Title: Fluoride Concentration in Saliva and Biofilm Fluid Following the Application of Three Fluoride Varnishes.

Short title: Fluoride in Saliva and Biofilm after Varnish Application.

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Abstract

Objective: Most of the commercially available fluoride varnishes (FV) have not been evaluated for their cariostatic properties. Consequently, the aim of this in vivo study was to investigate intra-oral fluoride retention and clearance patterns from three different FV.

Methods: Eighteen subjects (7-11 years) participated in a laboratory analyst-blinded, randomized, crossover study comparing the ability of 5% sodium fluoride varnishes (CavityShield-CS, Enamel Pro-EP, Vanish-V) to enhance fluoride concentrations in biofilm fluid, centrifuged and whole saliva over a period of 48h after a single FV application.

Results: Similar fluoride concentration × time patterns were noted for all investigated FV and studied variables, with the highest fluoride concentrations observed for the first biological sample collected after FV application (30min). Mean±SE (area under fluoride clearance curve) values were (µg F/g or ml × min): biofilm fluid – CS (472±191), EP (423±75), V (1264±279); centrifuged saliva – CS (42±7), EP (19±3), V (41±8); whole saliva – CS (68±11), EP (64±10), V (60±7). V delivered more fluoride to biofilm fluid than CS (p=0.0116) and EP (p=0.0065), which did not differ (p=0.27). For centrifuged saliva, CS and V were not significantly different (p=0.86), but resulted in higher fluoride

retention than EP ($p<0.0008$). No significant differences among FV were observed for whole saliva ($p=0.79$).

Conclusion: The present study has shown that FV vary in their ability to deliver fluoride intra-orally potentially related to formulation differences. To what extent the present findings relate to clinical efficacy remains, however, to be determined.

Key words: Fluoride varnish, saliva, biofilm fluid, caries.

Clinical Significance

Clinical research that investigates fluoride release patterns into saliva and biofilm fluid from different FV products is insufficient. More research is needed to investigate different FV formulations for their efficacy in order to help clinicians make better evidence based treatment choices.

Introduction

Topical fluorides have been shown to be efficacious in the prevention of caries [1] with fluoride varnishes (FV) containing 5% sodium fluoride being recommended as the primary choice for in-office dental caries prevention [2]. A large number of FV with varying fluoride concentrations and compounds, vastly different formulations and often containing ingredients being attributed cariostatic properties in their own right (e.g. xylitol, calcium compounds) are now commercially available. Comparative caries clinical

trials of these different FV, however, have not been conducted to date and only very few FV (three according to the present authors' review) have ever been evaluated for their efficacy clinically.[3]

As caries clinical trials are not only time- and resource-consuming but also put an unnecessary burden on participants, the study of clinically validated surrogate measures for efficacy is now commonly used by researchers. Current models on the cariostatic action of fluoride emphasize the significance of maintaining cariostatic levels of fluoride in oral fluids, namely saliva and dental biofilm [4]. Therefore, studying fluoride concentration increase in saliva and dental biofilm following the administration of topical fluoride is one way to relate to potential clinical efficacy as it can be indicative of fluoride levels in the aqueous phase available for interaction with the tooth structure during de- and remineralization [5, 6].

Very few studies on the kinetics of fluoride in saliva and biofilm following FV application have been reported. Two studies reported significant elevations in whole saliva fluoride concentration that persisted for up to 6h [7] or 24h [8], respectively. A fluoride dose-response was also established [7]. The sole study concerned with fluoride in biofilm was able to demonstrate slightly increased fluoride concentrations for up to 30 days after application which depended on the type of varnish. This study also suggested different patterns of fluoride retention for different varnishes [9]. The two comparative studies [7, 9], however, investigated FV of different fluoride concentrations and compounds (i.e. 5% sodium fluoride [2.26% F], difluorosilane [0.1% F], sodium and calcium fluoride [6% F]), thereby limiting conclusions that can be drawn.

As there is a considerable gap in our understanding of how different FV formulations of identical fluoride concentration affect intra-oral fluoride retention, the present study aimed to investigate fluoride concentrations in biofilm, centrifuged and whole saliva as surrogate measures for cariostatic efficacy after a single application of three inherently different 5% sodium fluoride FV over a period of 48h after a single application.

