flor Opal</td>
<td>41.0 (7.4)</td>
<td>A,B,C</td>
</tr>
<tr>
<td>Kolorz</td>
<td>44.6 (9.3)</td>
<td>A,B</td>
</tr>
<tr>
<td>MI</td>
<td>42.2 (7.6)</td>
<td>A,B</td>
</tr>
<tr>
<td>Vella</td>
<td>39.6 (6.8)</td>
<td>A,B,C</td>
</tr>
<tr>
<td>Nupro</td>
<td>49.6 (10.8)</td>
<td>A</td>
</tr>
<tr>
<td>PreviDent</td>
<td>43.3 (9.8)</td>
<td>A,B,C</td>
</tr>
<tr>
<td>Sparkle</td>
<td>51.8 (16.6)</td>
<td>A,D</td>
</tr>
<tr>
<td>Vanish</td>
<td>49.2 (7.6)</td>
<td>A,D</td>
</tr>
<tr>
<td>Waterpik</td>
<td>49.8 (11.4)</td>
<td>A,B,C</td>
</tr>
<tr>
<td>O-a</td>
<td>29.3 (9.5)</td>
<td>C,D</td>
</tr>
<tr>
<td>O-b</td>
<td>28.3 (7.0)</td>
<td>B</td>
</tr>
<tr>
<td>O-c</td>
<td>28.3 (7.0)</td>
<td>B,D</td>
</tr>
<tr>
<td>O+</td>
<td>37.8 (8.7)</td>
<td>A,B,C</td>
</tr>
</tbody>
</table>

*Different letters highlight statistically significant differences.
Table 9. Cumulative fluoride release and peak fluoride concentration (n=12).

<table>
<thead>
<tr>
<th>Fluoride Varnish</th>
<th>Cumulative Fluoride Release [µg/ml] Mean (SD)</th>
<th>Peak Fluoride Concentration [µg/ml] Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butler White</td>
<td>0.50 (0.15)</td>
<td>0.17 (0.09)</td>
</tr>
<tr>
<td>CavityShield</td>
<td>1.97 (0.14)</td>
<td>1.28 (0.11)</td>
</tr>
<tr>
<td>Duraflor Halo</td>
<td>1.82 (0.54)</td>
<td>0.70 (0.22)</td>
</tr>
<tr>
<td>Duraphat</td>
<td>2.64 (0.53)</td>
<td>0.97 (0.12)</td>
</tr>
<tr>
<td>Enamel Pro</td>
<td>9.20 (1.71)</td>
<td>5.44 (1.05)</td>
</tr>
<tr>
<td>Flor Opal</td>
<td>8.20 (1.91)</td>
<td>4.37 (0.85)</td>
</tr>
<tr>
<td>Kolorz</td>
<td>2.91 (0.46)</td>
<td>1.00 (0.11)</td>
</tr>
<tr>
<td>MI</td>
<td>14.97 (2.38)</td>
<td>9.71 (1.40)</td>
</tr>
<tr>
<td>Vella</td>
<td>4.91 (2.02)</td>
<td>2.57 (2.17)</td>
</tr>
<tr>
<td>Nupro</td>
<td>6.96 (1.26)</td>
<td>1.69 (0.43)</td>
</tr>
<tr>
<td>PreviDent</td>
<td>2.82 (0.59)</td>
<td>0.98 (0.09)</td>
</tr>
<tr>
<td>Sparkle</td>
<td>2.19 (0.47)</td>
<td>0.95 (0.15)</td>
</tr>
<tr>
<td>Vanish</td>
<td>2.63 (0.53)</td>
<td>1.28 (0.09)</td>
</tr>
<tr>
<td>Waterpik</td>
<td>3.07 (0.81)</td>
<td>0.93 (0.30)</td>
</tr>
<tr>
<td>O-a</td>
<td>2.15 (0.56)</td>
<td>1.20 (0.30)</td>
</tr>
<tr>
<td>O-b</td>
<td>0.85 (0.17)</td>
<td>0.71 (0.16)</td>
</tr>
<tr>
<td>O-c</td>
<td>0.81 (0.55)</td>
<td>0.55 (0.51)</td>
</tr>
<tr>
<td>O+</td>
<td>0.49 (0.16)</td>
<td>0.37 (0.16)</td>
</tr>
</tbody>
</table>
Table 10. Clinical study test products.