Subjects, Materials and Methods

Ethical Aspects and Subjects

The study protocol for this laboratory analyst-blind, randomized, cross-over, three-period study was reviewed and approved by the Indiana University Institutional Review Board, #1409221212. The study was conducted at Little Flower Catholic School, Indianapolis, IN, USA, in children age seven to eleven years. Informed consent (parents) and assent (children) were obtained from all study subjects prior to screening. All subjects received oral soft and hard tissue examinations throughout the study. In order to participate in the study, subjects had to meet the following inclusion criteria: be between seven and eleven years old, have good general and oral health, have at least 16 teeth with no cavitated carious lesions, and have no oral soft tissue lesions or active periodontal disease including severe gingivitis. Subjects also had to understand and be willing and able to comply with the instructions provided during the study which included abstention from eating for one hour prior to and for the two-hour duration of the test visit. Potential subjects were excluded if they had known or suspected allergy or hypersensitivity to FV or any of their listed ingredients, were taking fluoride supplements or other fluoride

products for medical purposes (except for fluoride naturally occurring in the diet), and were taking prescription antibiotics.

Power Calculation

Based on a previous study on adults [8], with a sample size of 16 subjects, the study had a 80% power to detect a difference of 1.5 for log (area under the curve [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 was 2.0. To account for 10% dropout, the study enrolled 18 subjects.

Fluoride Varnishes and Washout Toothpaste

No experimental fluoride varnish was used in this study. Fluoride varnishes contained a standard fluoride level of 5% sodium fluoride, were supplied in single dose packages, purchased from a provider of professional healthcare products and used at least six months prior to expiration. Fluoride concentration of the FV was not determined prior to use. FV packages were weighed before and after treatment application in order to calculate the amount of varnish applied. Pertinent information about the three FV used in this study can be found in Table 1. Subjects were asked to use fluoride-free toothpaste (Fluoride Free Children's Toothpaste, Tom's of Maine, Kennebunk, ME, USA) for a washout period of two 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 reported that baseline fluoride values returned to values that are very close to baseline following a washout period of two weeks [8].

Randomization Procedures and Blinding

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. Due to the uniqueness of each FV (color, flavor, and handling properties) the study investigator had the capability to discriminate between varnishes and was therefore blinded only 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 eligible subjects were 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 at home on any morning a biofilm sample was collected.

Subjects provided a baseline (BL) five-minute, non-stimulated saliva sample, followed by collection of an interproximal/buccal biofilm 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, and third/lingual of anterior teeth. The exact amount applied clinically was not standardized per se, but care was taken to cover all aforementioned tooth surfaces and the weight of applied FV determined (see above). The FV treatment was allowed to set and immediately thereafter, saliva samples were collected at 30, 60, and 120 min, and at 24h and 48h following the treatment. Approximately 1 mg of interproximal/buccal biofilm was collected immediately after each saliva sample. Subjects remained at school

throughout the treatment visits. At the end of each visit, 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, OH, USA). A two 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 were collected at baseline and immediately following treatment at 30, 60, and 120 min and at 24h and 48h. 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 were 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-minute collection period all remaining saliva was expectorated into the plastic vial. Saliva samples were stored at -20°C for later fluoride analysis.

Biofilm Collection Procedure

Immediately before dental biofilm collection, subjects were instructed to swallow all remaining saliva and keep their mouth open. Approximately 1 mg of dental biofilm was collected from the interproximal and buccal surfaces of teeth of all four quadrants. Biofilm samples were collected using a standardized protocol. Pooled biofilm samples were collected using a stainless steel periodontal scaler (S. McCall 17/18, Hu- Friedy, Illinois, USA) 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 biofilm sample was transferred into a plastic strip.

Biofilm Sample Preparation

Prior to biofilm sample collection, special centrifuge tubes were constructed by heat sealing 10 microliter (µl) micropipette tips. They were filled with heavy mineral oil (Mineral Oil, Heavy (USP/FCC) Fisher Chemical, Fisher Scientific, USA). Microcentrifuge tubes containing the plastic strip and biofilm sample were centrifuged for 10 min at 10,000 rpm (4,000*g*) at 4°C [10]. Partially oil-filled fine glass micro pipettes were used to recover small aliquots (approx. 5 nl) from the centrifuged tube under a microscope.

Biofilm Fluid Fluoride Analysis

The micro analytical method was used to analyze biofilm fluid samples for fluoride content [11]. Samples were placed, under mineral oil, on the surface of a specially constructed inverted F electrode. Mineral oil was used to prevent evaporation. Total ionic strength adjusting solution (TISAB III) was added to the samples in a ratio of 9:1. 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 biofilm fluid sample. Biofilm fluid fluoride was expressed as µg F/ml which was calculated by comparison to a standard fluoride curve, constructed the same day of the analysis.

Saliva Analysis

Each saliva sample was analyzed as whole and centrifuged saliva for fluoride concentration. A 1.4 ml aliquot of each thawed and vortexed saliva sample was centrifuged for 10 min at 10,000 rpm (4,000 *g*) at 7°C (centrifuged saliva) or analyzed for fluoride content without centrifugation (whole saliva). Analysis of saliva was conducted using a modification of the hexamethyl-disiloxane (HMDS) microdiffusion method [12] as modified recently [13]. 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), 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 (H_2SO_4) 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 NaOH. The trap was recovered and buffered to pH 5.2 with 25 μl acetic acid (CH_3COOH.). 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.

Statistical Analysis

For saliva, a second analyst examined 10% of the samples to assess inter-examiner agreement. 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 were made if the treatment main effect was 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 natural log-transformed data to satisfy the model assumptions, and results are presented after transformation back to the original scale. 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. No adverse events were reported. After all biological samples were collected and analyzed for fluoride, a thorough data review was conducted and potential outliers due to, for example, protocol violations, identified. Two subjects were most likely exposed to a fluoride source other than the applied FV close to saliva sampling at 24h. Data analyses

were performed with and without potential 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) of the fluoride measurements were both acceptable. The weight of applied FV (Table 2) varied between products, as considerably less CS was necessary to coat all tooth surfaces than EP and V ($p<0.0001$), however, applied amounts of EP and V were not different from each other ($p=0.23$).

Fluoride Data

Fluoride levels found in biofilm fluid, centrifuged and whole saliva are shown in Figure 4. The baseline fluoride values ranged from 0.20 to 0.80 ppm in biofilm fluid and from 0.02 to 0.03 ppm in centrifuged saliva. In general, all values returned to baseline or close to baseline levels 24h after FV treatments.

Fluoride Retention (Area Under the Curve; AUC)

The fluoride levels found over time can be found in Figure 1 (biofilm fluid), Figure 2 (centrifuged saliva) and Figure 3 (whole saliva). V had significantly higher biofilm fluid fluoride AUC than CS and EP ($p<0.02$) but CS and EP were not significantly different from each other. EP had significantly lower centrifuged saliva fluoride AUC than CS and V ($p<0.0008$) but CS and V had nearly identical values and were not significantly different from each other ($p=0.86$). No significant treatment effect was found for whole saliva fluoride AUC ($p=0.79$).

Time Point Comparisons

Comparisons among FV at specific time points were also investigated. The time-by-treatment interaction was significant only for biofilm fluid fluoride and centrifuged saliva. At 30 minutes, 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 minutes 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 minutes ($p=0.11$), 24 hours ($p=0.55$), or 48 hours ($p=0.40$). EP had significantly lower centrifuged saliva fluoride than CS and V at 30 min ($p\leq0.0033$), at 60 min ($p<0.0001$), and at 120 min ($p\leq0.005$); EP was not significantly different ($p\geq0.49$) from CS or V at baseline, 24h, or 48h. CS and V did not have significantly different centrifuged saliva fluoride regardless of time ($p=0.89$).

Centrifuged Saliva

For all FV under investigation, centrifuged saliva fluoride was significantly higher at 30 min than at any other time ($p\leq0.0001$), followed by 60 min ($p\leq0.0001$) and 120 min ($p\leq0.0001$), with no significant differences among baseline, 24h, and 48h.

Biofilm Fluid

It was found that at 30 min EP had significantly lower biofilm fluid fluoride than CS and V ($p<0.05$) but CS and V were not different from each other ($p=0.16$). At 60 min, EP and CS had significantly lower biofilm fluid fluoride than V but EP and CS were not different from each other. No treatment effect was found at baseline ($p=0.88$), 120 min ($p=0.11$), 24h ($p=0.55$), or 48h ($p=0.40$). For CS, biofilm 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, 24h, and 48h ($p=0.19$ for baseline vs. 24h, $p=0.59$ for baseline vs. 48h, $p=0.41$ for 24 vs. 48h) or between 60 and 120 min ($p=0.70$). For EP, biofilm fluid fluoride was significantly higher for 30, 60, and 120 min compared to baseline, 24h, and 48h ($p<0.0001$), while there were no differences among baseline, 24h, and 48h ($p=0.18$ for baseline vs. 24h, $p=0.65$ for baseline vs. 48h, $p=0.33$ for 24 vs. 48h) 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, biofilm fluid fluoride was significantly higher for 30, 60, and 120 min compared to baseline, 24h, and 48h ($p<0.0001$), and higher for 30 than 120 min ($p=0.0030$), while there were no differences among baseline, 24h, and 48h ($p=0.44$ for baseline vs. 24h, $p=0.82$ for baseline vs. 48h, $p=0.24$ for 24 vs 48h) 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 strongly correlated for CS ($r=0.84$; $p=<.0001$), EP ($r=0.82$; $<.0001$), and V ($r=0.88$; $<.0001$). Biofilm 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 biofilm fluid fluoride was rarely associated with whole or centrifuged saliva fluoride at the individual time points.