<table>
<thead>
<tr>
<th>Fluoride Varnish</th>
<th>Manufacturer</th>
<th>Fluoride Source and Concentration</th>
<th>Carrier</th>
<th>Other Active Ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enamel Pro</td>
<td>Premier Dental</td>
<td>5% NaF</td>
<td>Rosin</td>
<td>Amorphous calcium phosphate (ACP), Xylitol</td>
</tr>
<tr>
<td>CavityShield</td>
<td>3M ESPE</td>
<td>5% NaF</td>
<td>Colophony, Polyamide Resin</td>
<td>N/A</td>
</tr>
<tr>
<td>Vanish</td>
<td>3M ESPE</td>
<td>5% NaF</td>
<td>Pentaerythritol glycerol ester of colophony resin</td>
<td>Functionalized tri-calcium phosphate (fTCP), Xylitol</td>
</tr>
</tbody>
</table>
Table 11. Treatment randomization schedule.

<table>
<thead>
<tr>
<th>Randomization number</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CavityShield</td>
<td>Vanish</td>
<td>Enamel Pro</td>
</tr>
<tr>
<td>2</td>
<td>Enamel Pro</td>
<td>Vanish</td>
<td>CavityShield</td>
</tr>
<tr>
<td>3</td>
<td>Vanish</td>
<td>Enamel Pro</td>
<td>CavityShield</td>
</tr>
<tr>
<td>4</td>
<td>Vanish</td>
<td>CavityShield</td>
<td>Enamel Pro</td>
</tr>
<tr>
<td>5</td>
<td>CavityShield</td>
<td>Enamel Pro</td>
<td>Vanish</td>
</tr>
<tr>
<td>6</td>
<td>Enamel Pro</td>
<td>CavityShield</td>
<td>Vanish</td>
</tr>
<tr>
<td>7</td>
<td>Vanish</td>
<td>CavityShield</td>
<td>Enamel Pro</td>
</tr>
<tr>
<td>8</td>
<td>Enamel Pro</td>
<td>Vanish</td>
<td>CavityShield</td>
</tr>
<tr>
<td>9</td>
<td>CavityShield</td>
<td>Enamel Pro</td>
<td>Vanish</td>
</tr>
<tr>
<td>10</td>
<td>CavityShield</td>
<td>Vanish</td>
<td>Enamel Pro</td>
</tr>
<tr>
<td>11</td>
<td>Enamel Pro</td>
<td>CavityShield</td>
<td>Vanish</td>
</tr>
<tr>
<td>12</td>
<td>Vanish</td>
<td>Enamel Pro</td>
<td>CavityShield</td>
</tr>
</tbody>
</table>
Table 12. Mean amount of treatment applied in g and SD

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>18</td>
<td>0.13 (0.04)$^a$</td>
</tr>
<tr>
<td>EP</td>
<td>18</td>
<td>0.24 (0.06)$^a$</td>
</tr>
<tr>
<td>V</td>
<td>18</td>
<td>0.27 (0.11)$^a$</td>
</tr>
</tbody>
</table>