Whole saliva fluoride AUC was moderately correlated (r=0.62) with the amount of FV applied for EP. None of the other fluoride AUC measurements were associated with the amount of FV applied.

Discussion

The present in vivo study aimed to compare fluoride retention and clearance from three commercially available FV. The design took into account previous findings [7, 9] by simultaneously studying fluoride in biofilm and saliva rather than in isolation. The study was conducted in children (7-11 years) as they represent the population in which FV are being commonly used for caries prevention. The FV were chosen based on their varying performances in a range of in vitro studies [14, 15], and because of their different composition (Table 1). They present, however, comparable amounts of active ingredient as all contained 5% sodium fluoride (equates to 2.26% or 22,600 ppm or 1.19 M fluoride). The 'clinical reference' FV, Duraphat, was not included as it was not commercially available in single dosage packaging at the time the study was planned and conducted, and as a recent in vivo pilot study showed virtually no difference in fluoride release into centrifuged and whole saliva compared to V [16, 17]. The choice of biomarkers for cariostatic efficacy, fluoride in biofilm fluid and centrifuged saliva, was based on the mode of action of other topical fluorides [4, 6]. Fluoride in whole saliva was also investigated to understand the potential role of formulation components (e.g. calcium compounds) on fluoride sequestration in line with a recent study [18]. The most commonly used biomarker for FV efficacy in the past was enamel fluoride uptake [19, 20]. However it was not studied here due to its invasive nature and as the present study

was also concerned with fluoride clearance patterns in addition to its retention. It should also be noted that the role of tooth-bound fluoride in the caries process is limited especially in relation to extrinsically provided fluoride, such as that present in biofilm fluid and therefore the choice had been made to study this variable [21].

The present study has shown that commercially available FV differ in their ability to fluoridate oral reservoirs. An approximate three-fold difference in mean biofilm fluid fluoride AUC was noted between two of the tested FV (V vs. EP) which was also mirrored, albeit to a lesser extent, in centrifuged saliva. Fluoride release patterns into biofilm fluid and saliva were, however, very similar among FV with the highest fluoride concentration being observed for the first post-application sample that was collected. Likewise, no differences were observed for whole saliva.

The significantly lower levels of fluoride in both centrifuged saliva and biofilm fluid from EP may be related to the authors' subjective finding of its lower viscosity compared to the other FV under investigation (viscosity differences also explain the varying amounts of applied FV). EP adhered less to teeth in the process of application, is therefore more likely to seep away from the application zone and, consequently, may be swallowed before all of its fluoride is released. Another potential explanation could lie in its composition (Table 1). EP contains calcium and phosphate providing a source of ionic calcium. Upon contact of EP with saliva, calcium and fluoride are likely to be ionized and capable of forming compounds of poor solubility. This may explain the present results for whole saliva which did not mirror those of centrifuged saliva. Analysis of biofilm solids, not conducted presently, could have further confirmed this hypothesis.

We must keep in mind that analysis of the supernatant after centrifuging saliva has the advantage of eliminating any protein globules that act as an interference in the highly sensitive F ion selective electrode.[22] As we can see from our own findings that differences between FV were hard to assess when whole saliva was investigated in comparison to centrifuged saliva. However, we must keep in mind that while we are eliminating interferences, we are missing a significant portion of fluoride that resides in the sediment. Therefore, some researches prefer to analyze the sediment in addition to analyzing the supernatant.[23]

The findings for EP in comparison to the other tested FV highlight discrepancies between laboratory [14, 24, 25] and the present in vivo observations. Our results are in agreement with Downey et al. where we both believe that EP's consistency specifically its low viscosity resulted in rapid dissociation of the aforementioned FV in the oral environment before it could reach its potential. This is in contrast with its behavior in laboratory settings where we believe that EP was more effective in fluoride release due to the controlled and confined settings in an in vitro experimental design.[3] Because of this, caution is recommended when drawing conclusions about clinical efficacy from in vitro studies. However, even the present clinical findings need to be validated as surrogate measures for clinical efficacy for caries prevention. Our understanding of the mode of action of FV is still poor and is largely derived from other topical fluorides which are applied more frequently and contain significantly less fluoride, highlighting the need for longitudinal, mechanistic studies.

It should be noted here that FV was not directly applied to areas from which biofilm was later collected (in line with a recent mechanistic study [26]; i.e. fluoride had

to migrate from FV applied in the vicinity of biofilm to the collection sites. While this may be considered a flaw in the study design, the prolonged retention of FV on the tooth surface makes it otherwise impossible to study immediate effects of FV on biofilm fluoridation. However, as FV are rather viscous their penetration into pits, fissures and to proximal surfaces is limited. Fluoride delivered to these areas from FV is likely due to migration, thereby supporting the presently chosen design.

Another noteworthy finding of the present study is the significantly higher biofilm fluid fluoride AUC for V. This varnish contains fTCP, a promising calcium compound to promote enamel fluoridation and remineralization [27]. The calcium added to the formulation possibly acts as a scavenger for fluoride and thereby may aid in increasing the amount of fluoride reservoirs in biofilm fluid [28, 29]. Although similar results could have been expected between V and EP, fTCP is only sparingly soluble thereby limiting the release of ionic calcium. Inherent formulation differences between EP and V may further explain their different abilities to deliver fluoride, albeit to an unknown extent. In hindsight, a study on experimental FV formulations varying in fewer formulation parameters than those evaluated presently would have been advantageous as firmer conclusions about the potential role of formulation excipients could have been drawn. However, the tested FV are commercially available and being used by dental professionals in the prevention of caries in their patients, thereby adding translational value to the present study.

Fluoride release patterns somewhat mirrored those of other topical fluoride delivery vehicles [30]. A greater tenacity for fluoride retention in comparison to dentifrices was noted nonetheless which may be explained by the varnish excipients

providing some sort of a slow-release delivery system. Pharmacokinetic modeling was, however, not considered presently as no data were collected between 0 and 30 min, and 2 and 24h time points. The concentration of fluoride in saliva was only moderately correlated with biofilm fluid fluoride at the examined time points. A previous study found a strong linear correlation between fluoride concentrations in saliva and biofilm fluid at 30 and 60 min after administration of a sodium fluoride rinse [31]. The strong correlation may be attributed to differences in the delivery method. Rinses are swished in the mouth and thereby interact to a greater extent with the oral soft tissues, a major site for fluoride retention [32], whereas FV are applied only to the teeth and the fluoride needs to be released from a resin matrix.

The present study has shown that FV vary greatly in their ability to deliver fluoride intra-orally which may be explained by differences in their formulations. To what extend the present findings relate to clinical efficacy remains, however, to be determined. In light of the aforementioned and present clinical findings, there is an urgent need to not only develop efficacy guidelines for FV to set minimum standards but also to support research on how to further improve FV formulations.

Declaration of interests

The authors declare that they have no conflict of interest. The present study was solely funded through Indiana University School of Dentistry Research Incentive Funds.

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Legends

Figure 1. Mean concentration of fluoride release into biofilm fluid from fluoride varnishes as a function of time (log10 scale for better clarity). Mean +/- SE.

Figure 2. Mean concentration of fluoride release into centrifuged saliva from fluoride varnishes as a function of time (log10 scale for better clarity). Mean +/- SE.

Figure 3. Mean concentration of fluoride release into whole saliva from fluoride varnishes as a function of time (log10 scale for better clarity). Mean +/- SE.

Figure 4. Means and standard errors of fluoride concentrations (AUC) in biofilm fluid, centrifuged and whole saliva (log10 scale for better clarity).