*Different letters highlight statistically significant differences*
Table 13. Mean concentration (SE) of fluoride in whole saliva, centrifuged saliva and plaque fluid.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>CS</th>
<th>EP</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time</td>
<td>N</td>
<td>Mean (SE)</td>
</tr>
<tr>
<td>Whole Saliva F</td>
<td>AUC</td>
<td>17</td>
<td>83 (14)</td>
</tr>
<tr>
<td></td>
<td>BL</td>
<td>18</td>
<td>0.07 (0.03)</td>
</tr>
<tr>
<td></td>
<td>30min</td>
<td>17</td>
<td>20.86 (3.46)</td>
</tr>
<tr>
<td></td>
<td>60min</td>
<td>17</td>
<td>8.08 (1.02)</td>
</tr>
<tr>
<td></td>
<td>120min</td>
<td>18</td>
<td>5.57 (1.05)</td>
</tr>
<tr>
<td></td>
<td>24hr</td>
<td>17</td>
<td>0.05 (0.01)</td>
</tr>
<tr>
<td></td>
<td>48hr</td>
<td>16</td>
<td>0.07 (0.04)</td>
</tr>
<tr>
<td>Centrifuged</td>
<td>Saliva F</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td>17</td>
<td>51 (7)</td>
</tr>
<tr>
<td></td>
<td>BL</td>
<td>18</td>
<td>0.03 (0.01)</td>
</tr>
<tr>
<td></td>
<td>30min</td>
<td>18</td>
<td>18.77 (2.53)</td>
</tr>
<tr>
<td></td>
<td>60min</td>
<td>18</td>
<td>6.38 (0.77)</td>
</tr>
<tr>
<td></td>
<td>120min</td>
<td>17</td>
<td>3.15 (0.48)</td>
</tr>
<tr>
<td></td>
<td>24hr</td>
<td>18</td>
<td>0.02 (0.00)</td>
</tr>
<tr>
<td></td>
<td>48hr</td>
<td>17</td>
<td>0.07 (0.06)</td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>--------------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Plaque F</td>
<td>17 1237 (440) 6.16 (0.40)</td>
<td>17 525 (82) 6.05 (0.18)</td>
<td>18 1916 (468) 7.14 (0.22)</td>
</tr>
<tr>
<td>BL</td>
<td>17 0.2 (0.0) -1.83 (0.10)</td>
<td>18 0.8 (0.6) -1.68 (0.28)</td>
<td>17 0.5 (0.4) -1.79 (0.26)</td>
</tr>
<tr>
<td>30min</td>
<td>17 187.2 (41.9) 4.65 (0.34)</td>
<td>17 58.1 (9.2) 3.40 (0.45)</td>
<td>18 685.9 (205.7) 5.49 (0.49)</td>
</tr>
<tr>
<td>60min</td>
<td>17 127.0 (39.5) 3.32 (0.59)</td>
<td>18 43.2 (9.0) 2.85 (0.48)</td>
<td>18 171.4 (38.0) 4.69 (0.25)</td>
</tr>
<tr>
<td>120min</td>
<td>17 90.4 (35.0) 3.15 (0.52)</td>
<td>18 37.5 (6.4) 3.29 (0.24)</td>
<td>18 124.9 (33.5) 4.16 (0.31)</td>
</tr>
<tr>
<td>24hr</td>
<td>16 0.3 (0.1) -1.45 (0.18)</td>
<td>18 0.5 (0.1) -1.32 (0.23)</td>
<td>18 0.3 (0.1) -1.58 (0.14)</td>
</tr>
<tr>
<td>48hr</td>
<td>16 0.2 (0.0) -1.69 (0.11)</td>
<td>18 0.4 (0.2) -1.55 (0.23)</td>
<td>18 0.2 (0.0) -1.86 (0.13)</td>
</tr>
</tbody>
</table>
Figure 1. Mean change in surface microhardness ($\Delta$VHN) as a function of fluoride varnish treatment. Significant differences between varnishes are highlighted by different letters. Error bars denote standard deviations.
**Figure 2.** Fluoride release (log10 scale for better clarity) from fluoride varnishes into saliva as a function of time (n=1).
Figure 3. Enamel fluoride uptake (EFU; consecutive etches and combined data) as a function of fluoride varnish treatment. EFU was normalized per ml of acid etch solution. The dashed, horizontal line represents the lowest fluoride concentration of the calibration curve. Values lower than 0.01 µg/ml were calculated based on extrapolation of the calibration curve.
Figure 4. Illustration of 12 well microtiter plate lid with mounted acrylic blocks and specimens.
Figure 5. Enamel specimen (5 × 5 mm) with microhardness indentations and microdrill holes.
Figure 6. Mean change in surface microhardness ($\Delta VHN_{\text{post - lesion}}$) as a function of fluoride varnish treatment. Significant differences between varnishes are highlighted by different letters. Error bars denote standard deviations.
Figure 7. Mean change in surface microhardness ($\Delta VHN_{(art-lesion)}$) as a function of fluoride varnish treatment. Significant differences between varnishes are highlighted by different letters. Error bars denote standard deviations.
**Figure 8.** $F_{\text{total}}$ from fluoride varnishes into saliva as a function of time. Different letters highlight significant differences between varnishes. Error bars denote standard deviations.
Figure 9. $[\text{F}]_{\text{max}}$ the highest concentration of fluoride at any given time point as a function of fluoride treatment. Different letters highlight significant differences between varnishes. Error bars denote standard deviations.
Figure 10. Enamel fluoride uptake (EFU) as a function of fluoride varnish treatment.

Different letters highlight significant differences between varnishes. Error bars denote standard deviations.
**Figure 11.** Mean concentration of fluoride release into whole saliva from fluoride varnishes as a function of time (log10 scale for better clarity).
Figure 12. Mean concentration of fluoride release into centrifuged saliva from fluoride varnishes as a function of time (log10 scale for better clarity).
Figure 13. Mean concentration of fluoride release into plaque fluid from fluoride varnishes as a function of time (log10 scale for better clarity).
REFERENCES
52. Downey DJ. Salivary Fluoride Concentration Following the Application of Three Different 5% NaF Varnishes [Unpublished Thesis]: University of Michigan; 2013.
Laila Adel Al Dehailan

Educational Qualifications

2010-2016 PhD in Dental Science, Indiana University.

2010 Master of Science in Dentistry from Indiana University School of Dentistry (IUSD), major in Operative Dentistry, minor in Dental Materials and minor in Preventive Dentistry.

2005 Bachelor degree in Dental Surgery, Major: General Dentistry, B.D.S., King Saud University (KSU), Riyadh, Saudi Arabia.

Professional Experience:


2011-2015 Bench instructor for pre-doctoral dental students, Indiana University School of Dentistry (IUSD), Indianapolis, IN, USA.

2012-2013 Facilitator in Critical Thinking Skills (CTS) sessions for pre-doctoral dental students at Indiana University School of Dentistry.

2010-2011 Representative of PhD of Dental Sciences Program to Advanced Graduate Organization meetings at Indiana University School of Dentistry.

2009-2010 Representative of Graduate Operative Program to the Graduate Student Professional Conduct Committee at Indiana University School of Dentistry.

2008-2010 Representative of Graduate Operative Program to Advanced Graduate Organization meetings at Indiana University School of Dentistry.

2007-present Teaching assistant, Restorative Dental Sciences Department, King Faisal University, College of Dentistry, Dammam, Saudi Arabia.

2006-2007 Locum Staff Dentist at National Guard Health Affairs, Imam Abdulrahman Bin Faisal Program Hospital, Dammam, Saudi Arabia (Joint Commission International accredited).

2005-2006 Internship program, King Abdul Aziz Air base Hospital, Dhahran, Saudi Arabia.
Awards:

2016  Recipient of IUPUI Chancellor’s Scholars/ Chancellor’s Academic Honor Award.

2015  Recipient of IUPUI Chancellor’s Scholars/ Chancellor’s Academic Honor Award.

2010, 2011, 2013  Recipient of Educational Enhancement Grant from Indiana–University-Purdue University-Indianapolis, Indiana, USA.

2013  Recipient of Educational Enhancement Grant from Indiana–University-Purdue University-Indianapolis, Indiana, USA.

2008  Recipient of a full scholarship from King Faisal University, College of Dentistry to pursue graduate studies.

Presentations and Publications:


2015  Oral Presentation and published abstract, “In-Vitro Investigation of Anti-Caries Efficacy of Fluoride Varnishes”, International Association of Dental Research (IADR), Boston, MA.

2015  Poster Presentation, “In-Vitro Investigation of Anti-Caries Efficacy of Fluoride Varnishes”, IUSD Research day, Indianapolis, USA.

2015  Poster Presentation and published abstract. “Effects of Air-Abrasion Parameters and Aging Conditions of Zirconia Bonding”, International Association of Dental Research (IADR), Boston, MA.

2014  Oral Presentation and published abstract, “An In-Vitro Study to Determine the Anti-Caries Efficacy of Fluoride Varnishes” American Association of Dental Research (AADR), Charlotte, NC.

2013  Oral Presentation and published abstract, “The Effect of Nano-Filled Resin Coating on Fluoride Release in a New Conventional Glass Ionomer Cement” International Association of Dental Research (IADR), Seattle, WA.
2013 Poster Presentation, “An In-Vitro Study to Determine the Anti-Caries Efficacy of Fluoride Varnishes”. IUPUI Research day, Indianapolis, USA.

**Professional Memberships:**

2010-present Member of International Association for Dental Research & American Association for Dental Research (IADR/AADR).

2009-2010 Member of The Academy of Operative Dentistry, USA.

2007-2008 Member of the National Commission for Academic Accreditation and Assessment of the Higher Council of Education, Saudi Arabia.

2005-2008 Member of the Saudi Cancer Foundation, Saudi Arabia.

2005-2008 Member of Saudi Dental Society, Saudi Arabia.