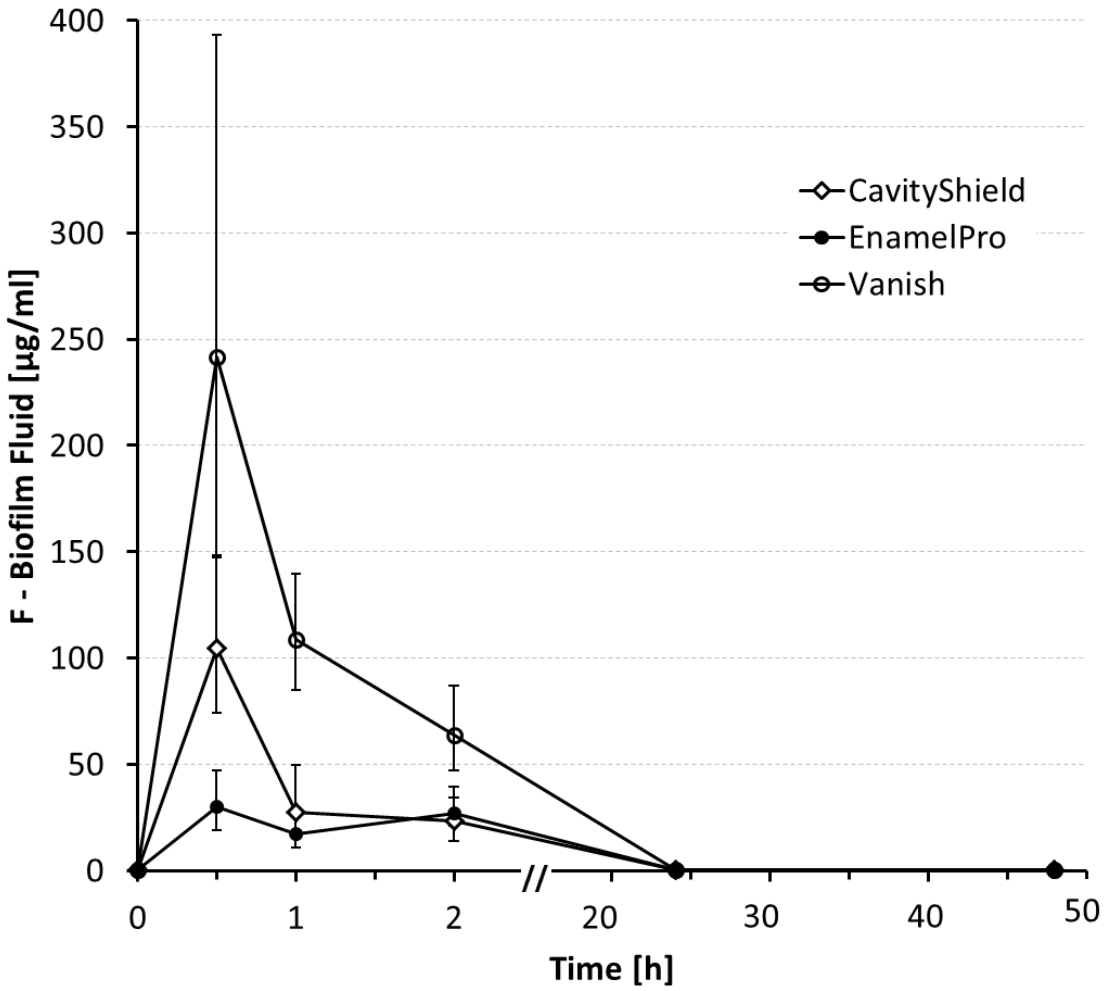


Figure 1

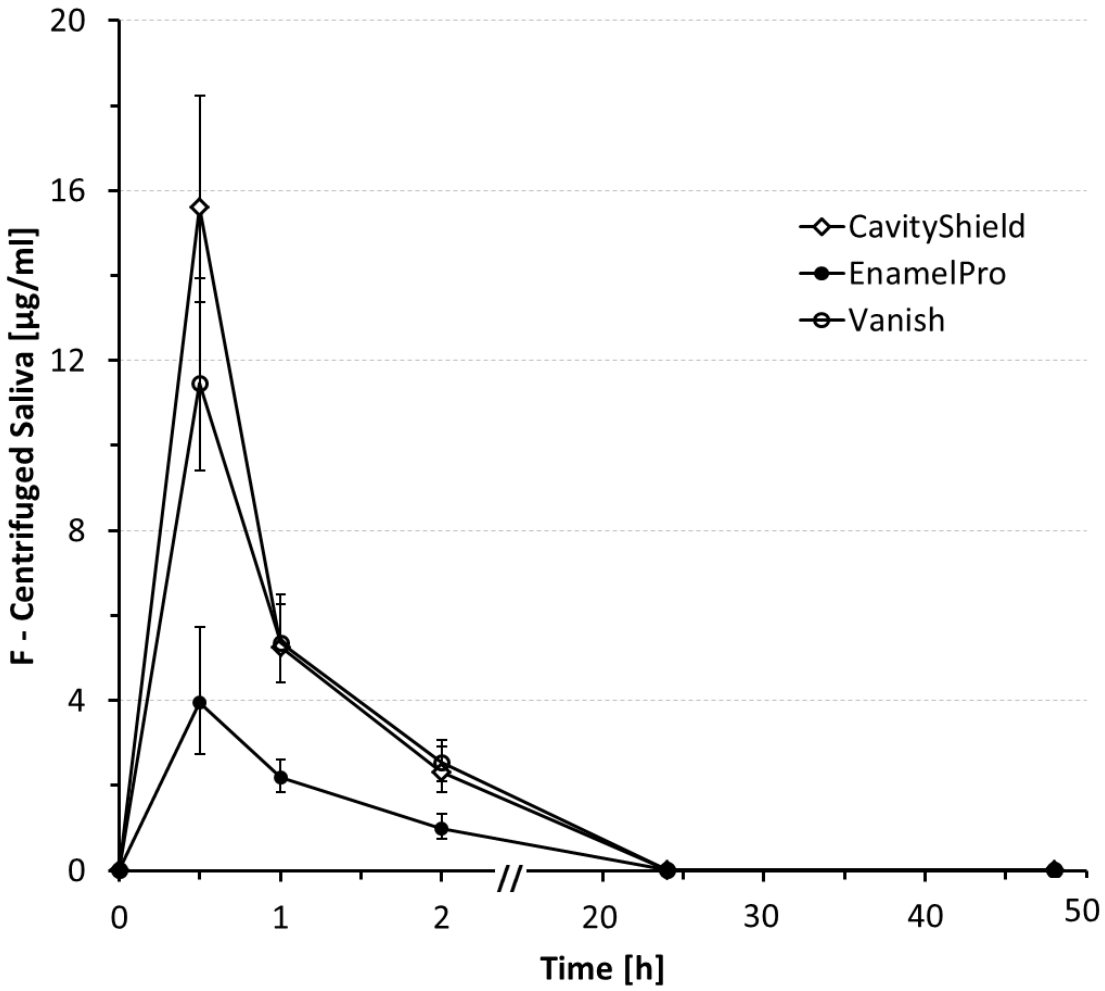


Figure 2

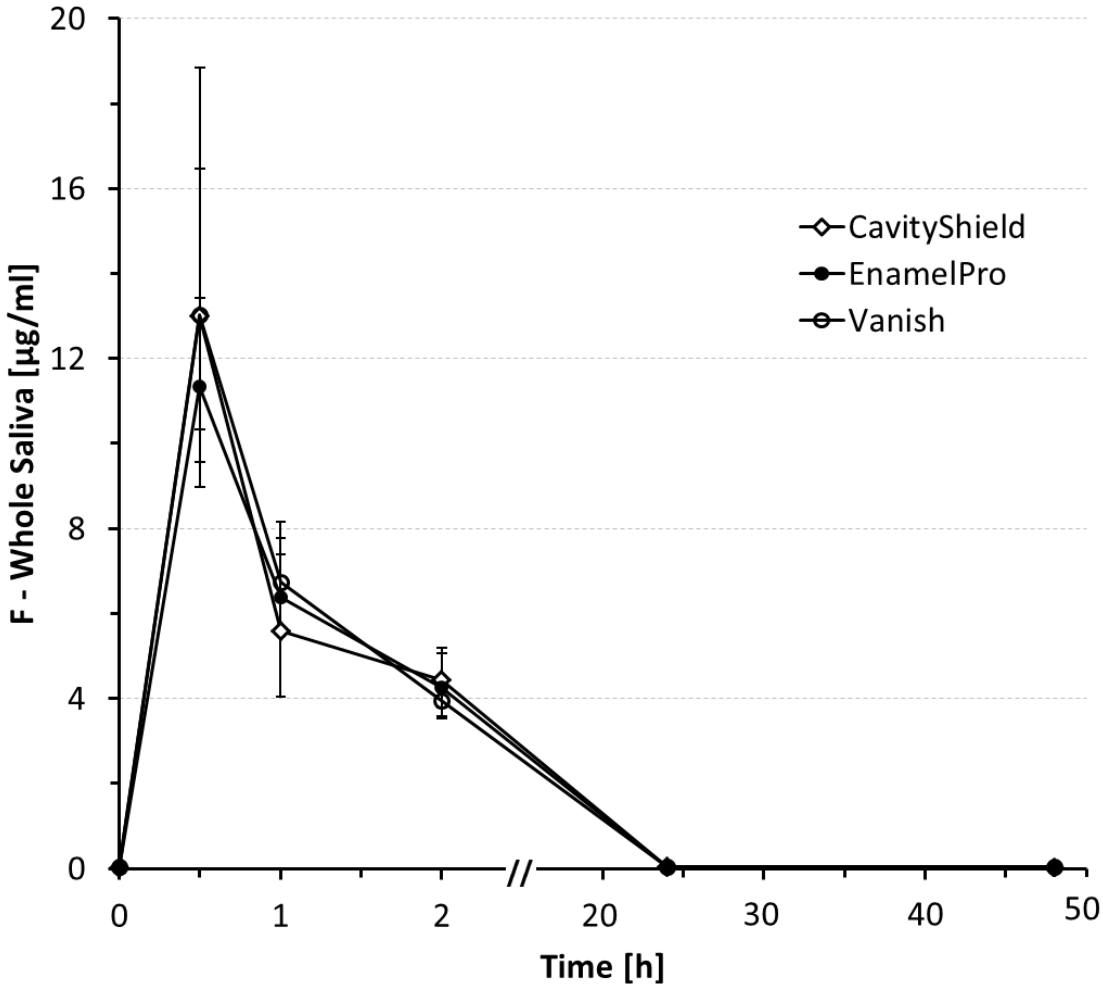
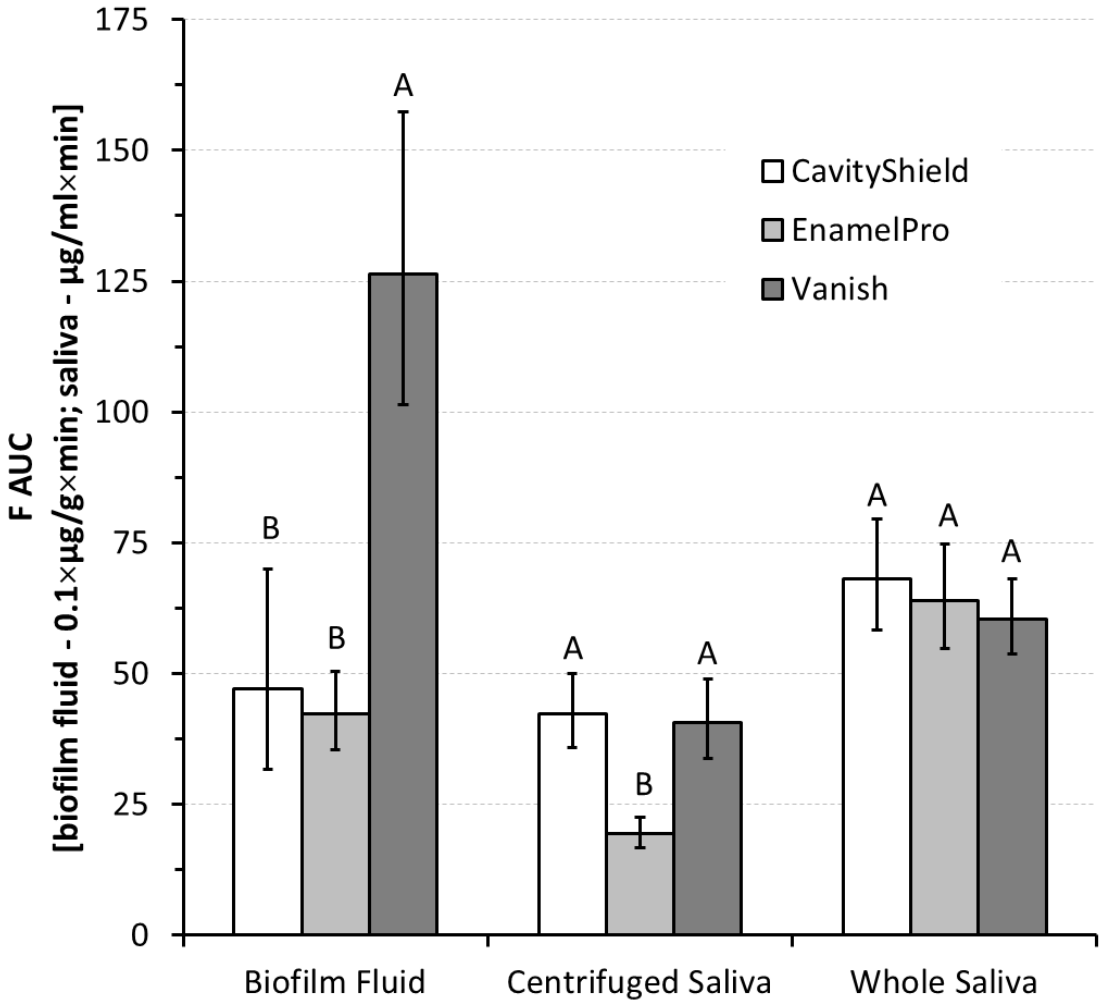


Figure 3



Error bars = standard errors

Different letters highlight statistically significant differences

Figure 4

Table 1. Test products.

Fluoride Varnish	Manufacturer	Fluoride Source and Concentration	Carrier	Other Active Ingredient
Enamel Pro	Premier Dental	5% NaF	Rosin	Amorphous calcium phosphate (ACP), Xylitol
CavityShield	3M ESPE	5% NaF	Rosin, Polyamide Resin	N/A
Vanish	3M ESPE	5% NaF	Pentaerythritol glycerol ester of Rosin	Functionalized tri-calcium phosphate (fTCP), Xylitol

Table 2. Mean amount of treatment applied in grams and SD

Table 2. Mean amount of FV applied per subject

Fluoride Varnish	N	Grams, Mean (SD)
CS	18	0.13 (0.04)[b]
EP	18	0.24 (0.06)[a]
V	18	0.27 (0.11)[a]

Different letters highlight statistically significant differences